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EFFECTS OF INTENSE EXERCISE ON CHANGE OF FUNCTION AND PATHOLOGY OF LIVER AND PANCREAS IN RATS

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A Thesis Submitted in Partial Fulfillment of the Requirements

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การศึกษานี้เพื่อศึกษาผลของ การเพิ่มขึ้นของความหนักของการออกกำลังกายอย่างหนักแบบเฉียบพลันที่ส่งผลต่อการเปลี่ยนการทำงานและพยาธิสภาพในตับและตับอ่อนในหนู โดยทำการทดลองในหนูขาวเพศผู้สายพันธุ์ Spraque-Dawley แบ่งโดยการสุ่มออกเป็น 5 กลุ่ม กลุ่มที่ 1 คือกลุ่มควบคุมปกติที่ไม่ได้รับการออกกำลังกาย กลุ่มที่ 2 คือกลุ่มที่ออกกำลังกายโดยการวิ่งบนลู่วิ่งที่ความหนัก 75% ของประสิทธิภาพการใช้ออกซิเจนสูงสุด (VO_{2max}) และเก็บตัวอย่างทันทีหลังเสร็จสิ้นการออกกำลังกาย กลุ่มที่ 3 คือกลุ่มที่ออกกำลังกายโดยการวิ่งบนลู่วิ่งที่ความหนัก 75% VO_{2max} ทำการเก็บตัวอย่างภายหลังการออกกำลังกายเป็นเวลา 6 ชั่วโมง กลุ่มที่ 4 คือกลุ่มที่ออกกำลังกายโดยการวิ่งบนลู่วิ่งที่ความหนัก 90% VO_{2max} และเก็บตัวอย่างทันทีหลังเสร็จสิ้นการออกกำลังกาย กลุ่มที่ 5 คือกลุ่มที่ออกกำลังกายโดยการวิ่งบนลู่วิ่งที่ความหนัก 90% VO_{2max} ทำการเก็บตัวอย่างภายหลังการออกกำลังกายเป็นเวลา 6 ชั่วโมง เมื่อสิ้นสุดการทดลองจะทำการเก็บตัวอย่างเลือด ตับ และตับอ่อน ผลการทดลองพบว่าระดับของ alanine aminotransferase (ALT) ในหนูกลุ่มที่ 2 และกลุ่มที่ 4 และ ระดับ aspartate aminotransferase (AST) ในหนูกลุ่มที่ 4 เพิ่มสูงขึ้นอย่างมีนัยสำคัญ เมื่อเทียบกับกลุ่มควบคุมปกติ ระดับของเอนไซม์ lipase เพิ่มสูงขึ้นอย่างมีนัยสำคัญในหนูกลุ่มที่ 4 เมื่อเทียบกับกลุ่มควบคุมปกติ พยาธิสภาพของตับ ในหนูกลุ่มที่ออกกำลังกายทุกกลุ่ม พบว่า เซลล์ตับบวม มีการอักเสบ และการตายของเซลล์ตับเมื่อเทียบกับกลุ่มควบคุมปกติ พยาธิสภาพของตับอ่อน ในหนูกลุ่มที่ออกกำลังกายทุกกลุ่ม พบว่า มีการบวมของเซลล์ตับอ่อน เมื่อเทียบกับกลุ่มควบคุมปกติ

ผลการทดลองครั้งนี้สรุปได้ว่า การออกกำลังกายอย่างเฉียบพลันที่ระดับความหนัก 75% และ 90% VO_{2max} ส่งผลให้ระดับเอนไซม์ในตับ ตับอ่อนเพิ่มขึ้น และเกิดการเปลี่ยนแปลงของพยาธิสภาพในเซลล์ตับ และตับอ่อนร่วมด้วย

สาขาวิชา.....เวชศาสตร์การกีฬา.....ลายมือชื่อนิสิต.....

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ADVISOR : ASSOC.PROF. DUANGPORN THONG-NGAM, M.D.,

CO-ADVISOR : ASSOC.PROF. ONANONG KULAPUTANA, M.D., Ph.D., 81 pp.

The study was conducted to investigate the effect of two acute exercise intensities on changes of function and pathology in liver and pancreas. Male Sprague-Dawley rats were randomly divided into five groups; Group 1 (normal control): no exercise, Group 2 (exercise 75% VO_2max): running on treadmill at 75% VO_2max and sacrified immediately after exercise, Group 3 (exercise 75% VO_2max + 6 hours group): running on treadmill at 75% VO_2max and sacrified at six hours after exercise, Group 4 (exercise 90% VO_2max): running on treadmill at 90% VO_2max and sacrified immediately after exercise, Group 5 (exercise 90% VO_2max + 6 hours group): running on treadmill at 90% VO_2max and sacrified at six hours after exercise. Blood, liver and pancreas samples were collected at the end of the study. The results showed that level of aspartate aminotransferase (AST) in Group 4 and alanine aminotransferase (ALT) in Group 2 and Group 4 were increased significantly as compared with normal control. Serum enzyme lipase was increased significantly in Group 4 as compared with normal control. Liver histopathology in all groups of exercise showed hepatocyte edema and necroinflammation. Pancreas showed congestion and edema in all groups of exercise.

Our data indicated that high-intensity exercise at 75% and 90% VO_2max caused the increase of serum and enzyme in liver and pancreas. In addition,such levels of exercise also caused histopathology change in liver and pancreas.

Field of study.....Sports Medicine..... Student's Signature.....

Academic year.....2008..... Advisor's Signature.....

Co-Advisor's Signature.....

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LIST OF ABBREVIATIONS

ALT	=	Alanine aminotransferase
AST	=	Aspartate aminotransferase
BW	=	Body weight
GCs	=	Glucocorticoid hormones
GH	=	Growth hormone
IGF-1	=	Insulin-like growth factor–1
SD	=	Standard deviation of the mean
TB	=	Total bilirubin
VO_{2max}	=	Maximal oxygen consumption

CHAPTER I

INTRODUCTION

Background and Rationale

The liver is an organ present in vertebrates and some other animals. It plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, and detoxification. This organ also is the largest gland in the human body. It weighs about 3 lb (1.36 kg) (Buranasiri, 1982). It lies below the diaphragm in the thoracic region of the abdomen. It has an integral role in the physiology of exercise. This organ supplies energy substrates to peripheral tissues via the Cori cycle and glycogen catabolism, plays a central role in lipid metabolism (producing ketone bodies by oxidation of fatty acids), and is important for detoxification. In addition, the liver releases many biologically active molecules into the circulation such as growth factors. Furthermore, almost all nutrients are carried to the liver by the portal circulation immediately after absorption from the intestine, and then are distributed throughout the body after hepatic processing (Aoi et al., 2004).

The pancreas is a glandular organ in the digestive and endocrine systems of vertebrates. In human, the pancreas is a yellowish organ about 7 inches (17.8 cm) long and 1.5 inches. (3.8 cm) wide. It lies beneath the stomach and is connected to the small intestine at the duodenum (Roysomut, 1992). It is both exocrine (secreting pancreatic juice containing digestive enzymes) and endocrine (producing several important hormones, including insulin, glucagon, and somatostatin) (Kritsanama, 1995).

Physical exercise increases the blood flow of working skeletal muscles, while it decreases the total hepatic blood flow (Rowell et al., 1964) and portal venous blood flow (Ohnishi et al., 1985) as well. Hepatic microcirculation is regulated by pre- and post-portal mechanisms. The liver receives the majority of its blood supply from the portal vein rather than the hepatic artery. The blood flow through the portal vein reflects the blood flow through the splenic vein from the spleen and the inferior and the superior mesenteric veins from the intestine. These splanchnic organs are vasoconstricted by the release of catecholamine (Pawlik et al., 1976).

During and high intensity exercise hepatic microcirculation was regulated by endothelin-1 rather than catecholamine. It was a decrease in supplying blood flow to the liver occuring at high intensity exercise causes liver damage (Yano et al., 1997). In the liver, different regions in the lobule have different functions. There are numerous important structural, biochemical, and functional differences between the various regions of the liver lobule (Lemasters et al., 1983). Based on the lobular distribution of hepatic enzymes periportal hepatocytes are adapted to gluconeogenic and oxidative process, whereas pericentral hepatocytes are specialized for glycolysis. Swelling of the mitochondria was observed in pericentral hepatocytes after exhaustion exercise (Yano et al., 1997). It is well known that high intensity exercise greatly reduces hepatic blood flow (Rowell et al., 1964). In human, the hepatic function is a perfused by liver after an acute exercise. Some authors have reported an altered liver function in strenuous exercise. A reduced extraction of propranolol, increased liver injury (Nagel et al., 1990) and the released of liver-specific enzymes (Fojt et al., 1976). An acute physical exercise in rats decreased the hepatocyte volume and that this volume change is not entirely linked to a decrease in hepatic glycogen level (Latours et al., 1999). The reduction in blood flow in the liver causes hypoxia of hepatocytes, and eventually induces their necrosis, thus causing cell damage. These factors suggest that strenuous exercise might cause hepatic necrosis or ischemic reperfusion.

However, there has been no histological study which accounts for the effects of intensity acute exercise on change of function and pathology of liver and pancreas in rats. Therefore, the present study was designed to investigate the effects of two different exercise intensites on hepatic and pancreatic function including biochemical changes and pathology in rats immediately after exercise and reperfusion.

Research questions

1. How do two vigorous exercise levels affect functions and histopathology of the liver in rats?

2. How do two vigorous exercise levels affect functions and histopathology of the pancreas in rats?

Objectives

1. To study the effect of vigorous exercise intensities level on functions and histopathology in rat liver.
2. To study the effect of vigorous exercise intensities level on functions and histopathology in rat pancreas.

Hypotheses

1. Changes of biochemical markers of liver and pancreas in rats are related to the level of exercise intensity.
2. Changes of pathology in the liver and pancreas in rats are related to the level of exercise intensity.

Scope of research

This study is an animal experimental research in male Sprague-Dawleys rats.

The experimental procedures were carried out according to the guiding principle for the care and use of animal in the field of physiological sciences approved by the Ethics Committee, Faculty of Medicine, Chulalongkorn University, Bangkok, 10330, Thailand.

Assumptions

All experimental animals are not significantly different.

Expected benefits and applications

1. To understand the impact of the levels of exercise on function and pathology of the liver and pancreas.
2. Providing the preliminary data for further research in both animals and humans.

CHAPTER II

REVIEW LITERATURES

The liver

The liver is the largest gland in the body. It is positioned immediately beneath the diaphragm in the right side of the peritoneal cavity. In the human, it has two main lobes, the right lobe is much larger than the left lobe, although in rat, it has fours lobes (Wells, 1964). The lobes of the liver are made up of many functional units called lobules (Figure 1). A lobule consists of specialized epithelial cells, called hepatocytes or parenchymal cells arranged in irregular, branching, and interconnected plates around a central vein. Rather than capillaries, the liver has larger spaces lined by endothelium called sinusoids, through which blood passed. The sinusoids are also party lined with stellate reticuloendothelial (Kupffer's) cells. These phagocytes destroy worn-out white and red blood cells, bacteria, and other foreign matter in the blood draining from the gastrointestinal tract (Tortora and Grabowski, 2000).

The liver receives blood from two sources, the hepatic artery with oxygenated blood, and the hepatic portal vein with deoxygenated blood containing newly absorbed nutrients, drugs, and possibly microbes and toxin from the gastrointestinal tract. Branches of both hepatic artery and hepatic portal vein carry blood into liver sinusoids, where oxygen, most of the nutrients, and certain poisons are extracted by the hepatocytes. Products manufactured by the hepatocytes and nutrients needed by other cells are secreted back into the blood. The blood drain into the central vein. Central veins drain into larger veins often called sublobular veins and these in turn drain into the hepatic veins and empty their blood into the inferior vena cava (Figure 2). Branches of the hepatic portal veins, hepatic artery, and bile duct typically accompany each other in their distribution through the liver. Collectively, these three structures are called a portal triad or portal tract (Tortora and Grabowski, 2000).

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The liver is subdiving into functional lobule to regard as a unit of hepatocyte that region which is irrigated by a terminal branch of the distributing veins. This is called the hepatic acinus (Reppaport, 1956) (Figure 3). Cells in the hepatic acinus can be subdivided into 3 zones. Zone I called periportal area would be closet to the vessel and consequently the first to be affected by or to alter the incoming blood. Cell in zone II called midzone would be second to respond to the blood and zone III called centrilobular or periacinal area would be portal vein blood that has been previously alterd by cells in both zone I and II. The zonation is important in the description and interpretation of patterns of degeneration, regeneration, and specific toxic effects in the liver parenchyma relative to the degree or quality of vascular perfusion of the hepatic cells. As a result of the sinusoidal blood flow, the oxygen gradient, the metabolic activity of the hepatocytes, and the distributon of hepatic enzymes varies across the three zones. The distribution of liver damage due to ischemia and exposure to toxic substances can be explained using this zonal interpretation (Ross et al., 2003).

Cell in zone I are the first to receive oxygen, nutrients, and toxins from the sinusoids blood and are the first to show morphologic changes following bile duct occlusion (bile stasis). These cells are also the last to die if circulation is impaired and the first to regenerate. On the other hand, cells in zone III are the first to show ischemic necrosis (centrilobular necrosis) in situations of reduced perfusion and the first to show fat accumulation, They are the last to respond to toxic substances and bile stasis. Normal variations in enzyme activity, the number and size of cytoplasmic organelles, and the size of cytoplasmic glycogen deposite are also seen between zone I and III, Cells in zone II have functional and morphologic characteristics and respones intermediate to those of zones I and III (Ross et al., 2003).

Bilirubin

Bilirubin, the other major constituent of bile, does not play a role in digestion at all but instead is a waste product excreted in the bile. Bilirubin is the primary bile

pigment derived from the breakdown of worn-out red blood cells. The typical life span of a red blood cell in the circulatory system is 120 days. Worn-out red blood cells are removed from the blood by the macrophages that line the liver sinusoids and reside in other areas in the body. Bilirubin is the end product from degradation of the heme (iron-containing) part of the hemoglobin contained within these old red blood cells. This bilirubin is extracted from the blood by the hepatocytes and is actively excreted into the bile. Bilirubin is a yellow pigment that gives bile its yellow color. Within the intestinal tract, this pigment is modified by bacterial enzymes, giving rise to the characteristic brown colorof feces. When bile secretion does not occur, as when the bile duct is completely obstructed by a gallstone, the feces are grayish white. A small amount of bilirubin is normally reabsorbed by the intestine back into the blood and when it is eventually excreted un the urine, it is largely responsible for the urine's yellow color. The kidney cannot excrete bilirubin untill after it has been modified during its passage through the liver and intestine. If bilirubin formed more rapidly than it can be excreted, it accumulates in the body and causes jaundice. Patients with this condition appear, yellowish, with this color being seen most easily in the whites of their eyes. Jaundice can be brought about in three different way: (1) Prehepatic (the problem occurs before the liver) or hemolytic, jaundice is due to excessive breakdown (hemolysis) of red blood cells, which results in the liver being presented with more bilirubin than it is capable of excreting. (2) Hepatic (the problem is the liver) jaundice occurs when the liver is dieased and cannot deal with even normal load of bilirubin. (3) Posthepatic (the problem occurs after the liver) or obstructive, jaundice occurs when the bile duct is obstructed such as by a gallstone so that bilirubin cannot be eliminated in the feces (Peter, A., 2007).

In conclusion, the liver is the largest gland and most important metabolic organ in the body; it can be viewed as the body's major biochemical factory, It importance to the digestive system is its secretion of bile salts, which aid fat digestions and absorption. The liver also performs a wide variety of functions not related to digestion, Including the following:

1. Metabolic processing of the major categories of nutrients (carbohydrates, proteins, and lipids) after their absorption from the digestive tract.
2. Detoxifying or degrading body wastes and hormones as well as drugs and other foreign compounds.
3. Synthesizing plasma proteins, including those needed for blood clotting and those that transport steroid and thyroid hormones and cholesterol in the blood.
4. Storing glycogen, fats, iron, copper, and many vitamins.
5. Activating vitamin D, which the liver does in conjunction with the kidneys.
6. Removing bacteria and worn-out red blood cells, thanks to its resident macrophages.
7. Excreting cholesterol and bilirubin, the latter being a breakdown product derived from the destruction of worn-out red blood cells

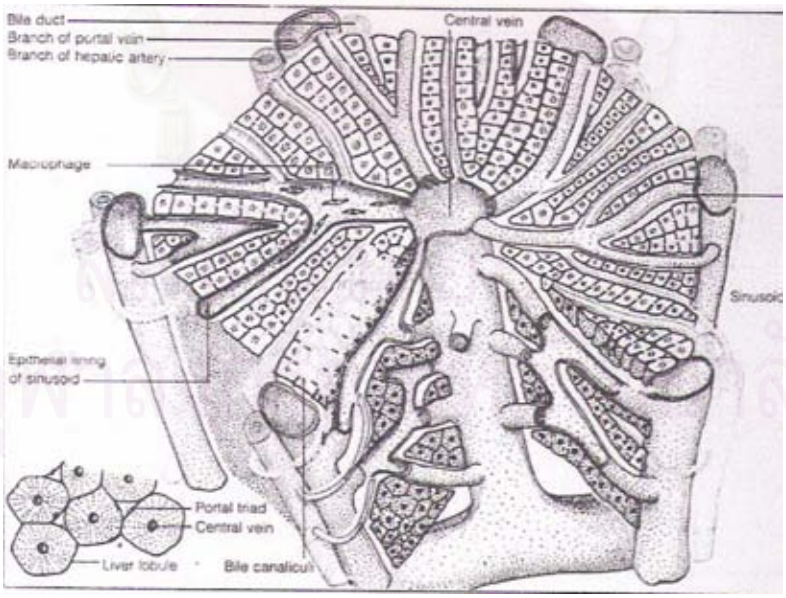


Figure 1. A liver lobule (Landay, 1980)

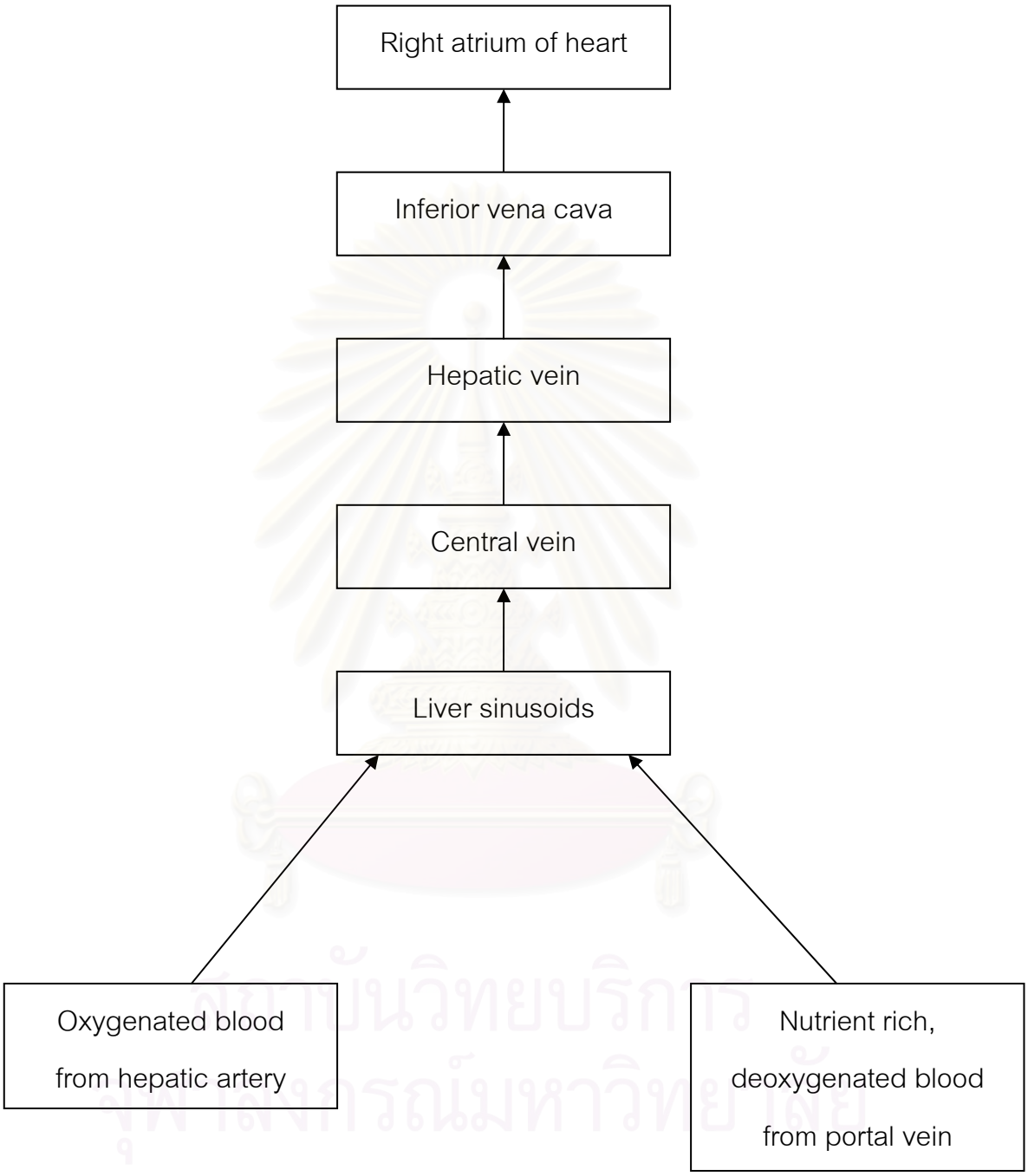


Figure 2. Blood flow through liver and return to the heart. (Tortora and Grabowski, 2000)

Exercise has various beneficial effects of liver function, enhancing both nutrient metabolism and antioxidant capacity. The liver has an integral role in the physiology of exercise. This organ supplies energy substrates to peripheral tissues via the Cori cycle and glycogen catabolism, plays a central role in lipid metabolism (producing ketone bodies by oxidation of fatty acids), and is important for detoxification. In addition, the liver releases many biologically active molecules into the circulation such as growth factors. Furthermore, almost all nutrients are carried to the liver by the portal circulation immediately after absorption from the intestine, and then are distributed throughout the body after hepatic processing (Aoi et al., 2004).

The pancreas

The pancreas is an elongated gland that lies behind and below the stomach, above the first loop of the duodenum. This mixed gland contains both exocrine and endocrine tissue. The predominant exocrine part consist of grapelike clusters of secretory cell that form sacs known as acini, which connect to ducts that eventually empty into the duodenum. The smaller endocrine part consist of isolated islands of endocrine tissue, the islets of Langerhans, which are dispersed throughout the pancreas. The most important hormones secreted by the islet cells are insulin and glucagon. The exocrine and endocrine pancreas are derived from different tissues during embryonic development and share only their location in common. Although both are involved with the metabolism of nutrient molecules, They have different functions under the control of different regulatory mechanisms (Peter, A., 2007).

The exocrine pancreas (Figure 2). secretes a pancreatic juice consisting of two components: (1) pancreatic enzymes actively secreted by the acinar cells that from the acini and (2) an aqueous alkaline solution actively secreted by the duct cells that line the pancreatic ducts. The aqueous (watery) alkaline component is rich in sodium bicarbonate ($NaHCO_3$).

Like pepsinogen, pancratic enzymes are stored within zymogen granules after being produced, then are released by exocytosis as needed. These pancreatic enzymes are important because they can almost completely digest food in the absence of all other digestive secretions. The acinar cells secrete three different types of pancreatic enzymes capable of digesting all three categories of foodstruffs: (1) proteolytic enzymes for protein digestion, (2) pancreatic amylase for carbohydrate digestion, and (3) pancreatic lipase for fat digestion (Peter, A., 2007).

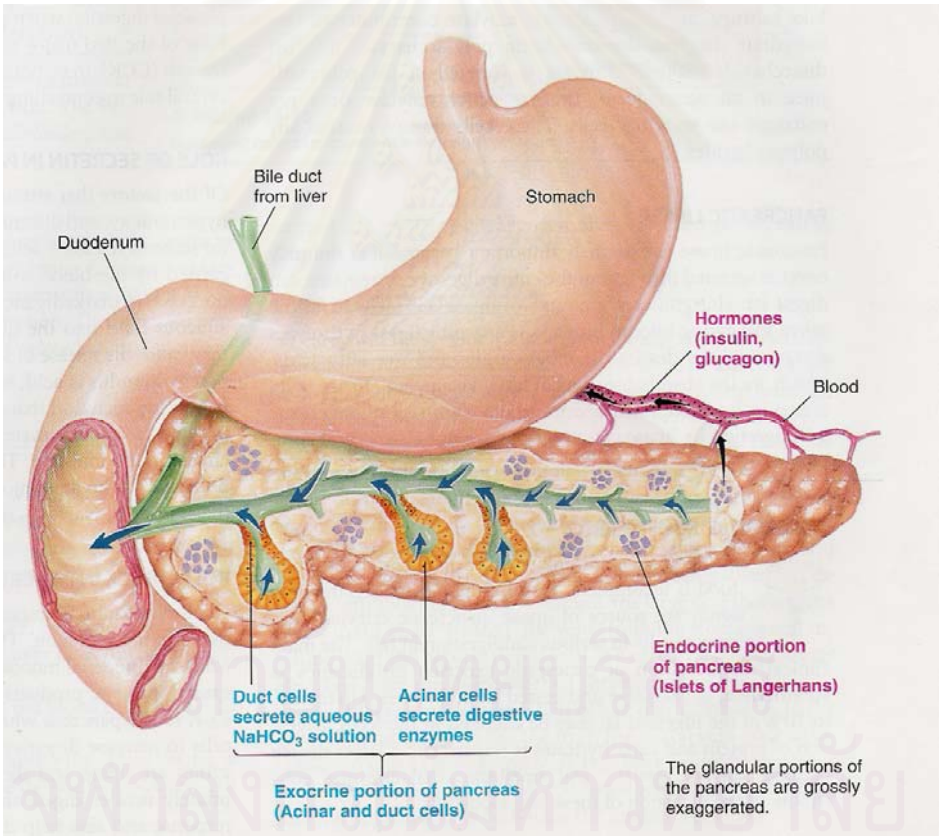


Figure 3. A pancreas, the exocrine and endocrine portion of the pancreas

Pancreatic amylase

Like salivary amylase, pancreatic amylase contributes to carbohydrate digestion by converting polysaccharides into the disaccharide maltose. Amylase is secreted in the pancreatic juice in an active form, because active amylase does not endanger the secretory cells. These cells do not contain any polysaccharides (Peter, A., 2007).

Pancreatic lipase

Pancreatic lipase is extremely important because it is not only enzymes secreted throughout the entire digestive system that can digest fat. Insignificant amounts of lipase are secreted in the saliva and gastric juice in humans. Pancreatic lipase hydrolyzes dietary triglycerides into monoglycerides and free fatty acids, which are the absorbable units of fat. Like amylase, lipase is secreted in its active form because there is no risk of pancreatic self-digestion by lipase. Triglycerides are not a structural component of pancreatic cells (Peter, A., 2007).

Maximum oxygen consumption of rats

The maximum oxygen consumption (VO_{2max}) also called maximal oxygen uptake, aerobic power or aerobic capacity. VO_{2max} of animals, including man, is considered to be the best single measure of aerobic capacity. VO_{2max} using a variety of exercise that activate the body's large muscle groups, provided the intensity and duration of effort are sufficient to maximize aerobic energy transfer. The usual exercise modes include treadmill running or walking, bench stepping, and stationary cycling. The VO_{2max} test consists of progressive increments in effort (graded exercise) to a point at which the subject simply refuses to continue exercising. Some researchers have termed this end point "exhaustion." Variation in VO_{2max} with different forms of exercise generally refects the quantity of muscle mass activated (Blomquist et al. 1982 and Lewis, et al. 1983). Studies that determined VO_{2max} for the same subjects during different exercise modes indicate that treadmill exercise usually produces the highest values. Bench-stepping has produced VO_{2max} scores indentical to treadmill values and significantly higher than values on a cycle ergometer (Kasch, et al. 1966). During arm-crank exercise, aerobic

capacity averages only about 70% of one's treadmill score (Toner, et al. 1983). For skilled but untrained swimmers, the VO_{2max} during swimming usually equals about 80% of treadmill values (Magel, et al.1967 and McArdle, et al. 1978). A definite test specificity emerges for this form of exercise because trained collegiate swimmers archieve VO_{2max} values swimming only 11% below treadmill values (McArdle, et al. 1971) and some elite swimmers equal or even exceed their treadmill scores during swimming tests (Magel, et al. 1967). Similarly, a distinct exercise specificity exists for competititive racewalkers who achieve a similar VO_{2max} during treadmill walking and running (Menier, et al. 1968). When competitive cyclists pedal at the rapid frequencies of competition, they too achieve VO_{2max} values equivalent to their treadmill VO_{2max} scores (Hagberg, et al. 1978 and Stromme, et al. 1977).

Treadmill exercise proves highly desirable for determining VO_{2max} in healthy subjects in the laboratory. One can easily quantify and regulate exercise intensity. Compared with other forms of exercise, the treadmill allows subjects to more easily meet one or more of the criteria for attaining VO_{2max}. Graded treadmill exercise after the intial rapid rise from the resting level, systolic blood pressure increase linearly with exercise intensity while diastolic pressure remains stable or decreases slightly at the higher exercise levels. Both sedentary and endurance trained subjects demonstrate similar blood pressure responses. During maximum exercise by healthy, fit men and women, systolic blood pressure may increase to 200 mmHg or higher, despite significantly reduced total peripheral resistance (Mitchell and Raven.1994). This level of blood pressure may most likely reflects the heart's large output of blood during maximal exercise by individuals with high aerobic capacity. Blood flow during exercise approximately 4 to 7 mL of blood flows each minute to each 100 g of muscle at rest. This flow increases steadily in grade exercise with active muscle receiving up to 50 to 75 mL per 100 g of tissue during each minute of maximum exertion (MacDougall, et al. 1979). Blood flown within active muscle is highly regulated. The greatest quantity of blood diverts to the oxidative portions of the muscle at the expense of those areas with high glycolytic capacity (Blyakhamn, et al. 1999). Thus, peak blood flow values in small

portion of active quadriceps muscle achieves a perfusion as high as 300 to 400 mL·100 g^{-1}· min^{-1} (Laughlin, et al. 1994, Lieber, 1992 and Lieber, et al. 1994). During large muscle activities such as running and cycling at maximum intensity, muscle blood flow accounts for 80% to 85% of the total cardiac output (Nielsen, et al. 2000).

Recents clinical and epidemiological studies suggest that beneficial effects of regular physical exercise may depend on intensity or amount of work performed during training (Lee et al., 2003). An aerobic exercise capacity measured as VO_{2max} or metabolic equivalents is a major predicator of all-cause mortality both in normal subjects and cardiovascular disease (Gulati et al., 2003). In contrast, current recommendations for prevention and rehabilitation range 40%-90% of VO_{2max} . Treadmill running at 85-90% of current VO_{2max} yielded substantially larger effects on physiological, cardiomyocyte contractility and aerobic fitness than moderate exercise at 65%-70% of VO_{2max}. In contrast, full effect of endothelial function was induced by regular exercise at moderate intensity, as endothelium function. It seems likely that beneficial effects of regular exercise result from several mechanisms that may depend differentially on intensity; those associated with myocardial function to require high intensity training over several weeks to be fully active, whereas endothelium effects may plateau at lower intensity (Kemi et al., 2005). The VO_{2max} normalized for body mass as a function of aerobic capacity has been reported to generally decline with age (Mazzeo et al., 1984). In addition, this decline in VO_{2max} with age is associated with a decrease in the running speed to elicit VO_{2max}. Since the relative VO_{2max} declinced linearly with age after 12 weeks of age it might continue to decrease during the lifetime of the rat. Accordingly, the decline in VO_{2max} during development is not likely to agree with the decline in VO_{2max} with age. It is generally accepted that a decrease in VO_{2max} with age might be related to atrophy or deterioration of skeletal and cardiac muscle (Mazzeo et al., 1984).

Furthermore, in sedentary rats, the period of peak of actomyosin ATPase and creatine kinase activity in the heart occurs at less than 8 week of age. The running speed to elicit VO_{2max} increased from 4 to 8 weeks of age, although the relative VO_{2max} declinced (Yano et al., 1995).

Endurance training increases cardiovascular capacity and quality of life, reduces mortality in patients with heart failure, increases exercise capacity as measured by VO_{2max} improves work economy, and enhances anaerobic threshold. Cardiac effects should reduce resting and submaximal heart rates (HR) and increased ventricular weights, volumes and myocyte hypertrophy.

In the adult rat treadmill-running model, the previously published training regimens used are known to elicit minor effects on ventricular mass and/or cardiac myocyte dimensions (Mokelke et al., 1997). Studies have shown a 0–20% increase in left ventricular weight and myocyte length (Moore et al.,1993). Several studies did not find any significant change in myocardial mass as a result of treadmill training in female rats and concluded that ventricular enlargement in female rats depends critically on the mode of training, with effects observed only with swim training (Gleeson et al., 1983). In contrast, treadmill training increases VO_{2max} and work economy by 10%–20% and reduces resting HR by 5% both in male and female rats (Musch et al., 1989). In female rats, 4 wk of endurance training increased left and right ventricular weights by 10% and 12%, and at 13 wk, the increases were 34% and 30%, respectively. The corresponding increases for trained male rats were 25% and 23% at 7 wk (Wisloff et al., 2000) VO_{2max} was reduced independent of muscle convective O_2 delivery in late middle-age animals and alterated within the skeletal contribute significantly to the decline in VO_{2max} with aging. Notably, a reduction in VO_{2max} prevailed after the smaller muscle in the older animals were taken into account, showing that qualitative impairments in aged muscles contribute to reduce VO_{2max} with aging and alterations in mitochondrail oxidative capacity play an important role in this decline in muscle VO_{2max} with aging (Hepple et al., 2003).

Types of exercise in rats

There are many types of exercise in rat protocol. Over the last few years, evidence has been gathered and shown that, there were two major types of exercise in rat; (1) running exercise (run on motor-driven treadmill) and (2) swimming exercise.

Running exercise on treadmill

Exercise on treadmill can predict the VO_{2max}. The VO_{2max} provides a quantitative measure of a person's capacity for aerobic adenosine triphosphate (ATP) resynthesis. This makes the VO_{2max} an important physiologic meaning in addition to its role in sustaining energy metabolism. High aerobic power requires the integrated and high-level response of diverse physiologic support systems. In rat exercise VO_{2max} can predict by adjusting grade (degree) and running velocity (Bedford et al., 1979)

Swimming exercise

In swimming training exercise, it is very hard to control the relative and absolute training intensities, and it has been suggested that swimming involves a significant learning component in rats. Swimming is potentially stressful to the rat and may induce substantial sympathetic stimulation, high catecholamine levels, and increase HR and blood pressure, which may confound the interpretation of observed effects (Wisloff et al.,2000). Intensity in swimming exercise can adjust by applied with a load proportional to rat body weight e.g. 0%, 5% and 6% for increasing intensity.

Table 1. Showed VO_{2max} of rats during exercise on treadmill (Bedford et al., 1979).

Stage	ml.kg-1.min-1	%of maximum
0,sitting	28.1 ± 2.0	33.3 ± 3.3
1,0° grade,8.2 m·min^{-1}	45.2 ± 2.5	52.9 ± 3.1
2,5° grade,15.2 m·min^{-1}	54.9 ± 3.6	64.0 ± 4.5
3,10° grade,19.3 m·min^{-1}	64.7 ± 2.6	76.0 ± 2.8
4,10° grade,26.8 m·min	78.6 ± 1.6	92.3 ± 2.8
5, maximum response	85.5 ± 2.3	100%

Exercise

Physical activity is defined as any form of muscular activity. Therefore, physical activity results in the expenditure of energy proportional to muscular work and is related to physical fitness. Exercise represents a subset of physical activity that is planned, with a goal of improving or maintaining fitness (Power SK.,2001)

Exercise or physical activity, or work of a fairly vigorous nature, lead to a number of important bodily changes. The changes in function of the body are brought about by both single and repeated bouts of exercise. A single bout exercise is sometimes called " acute exercise" whereas repeated bouts of exercise over several days or months is called "chronic exercise" or "exercise training" (Lamp DR., 1984)

The functional changes that occur when one exercises a single time are called response 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 stomach and the kidney. Each of

these will persist no longer than a few minutes after the exercise is over (Lamp DR.,1984, Fox LE., 1993)

Training has the major objective of facilitating 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. The training is influenced by many factors such as intensity, duration, frequency and mode of exercise (Lamp.,1984, Mcardle WD.,1996). Each of these is important for physical adaptations. To achieve the training improvement, these major factors must be considered (Robergs RA.,1997)

Intensity

Intensity refers to the level of stress achieved during the exercise period. Intensity is determined best from measurements of oxygen consumption, but indirect methods are heart rate, respiration rate, or from the rating of perceived exertion (RPE). Exercise sessions can be low intensity or high intensity. Low intensity exercise would be equal or 50% to 60% of an individual’s maximal heart rate, whereas 85% to 90% would relate to high intensity exercise. It is best to begin an exercise program at a low intensity and gradually increase the intensity over time (Lamp.,1984, Mcardle WD.,1996).

Duration

Duration refers to the length of the training session. Duration and intensity are inversely related; that is, if the intensity of the exercise is high, the duration is generally low, and vice versa. The duration of the exercise session can be affected by environmental factor (e.g., heat, humidity, altitude). It can also be affected by present fitness level or energy supply of an individual (Lamp.,1984, Mcardle WD.,1996).

Frequency

Frequency refers to the number of training sessions per week. It is recommended that individuals try to exercise 4 or 5 days per week. The frequency of exercise depends on the type of exercise performed and the fitness status and goals of the individual (Lamp.,1984, Mcardle WD.,1996).

Mode

Mode refers to the type of activity performed during the exercise session. Various modes of exercise can affect the components of fitness differently. Choosing the correct mode of exercise is important because it has a direct effect on the outcome (Lamp.,1984, Mcardle WD.,1996).

Physiological adaptations to training

The physiological adaptations that occur with chronic exposure to exercise improve both exercise capacity and efficiency. These adaptations are highly specific to the type of training (Wilmore JH., 1999).

The two contrasting types of training, anaerobic and aerobic, have difference consequences on these parameters anaerobic exercise (static or isometric) includes such activities as carrying a suitcase or weightlifting ; muscle tension develops. Although, there is little or no displacement of the object worked against aerobic exercise (dynamic or isotonic) includes running, walking and related sports; regular muscular activity occurs, but against a light load (Opie H.,1998).

Adaptations to anaerobic (sprint) 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. In training to enchance creatine phosphate (ATP-CP) energy transfer capacity, the activities selected must engage the specific muscles at the movement speed and power

output for which the experiment 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 magitude of anaerobic energy generated in glycolysis increases. To improve the capacity for energy transfer by the short-term lactid acid energy system, training must overload this aspect of energy metabolism (MaArdle W., 1996).

The anaerobic changes in skeletal muscle resulting from training involve increased capacities of (1) ATP-CP system; the capacity of the ATP-CP system is enchanced by two major biochemical changes: (a) increased levels of muscular stores of ATP and CP and (b) increased activities of key enzyme involved in the ATP-CP system [i.e, the enzymes myokinase (MK) and creatine kinase (CPK)] and (2) anaerobic glycolysis (i.e., lactic acid system; the glycolytic capacity is enhanced by increased the glycolytic enzyme activities i.e. phosphofructokinase (PFK) which is important in the early reactions of glycolysis (Fox LE., 1993).

Adaptations to aerobic (endurance) training

The aerobic, or oxygen, system release energy for ATP production from the breakdown mainly of carbohydrate and fat, and sometime of protein (Fox LE.,1993). The aerobic exercise is the activities to perform whole body activities for extended period of time (Prentice WE. 1999).

The effects of aerobic endurance training on the mechanisms of adaptation have been reviewed previously throughly with respect to the cardio-respiratory system, and skeletal muscle (Zuluagn M., 1995).

1. Cardio-respiratory system adaptations

Adaptations within the cardiovascular system with training may be considered as either central or peripheral in origin. Central adaptations include changes in cardiac output, blood volume and arterial oxygen carrying capacity. Peripheral adaptations

include skeletal muscle blood flow and capillarization. The functional significance of these changes may be assessed by examining their respective roles in the improvements in VO_{2max} and endurance performance after endurance training. Endurance training program that increase VO_{2max} involve a large muscle was in dynamic exercise (e.g., running, cycling or swimming) for twenty to sixty minutes per session three to five times per week at an intensity of about 50% to 85% VO_{2max} (American colleage of sports Medicine, 1998).

VO_{2max} is a value expressed quantitatively of person's capacity for aerobic resynthesis of ATP. It provides important information on the capacity of the long term energy system (Heyward VH.,1997). Since VO_{2max} is equal to the product of systemic blood flow (cardiac output) and systemic oxygen extraction (arteriovenous oxygen difference), changes in VO_{2max} would have to be due to changes in one or more of those variables (Figure 3). Endurance training induced increases in maximal cardiac output are due to an increase in stroke volume by both an increase in preload and a decrease in afterload. The increased in the arteriovenuous O_2 difference could be due to an elevation of the arterial content (higher hemoglobin or PO_2) or a decrease in the mixed venous oxygen content. The increase capacity of the muscle to extract O_2 following endurance training is believed to be due to the increase in capillary density and mitochondria number. The increase in capillary density in trained muscle accommodates the increase in muscle blood flow during exercise, decrease the diffusion distance to mitochondria and slows the rate of blood flow to allow time for diffusion to take place. The increases in mitochondria following endurance training favor O_2 transport from the capillary and contribute to the expanded a-v O_2 differences.

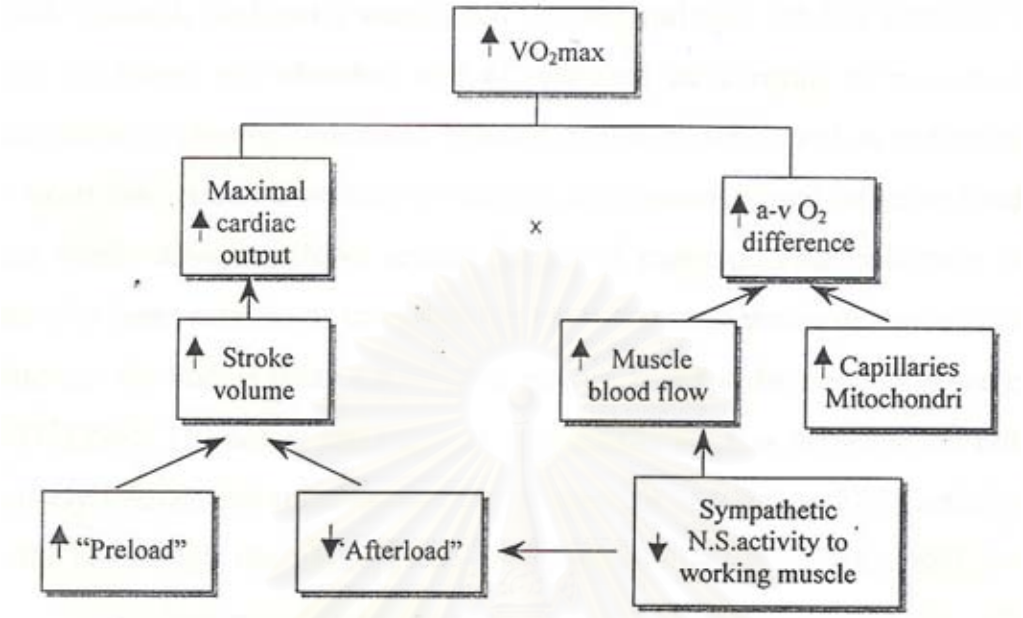


Figure 4. Summary of factors causing an increase in VO_{2max} with endurance training

Changes in heart size and heart rate are the cardiovascular adaptations which occur in response to training. In response to increase work demand, the heart's weight and volume and thus the left ventricle wall thickness and chamber size all increase as a result of a normal adaptation to chronic endurance training (Wilmore JH.,1999). A reduction in heart rate is an adaptation of training for a submaximal exercise load that nearly always follows several weeks of training. This reduction in exercise heart rate seems to enable to heart to pump the same amount of blood to the body's tissues at a lower energy cost for the heart (McArdle WD.,1996). The actual mechanisms responsible for this decrease are not entirely known, but training appears to increase parasympathetic activity in the heart while decreasing sympathetic activity (Wilmore JH.,1999).

2. Skeletal muscle adaptations

The aerobic adaptations occur in skeletal muscle mainly as a result of endurance-training programs (Fox LE.,1993). After endurance training, exercise at a given power output is characterized by a reduced decline in muscle phophagen (ATP and CP) and glycogen stores and a smaller accumulation of lactate in both muscle and blood. These changes were suggested originally to result from the increased mitochondrial volume and capillarization evidence with endurance training.

One of the most striking muscle skeletal muscle adaptations with endurance training is the marked increase in mitochondrial activities, reflecting an increase mitochondrial volume in skeletal muscle. Enzymes involved in the Kreb's cycle, electron transport chain and beta oxidation (fat metabolism) are all increase with endurance training. These changes are reversible, demonstrating a sharp decline after only 1 week of detraining. The molecular mechanisms underlying mitochondrial upregulation with training remain obscure, but may be associated with increases in 3'5' cyclic adenosine monophosphate (cAMP), or changes in CP stores in skeletal muscle with contractile activity (Zuluagn M.,1995).

Effect of exercise on liver

Numerous studies have demonstrated that exercise has a beneficial effect on liver function. Endurance training is known to improve the metabolic profile, including reduction of plasma triglycerides and elevation of high-density lipoprotein cholesterol (Gorski et al., 1990; Simonelli and Eaton, 1978). Endurance training has been shown to reduce hepatic accumulation of total fat and cholesterol in rats fed a high-fat diet (Gorski et al., 1990; Simonelli and Eaton, 1978). Training also leads to a reduction of glyconeogenesis and has a glycogen-sparing effect during exercise (Cartee and Farrar, 1988; Podolin et al., 1994). Prior training promotes oxidation of fat in muscle as an energy substrate during exercise. By limiting glycolysis, this facilitates maintenance of a normal blood glucose concentration, promotes glucose homeostasis, and has a hepatic glycogensparing effect (Cartee and Farrar, 1988; Podolin et al., 1994; Donovan and Sumida, 1997). Such glycogen sparing is particularly critical for resistance to exercise-induced hypoglycemia with aging, because hepatic capacity for gluconeogenesis and liver glycogen stores decline with age (Donovan and Sumida, 1997). In rats with streptozocin-induced diabetes, training also reduced hepatic catabolism of branched-chain amino acids, in association with the suppression of diabetes-induced branched-chain alpha-ketoacid dehydrogenase (BCKDH) complex expression in the liver (Li et al.,2001). Secretion of growth hormone in response to its hypothalamic releasing factor is especially enhanced by resistance exercise. Growth hormone regulates insulin-like growth factor (IGF)-1 mRNA expression in the liver during training and has been shown to induce a significant increase of hepatic exon 1-derived IGF-1 mRNA (Zanconato et al., 1994). Training also modulates antioxidant enzymes in the liver, reducing oxidative damage (Reddy Avula and Fernandes, 1999; Venditti and Di Meo, 1996, 1997). Furthermore, physical exercise promotes the oxidative metabolism of drugs. In humans and horses, regular exercise has been found to increase hepatic processing of antipyrine and enhance its clearance from the plasma (Dyke et al., 1998; Mauriz et al., 2000).

Effect of exercise on hepatic gene expression

Running training for 4 week increased expression of 105 genes and decreased expression of 86 genes, including genes with unknown functions (Aoi et al., 2004). Exercise has various beneficial effects on the liver, such as changes to the metabolism of nutrients including lipids, glucose, proteins (Aoi et al., 2004). The mechanisms of such effects are not fully known and gene expression may be involved. In skeletal muscle, it has been demonstrated that changes of gene expression are involved in various response to exercise. Muscle hypertrophy is associated with up-regulation of myosin heavy chain expression and down-regulation of myostatin (Roth et al., 2003). Insulin sensitivity is improved through increased expression of glucose transporter 4 and the insulin receptor (Kirwan and del Aguila, 2003). Also, expression of the genes involved in fatty acid synthesis is decreased by training (Fiebig et al., 2002). Changes of several genes related to metabolism were found in microarray study. AcylCoA synthetase expression was decreased. Exercise, including endurance training, increases the production of many anabolic factors, such as growth hormone and growth factors (Zanconato et al., 1994; Wideman et al., 2002), resulting in the elevation of protein synthesis. Frenkl et al. demonstrated an increase in constituents of hepatic microsomal monooxygenase system including p450 (Frenkl et al., 1980). Hepatic damage induced by severe exercise in less severe in trained rats (Venditti and Di Meo, 1996,1997). Addition, some of genes related to secretion, encoding proteins such as activin, secretory carrier membrane protein and fasting-induced adipose factor were regulated by training. Although, expression of several hepatic genes was altered by training, the changes could not explain all health benefits resulting from training (Aoi et al., 2004).

Effect of exercise on levels of insulin-like growth factor–1

Insulin-like growth factor–1 (IGF-1) is a peptide with a tertiary structure that consists of 70 amino acid residues and has a molecular mass of 7649 Da. Two main actions are attributable to this peptide: namely, a growth-related action and an anabolic action, with the latter being also included in the former. IGF-1 plays a role in growth by mediating the action of growth hormone (GH), therefore also being called somatomedin C, and promotes cell proliferation as well as synthesis of proteoglycans by cartilaginous, connective, and bone tissue. The anabolic role of IGF-1 consists of stimulating the uptake of amino acids and glucose by the cells, with an action similar to that of insulin, a fact that led to its being named insulin-like growth factor (Phillips et al., 1998).

The anabolic action of IGF-1 is compromised by specific physiological states such as diabetes, protein deficiency, physical exercise, and energy restriction, with these factors being considered as regulators of the secretion and biological activity of IGF-1 (Boni-Schnetzler M et al.,1991). Physical exercise in particular may influence the plasma and tissue IGF-1 concentration by mechanisms that are not completely understood. Zanconato et al., found a higher amount of IGF-1 mRNA in muscle of exercised in rats and Henriken et al., comparing the insulin-controlled and IGF-1-controlled glucose and amino acid transport systems, demonstrated that exercise significantly increased the actions of insulin and IGF-1 on the glucose transport systems. Plasma IGF-1 concentration seems to increase during both resistive and endurance exercise (Koziris LP et al.,1999), VO_{2max} and physical activity itself are correlated with resting plasma IGF-1 levels (Poehlman ET et al.,1994). It seems that this effect is observed more young persons (Roelen CA et al.,1997) because in elderly individuals, who normally present with reduced IGF-1 concentrations, exercise seems to have no effect. A progressive exercise intensity did not modify the plasma concentration of IGF-1 in elderly subjects trained for 6 months (Vitiello MV et al.,1997). However, in a study on young and elderly Guatemalan women, Porch et al., (Porch JV et al.,1997) confirmed a strong relationship between IGF-1 and age, although when they submitted these women to physical training no changes in IGF-1 were detected in either groups.

IGF-1 secretion related to exercise may not be linked to age among young or elderly adults. Thus, another correlation proposed would be that the duration of exercise and the evaluation of single activity sessions. Acute exercise and first 10 minutes of activity seem to provoke a temporary increase in plasma IGF-1 concentration, which rapidly returns to basal levels thereafter (Cappon J et al.,1994, Schwarz AJ et al.,1996).

However, there are still many variables to be studied to determine how exercise acts on IGF-1 secretion and to resolve conflicting results. Fedele et al., observed a significant 26-93% increase in the rate of muscle protein synthesis and a reduction in plasma IGF-1 levels in rats after acute exercise. Comparing plasma IGF-1 level during activity, a significant 12% increase in IGF-1 levels was found during progressive exercise until exhaustion on a bicycle ergometer, a significant 15% was found reduction during long-distance skiing, and there was no change during 20 minutes on a treadmill simulating a soccer game (Roelen CA., 1997). The increase in IGF-1 observed by Nguyen et al., seems to be related to the stimulus of short-duration intense exercise, which probably provokes an increase in growth hormone secretion, whereas during less intensive exercise growth hormone secretion is lower and, consequently, plasma IGF-1 levels do not increase.

Effect of exercise on glucocorticoid receptors

Glucocorticoid hormones (GCs) have many profound physiological and pharmacological effects on the body. Like other steroid hormones, glucocorticoids exert their biological effects by binding to specific intracellular receptors (glucocorticoid receptors, GR), members of a large family of ligand activated transcription factors. GR is proteins which rapidly turn over, with a very short half life (Guo et al., 1986). Genetic and biological data demonstrate that GR-hormone complexes interact with specific target DNAs, the glucocorticoid response to elements (GREs) , associated with particular genes transcription of which is altered in response to hormone (Yamamoto., 1985). Their concentrations are modulated by glucocorticoid hormones (Svec, 1985), cell cycle stage and circadian rhythums. The change of glucocorticoid receptors after acute

exercise have also been investigated and it showed profound decrease of glucocorticoid receptors in renal and myocardial cytosol in low intensity (swimming without an extra weight for 60 minutes) and high intensity (swimming with a weight equal to 6% of body mass for 60 minutes). It demonstrated that both acute exercise training chronic endurance training could lead to decrease in glucocorticoid receptors, which was in a training intensity and training load volume-dependent manner and the changes in glucocorticoid receptors during exercise training were reversible (Peijie et al., 2003). A decrease of GR concentrations leading to GCs resistance would decrease GCs feedback and may be a contributing mechanism in the pathogenesis of overtraining syndrome (Peijie et al., 2003)..

Effect of exercise on rat pancreas

The pancreatic enzymes synthesis and secretion have been reported to change in various physical and dietary conditions, including physical exercise (Minato, 2000). After acute exercise in dogs showed that decreased post prandial pancreatic secretion (Konturek et al., 1973). However, chronic swimming exercise or voluntary running in rats (Kugino et al., 1991) increases pancreatic enzyme activity and secretion (Zsinka et al., 1983). Endurance running training increased pancreatic protein content, pancreatic enzyme activity and basal amylase secretion in male Wistar rats and female Fischer 344 rats (Minato, 1997). Chronic exercise rats were exercised for 60 min, 5 d/wk during the experiment. After 8 wk of chronic endurance exercise increases pancreatic weight, protein content and enzyme activity through hypertrophy of acinar cells (Minato., 2000).

CHAPTER III

MATERIALS AND METHODS

In the present study, the effects of intense exercise on change of function and pathology of liver and pancreas in rats were studied. The animals were assigned into five groups primarily according to exercise intensities. The experimental procedure was studied in three parts. First, physiological characteristics in all animals groups were examined. Second, blood analyses including determinations of total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), enzyme amylase and enzyme lipase were performed. And third, histopathological study of the liver and pancreas in rats were investigated. All protocols and procedures employed in this study were reviewed and approved by the Committee on Human Rights Related to Animal Experimentation, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.

Animal preparation

Male Sprague-Dawley rats, weighing 180-200 grams, were purchased from the National Laboratory Animal Center at Mahidol University, Salaya, Nakorn Pathom, Thailand. The animals were allowed to rest for a week after arrival at the Animal center, Department of Physiology, Faculty of Medicine, Chulalongkorn University before being used in the experiment. The animals were kept in a room with controlled temperature at 25 ± 3°C under standard conditions (12 hour dark:12 hour light cycle), fed regular with dry rat chow ad libitum, and had freely access to drink water.

Exercise protocols

The treadmill exercise intensity was determined by the result of VO_{2max} tests, as reported previously (Bedford., 1979). The exercise protocol used in the present study consisted of running on a motorized treadmill model NO. Sportart 1190. Rats fed regular with dry rat chow ad libitum, and had freely access to drink water. The time of exercise stared from 1.00 p.m. to 6.00 p.m.

All rats were divided randomly into five groups as follows:

Group 1: (Control group) no exercise, n = 6

Group 2: (Exercise 75% VO_{2max} group) running at 75% VO_{2max} and necropsied immediately after exercise, n = 5

Group 3: (Exercise 75% VO_{2max} + 6 hours group) running at 75% VO_{2max} necropsied at six hours after exercise, n = 4

Group 4: (Exercise 90% VO_{2max} group) running at 90% VO_{2max} necropsied immediately after exercise, n = 5

Group 5: (Exercise 90% $VO_{2max\ x}$ + 6 hours group) running at 90% VO_{2max} necropsied at six hours after exercise 90% VO_{2max} , n = 4

Exercise 75% VO_{2max} group, initial exercise intensity speed was 8.2 $m \cdot min^{-1}$, 0° grade for 10 min. Exercise intensity was increased to 19.3 $m \cdot min^{-1}$, 10° grade and the running time was extended until a running time of 60 minuates. Rats in this group were sacrificed immediately for blood collection and histopathology.

Exercise 90% VO_{2max} group, intial exercise intensity was 8.2 $m \cdot min^{-1}$, 0° grade for 10 min. Exercise intensity was increased to 26.8 $m \cdot min^{-1}$, 10° grade and the running time was extened untill a running time of 60 minuates. Rats in this group were sacrificed immediately for blood collection and histopathology.

In the Exercise 75% VO_{2max} + 6 hours group and Exercise 90% VO_{2max} + 6 hours group, each rat was placed in a separate cage following the completion of exercise, and given ad libitum access to food and water for 6 hours before being sacrified.

Experimental protocol

In the present study, five- week-old rats, weighed 180-200 g, were used. The time of familiarized for 10 minuates at 8.2 $m \cdot min^{-1}$, 0° grade. After being familiarized with treadmill running for 1 time, rats were divided randomly into five groups as described in exercise protocol. Rats in each group were sacrificed and the samples were collected (diagram of the experiment was shown in Figure 5).

Three experimental steps were separately performed as follows:

Step 1: Physiological characteristics and exercise

All rats were used into the study if they were five-week-old and weighed 180-200 grams. The reasons that we selected such age and weight are based on the exercise intensities that have been reported privously (Bedford., 1979). Two vigorous exercise intensities (about 75% VO_{2max} and 90% VO_{2max}) were used in this study.

After being familiarized with treadmill running for 1 time about 10 minuates at 8.2 $m \cdot min^{-1}$, 0° grade. If, rats were running, Its divided randomly into five groups as described in exercise protocol, or not running, Its excluded from this study. About 10% of all rats remained for running exercise. Group exercising at 75% VO_{2max}, initial exercise intensity speed was 8.2 $m \cdot min^{-1}$, 0° grade 10 minuates for warm up period. And, adjust intensity increased to 19.3 $m \cdot min^{-1}$, 10° grade and the running time was extended until a running time of 60 minuates. Group exercising at 90% VO_{2max}, initial exercise intensity speed was 8.2 $m \cdot min^{-1}$, 0° grade 10 minuates for warm up period. And, adjust intensity increased to 26.8 $m \cdot min^{-1}$, 10° grade and the running time was extended until a running

time of 60 minuates. When the rats finnished exercise, all blood samples and histopathology were collected and analyzed.

Step 2: Blood analysis

In this study, the blood samples were used to determine function of liver and pancreas. On the day of the experiment, immediately (group 2 and 4) and six hours (group 3 and 5) after the end of exercise, all rats were anesthetized using intraperitoneal injection of an overdose (45 mg/kg BW) of sodium pentobarbital. Then the abdominal walls were opened. Blood was drawn by cardiac puncture using a disposable syringe with needle NO.21. Approximately 3 ml of blood was immediately collected into a dry tube and let clotted for 1 hour at room temperature. Blood samples was centrifuged at 3000 rpm at 4°C for 30 minutes to separate serum. Top serum layers was pipetted off into a microcentrifuge tube. All samples were stored at –80°C until the time for analysis. Serum was used for the specific measure levels of total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT), enzyme amylase and enzyme lipase. Serum samples were analyzed at the Laboratory Center, King Chulalongkorn Memorial Hospital.

Step 3: Histopathological examination

After blood collection, the entire liver and pancreas were dissected out quickly. The remaining liver and pancreas samples were fixed in 10% formalin solution at room temperature. They were processed by standard method, tissues were embedded in paraffin, sectioned at 5 μm, and stained with Hematoxylin-Eosin (H&E) and then picked up on glass slides for light microscopy. An experienced pathologist evaluated all samples while blinded to the experiment. All fields in each section were examined for grading of necroinflammation according to brunt et al. criteria (Brunt et al., 1999),

The hepatic necroinflammation was graded from 0 to 3; score 0 = no hepatocyte injury/inflammation, score 1 (mild) = sparse or mild hepatocyte injury/inflammation, score 2 (moderate) = noticeable hepatocyte injury/inflammation, score 3 (severe) = severe hepatocyte injury/inflammation.

The hepatocyte congestion and edema was graded from 0 to 3; score 0 = no congestion/edema hepatocyte, score 1 (mild) = mild congestion/edema hepatocyte, score 2 (moderate) = noticeable congestion/edema hepatocyte, score 3 (severe) = marked congestion/edema hepatocyte (Brunt et al., 1999),

. The pancreas congestion and edema was graded from 0 to 3; score 0 = no congestion/edema, score 1 (mild) = mild congestion/edema, score 2 (moderate) = noticeable congestion/edema, score 3 (severe) = marked congestion/edema.

Data Analysis

Most data were expressed as mean ± standard deviation of the mean (SD) using the SPSS version 14 for windows program. Data for histopathology findings were reported as number of rats found in each scoring category. Statistical comparisons between groups were analyzed by nonparametric Kruskal-Wallis and 2 Independent were done with Mann-Whitney correction were applicable. The p values less than 0.005 were considered significant.

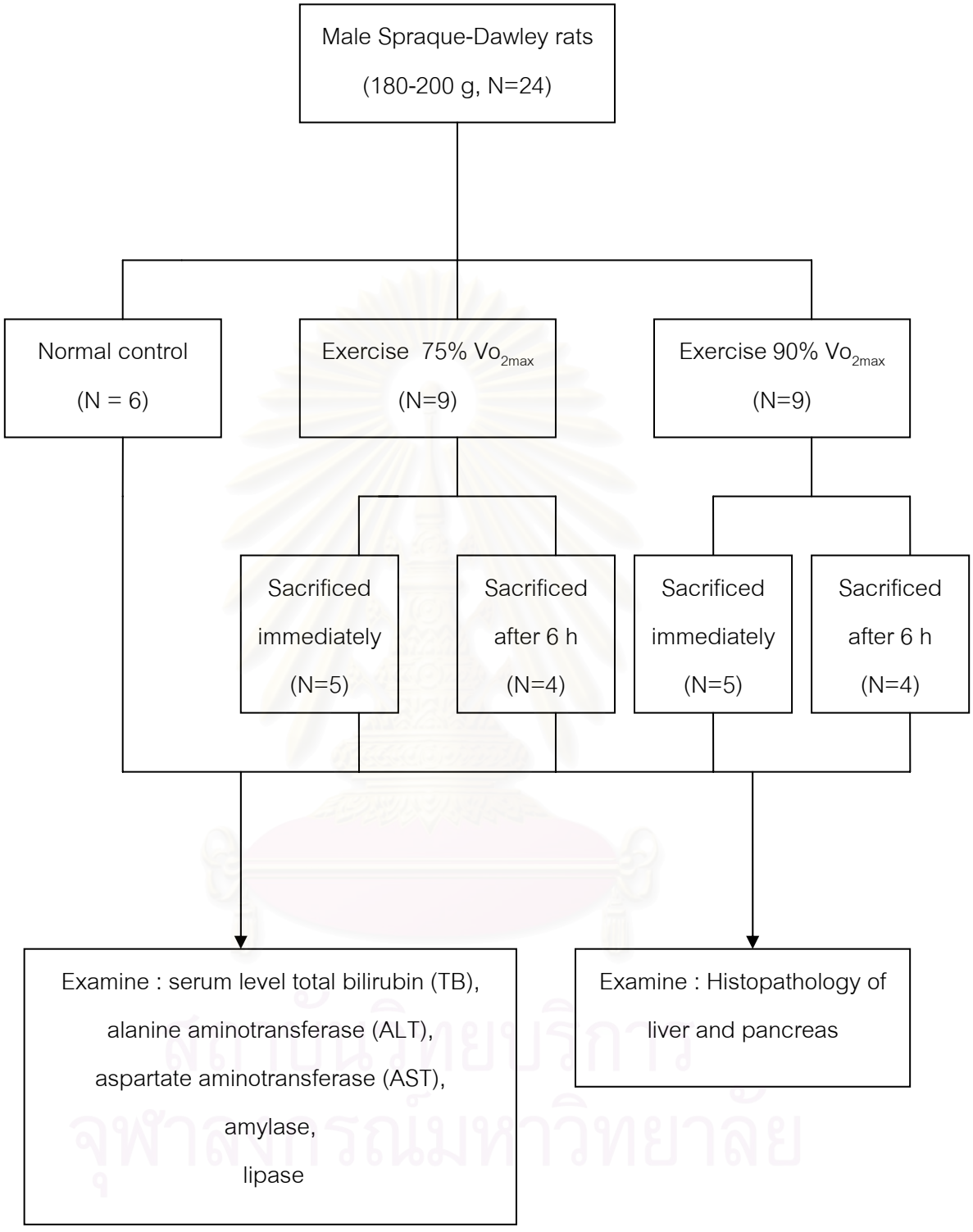


Figure 5. Experimental diagram

A

B

Figure 6.The rats employed exercise by running on a motorized treadmill.

CHAPTER IV

RESULTS

This chapter of results composed of three major parts which were served to examine the effects of intense exercise on change of function and pathology of liver and pancreas in rats. Three major parts of the result are reported in this chapter.

Part 1 Characteristics of animals

: Body weight (BW)

Part 2 Blood analysis (Serum biochemical parameters)

: Total bilirubin (TB)

: Alanine aminotransferase (ALT)

: Aspartate aminotransferase (AST)

: Serum enzyme amylase

: Serum enzyme lipase

Part 3 Histopathological study

: Hepatic congestion and necroinflammation

: Pancreas congestion and edema

1. Characteristics of animals

Body weight

The mean body weight of rats in normal control group was 182.83±2.48 g, exercise 75% VO_{2max} group was 190.60±8.47 g, exercise 75% VO_{2max} + 6 hr group was 195.75±4.34 g, exercise 90% VO_{2max} group was 196.00±3.74 g, and exercise 90% VO_{2max} + 6 hr was 189.00±8.04 g, respectively.

The mean body weight of rats in exercise 90% VO_{2max} group were significantly higher than in normal control group (196.00±3.74 g VS.182.83±2.48 g, p=0.004) (Table 2 and Figure 7).

Animals after exercise

After rats ran finish 60 minutes, its found very exhausted in all groups, especially in group exercising at 90% VO_{2max}. Moreover, group resting 6 hours after exercise most of rats died before collection blood and histopathology.

2. Serum biochemical parameters

Serum total bilirubin (TB)

The mean serum total bilirubin (TB) level in normal control was 0.08 ± 0.02 mg/dL. Group exercise 75% VO_{2max} was 0.13 ± 0.54 mg/dL. Group exercise 75% VO_{2max} + 6 hr was 0.13 ± 0.31mg/dL. Group exercise 90% VO_{2max} was 0.15 ± 0.35 mg/dL and group exercise 90% VO_{2max} + 6 hr was 0.08 ± 0.01mg/dL. The mean level serum total bilirubin was not changed statistically siginificant in any groups (Table 3 and Figure 8).

Serum aspartate aminotransferase (AST)

The mean of serum aspartate aminotransferase (AST) in normal controls was 131.16 ± 16.44 U/L. Group exercise 75% VO_{2max} was 221.20 ± 51.84 U/L. Group exercise 75% VO_{2max} + 6 hr was 180.25 ± 37.65 U/L. Group exercise 90% VO_{2max} was 267.4 ± 60.12 U/L. Group exercise 90% VO_{2max} + 6 hr was 134.75 ± 19.95 U/L.

The mean level of serum aspartate aminotransferase (AST) were increased significantly in group exercise 90% VO_{2max} than in normal group (267.4 ± 60.12 U/L VS. 131.16 ± 16.44 U/L, p=0.004, Table 4 and Figure 9).

Serum alanine aminotransferase (ALT)

The mean of serum alanine aminotransferase (ALT) in normal control was 40.50 ± 5.57 U/L. Group exercise 75% VO_{2max} was 81.60 ± 28.64 U/L. Group exercise 75% VO_{2max} +6 hr was 62.50 ± 15.26 U/L. Group exercise 90% VO_{2max} was 76.00 ± 22.54 U/L. Group exercise 90% VO_{2max} +6 hr was 46.75 ± 11.05 U/L.

The mean level of serum alanine aminotransferase (ALT) in exercise 75% VO_{2max} group and exercise 90% VO_{2max} were increased significantly when compared with normal control group (81.60 ± 28.64 U/L VS. 40.50 ± 5.57 U/L and 76.00 ± 22.54 U/L VS. 40.50 ± 5.57 U/L, $p<0.005$, respectively). (Table 5 and Figure 10).

Serum enzyme amylase

The mean of enzyme amylase in normal group was 2,991.16 ± 280.31 U/L. Group exercise 75% VO_{2max} was 2872.80 ± 346.19 U/L. Group exercise 75% VO_{2max} + 6 hr was 2418.00 ± 173.37 U/L. Group exercise 90% VO_{2max} was 2397.20 ± 698.66 U/L. And, group exercise 90% VO_{2max} + 6 hr was 2563.25 ± 128.47 U/L.

The mean level serum enzyme amylase was not changed statistically siginificant in any groups (Table 6 and Figure 11).

Serum enzyme lipase

The mean lipase levels in normal control was 9.83 ± 0.75 U/L. Group exercise 75% VO_{2max} was 11.60 ± 0.89 U/L. Group exercise 75% VO_{2max} + 6 hr was 12.75 ± 3.40 U/L. Group exercise 90% VO_{2max} was 28.80 ± 16.75 U/L. And, group exercise 90% VO_{2max} + 6 hr was 10.25 ± 0.50 U/L.

The mean serum enzyme lipase in group exercise 90% VO_{2max} was increased significantly than in normal group (28.80 ± 16.75 U/L VS. 9.83 ± 0.75 U/L, p=0.004, Table 7 and Figure 12).

3. Histopathological Examination

The histopathological grading for liver congestion, necroinframmation and pancreas congestion and edema were summarized in Table 8 and 9. Histopathological study demonstrated that both liver and pancreas sections in the control group had normal morphological appearances. The congested and edematous pathological changes of liver and pancreas were obviously found in all groups of exercise compared with the normal control group (Table 8-9 and Figure13-25).

Table 2. Mean body weight of rats in each group.

Group	Body weight (g.)
Normal control	182.83±2.48
Exercise 75% VO_{2max}	190.60±8.47
Exercise 75% VO_{2max} + 6 hours	195.75±4.34
Exercise 90% VO_{2max}	196.00±3.74 *
Exercise 90% VO_{2max} + 6 hours	189.00±8.04

Values expressed as mean ± standard deviation (SD)

*Significant difference (p=0.004) compared with normal control group.

Table 3. Mean serum total bilirubin (TB) in each groups.

Group	Serum total bilirubin (mg/dL)
Normal control	0.08 ± 0.02
Exercise 75% VO_{2max}	0.13 ± 0.54
Exercise 75% VO_{2max} + 6 hours	0.13 ± 0.31
Exercise 90% VO_{2max}	0.15 ± 0.35
Exercise 90% VO_{2max} + 6 hours	0.08 ± 0.01

Values expressed as mean ± standard deviation (SD)

No significant difference in each group.

Table 4. Mean serum aspartate aminotransferase (AST) in each group.

Group	Serum aspartate aminotransferase (U/L)
Normal control	131.16 ± 16.44
Exercise 75% VO_{2max}	221.20 ± 51.84
Exercise 75% VO_{2max} + 6 hours	180.25 ± 37.65
Exercise 90% VO_{2max}	267.4 ± 60.12 *
Exercise 90% VO_{2max} + 6 hours	134.75 ± 19.95

Values expressed as mean ± standard deviation (SD)

*Significant difference (p=0.004) compared with normal control group.

Table 5. Mean serum alanine aminotransferase (ALT) in each group.

Group	Serum alanine aminotransferase (U/L)
Normal control	40.50 ± 5.57
Exercise 75% VO_{2max}	81.60 ± 28.64 *
Exercise 75% VO_{2max} + 6 hours	62.50 ± 15.26
Exercise 90% VO_{2max}	76.00 ± 22.54 *
Exercise 90% VO_{2max} + 6 hours	46.75 ± 11.05

Values expressed as mean ± standard deviation (SD)

*Significant difference (p=0.004) compared with normal control group.

Table 6. Mean serum enzyme amylase in each group.

Group	Serum enzyme amylase (U/L)
Normal control	2,991.16 ± 280.31
Exercise 75% VO_{2max}	2,872.80 ± 346.19
Exercise 75% VO_{2max} + 6 hours	2,418.00 ± 173.37
Exercise 90% VO_{2max}	2,397.20 ± 698.66
Exercise 90% VO_{2max} + 6 hours	2,563.25 ± 128.47

Values expressed as mean ± standard deviation (SD)

No significant difference in each group.

Table 7. Mean serum enzyme lipase in each groups.

Group	Serum enzyme lipase (U/L)
Normal control	9.83 ± 0.75
Exercise 75% VO_{2max}	11.60 ± 0.89
Exercise 75% VO_{2max} + 6 hours	12.75 ± 3.40
Exercise 90% VO_{2max}	28.80 ± 16.75 *
Exercise 90% VO_{2max} + 6 hours	10.25 ± 0.50

Values expressed as mean ± standard deviation of the mean ± SD

*Significant difference (p=0.004) compared with normal control group.

Table 8. Summary of the level of congestion, edema and necroinflammation of the liver.

Group	Number	Level of congestion and edema				Level of necroinflammation			
		0	1	2	3	0	1	2	3
Normal control	6	6	-	-	-	6	-	-	-
Ex.75% VO_{2max}	5	-	5	-	-	-	4	1	-
Ex.75% VO_{2max} + 6 hr	4	-	4	-	-	-	2	1	1
Ex. 90% VO_{2max}	5	-	3	1	1	-	3	1	1
Ex.90% VO_{2max} + 6 hr	4	-	2	-	2	-	2	2	-

The severity of congestion and edema was leveled by:

0 = no congestion and edema

1 = mild congestion and edema

2 = moderate congestion and edema

3 = severe congestion and edema

The severity of necroinflammation was leveled by:

0 = no hepatocyte injury/inflamammation

1 = Sparse or mild hepatocyte injury/inflamammation

2 = noticeable hepatocyte injury/inflammation

3 = severe hepatocyte injury/inflammation

Table 9. Summary of the level of congestion and edema of pancreas.

Group	Number	Level of congestion and edema			
		0	1	2	3
Normal control	6	6	-	-	-
Exercise 75% VO_{2max}	5	-	5	-	-
Exercise 75% VO_{2max} + 6 hr	4	-	3	1	-
Exercise 90% VO_{2max}	5	-	3	-	2
Exercise 90% VO_{2max} + 6 hr	4	-	4	-	-

The severity of congestion and edema was leveled by:

0 = no congestion and edema

1 = mild congestion and edema

2 = moderate congestion and edema

3 = mark congestion and edema

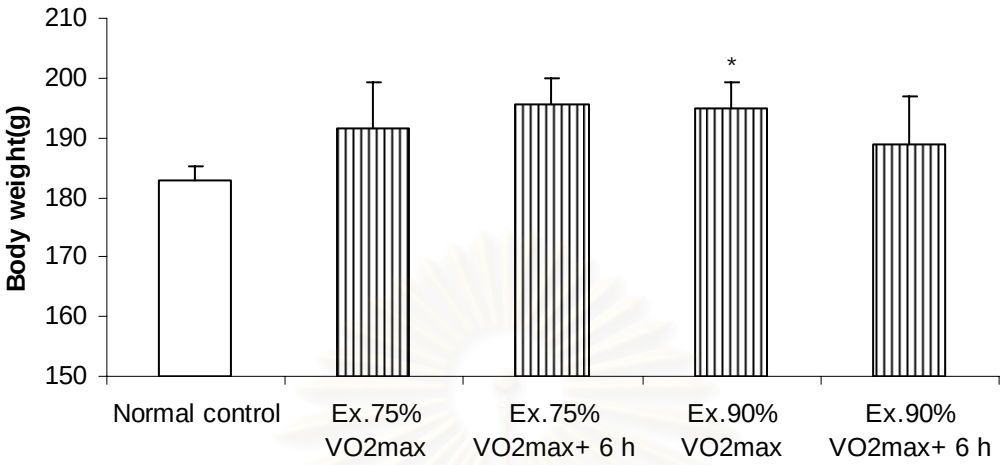


Figure 7. The body weight (g) of all groups (mean ± SD)

*Significant difference (p=0.004) compared with normal control group

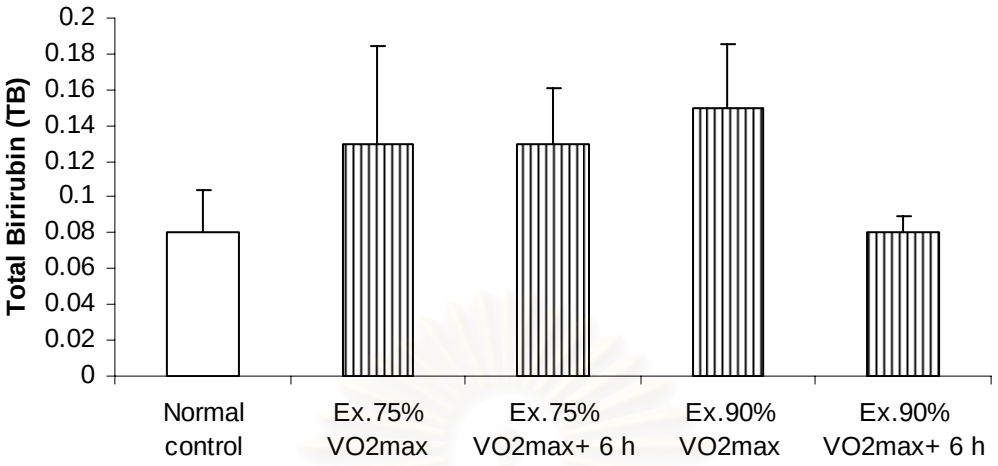


Figure 8. Effects of intense exercise on serum total biririubin (TB) (mean $\pm$ SD)

No significant difference in each group.

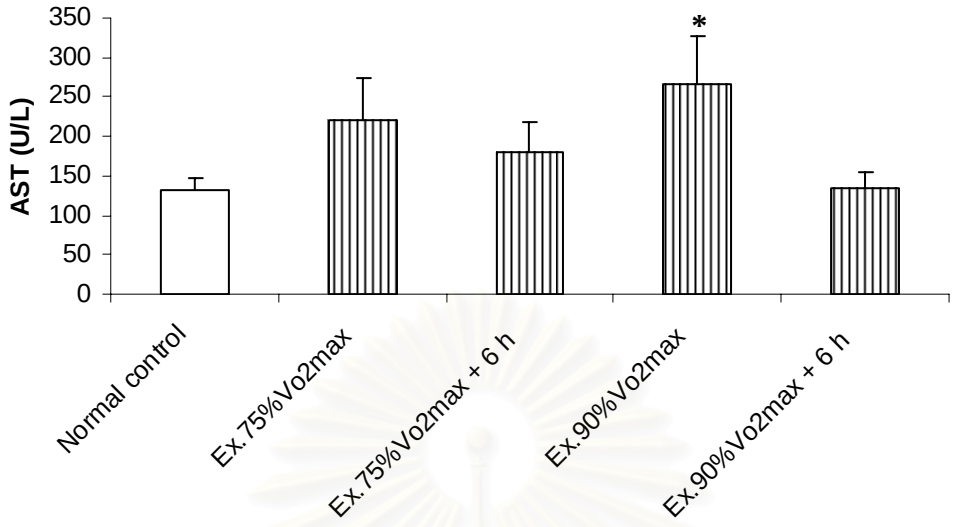


Figure 9. Effect of exercise on serum AST (U/L) (mean $\pm$ SD)

*Significant difference (p=0.004) compared with normal control group

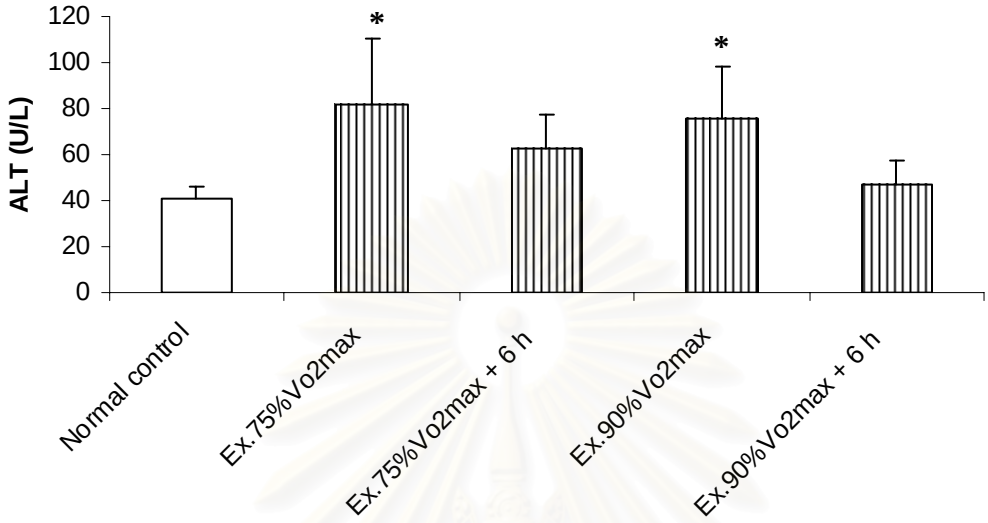


Figure 10. Effect of intense exercise on serum ALT (U/L) (mean ± SD)

*Significant difference (p=0.004) compared with normal control group

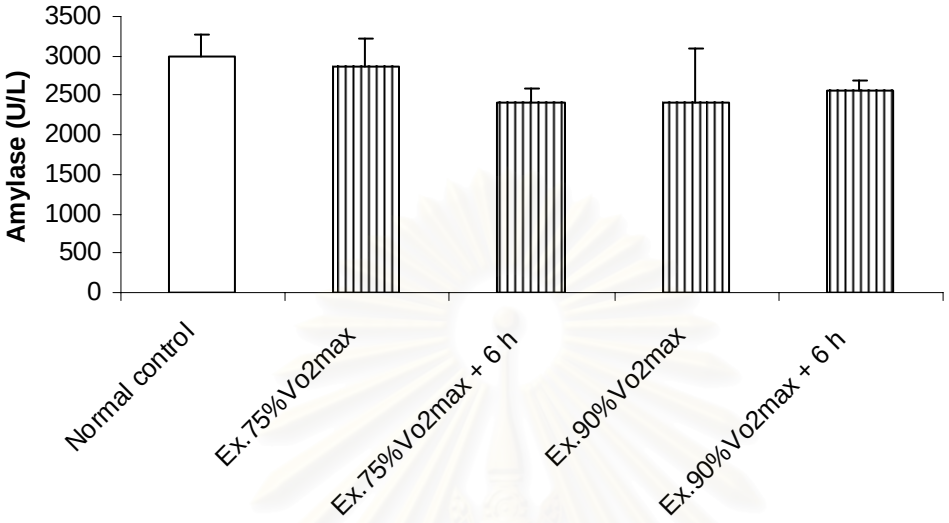


Figure 11. Effect of exercise on seum enzyme amylase (U/L) (mean ± SD)

No significant difference in each group.

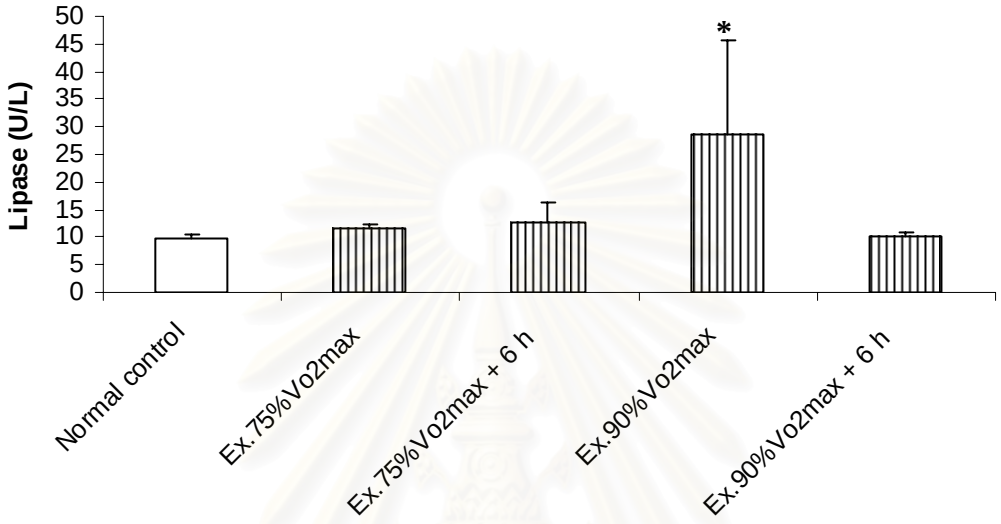


Figure 12. Effect of intense exercise on seum enzyme lipase U/L) (mean ± SD)

***** Significant difference (p=0.004) compared with normal control group

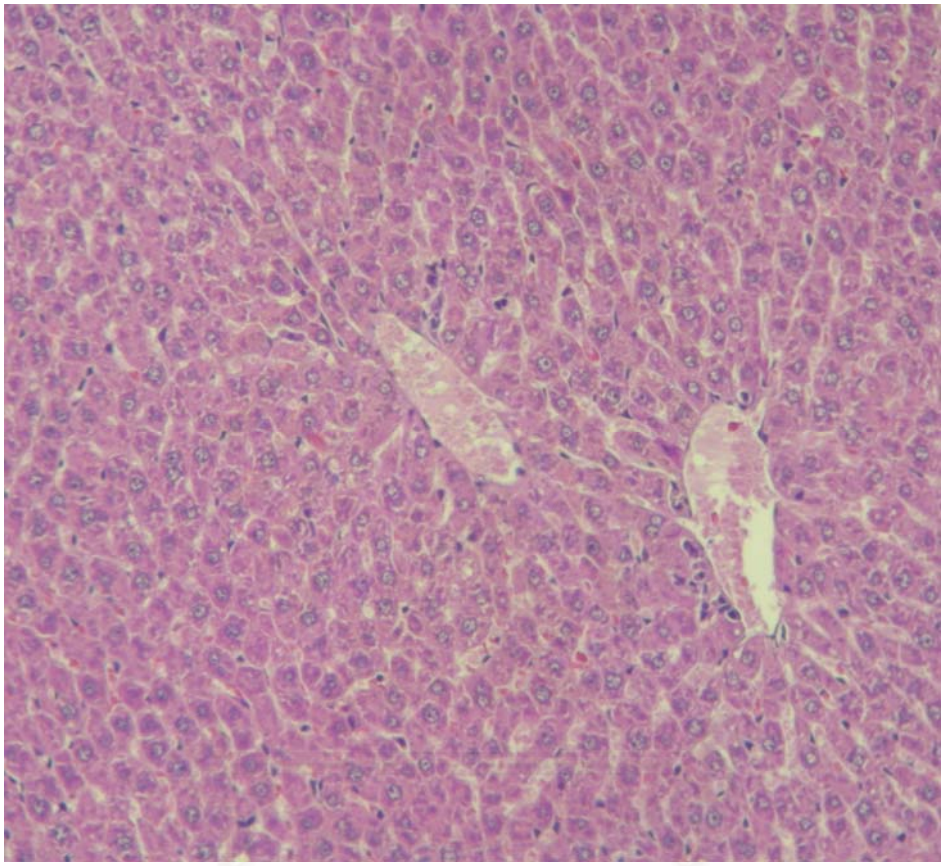


Figure 13. Liver section (H&E, 100X) in normal control group.

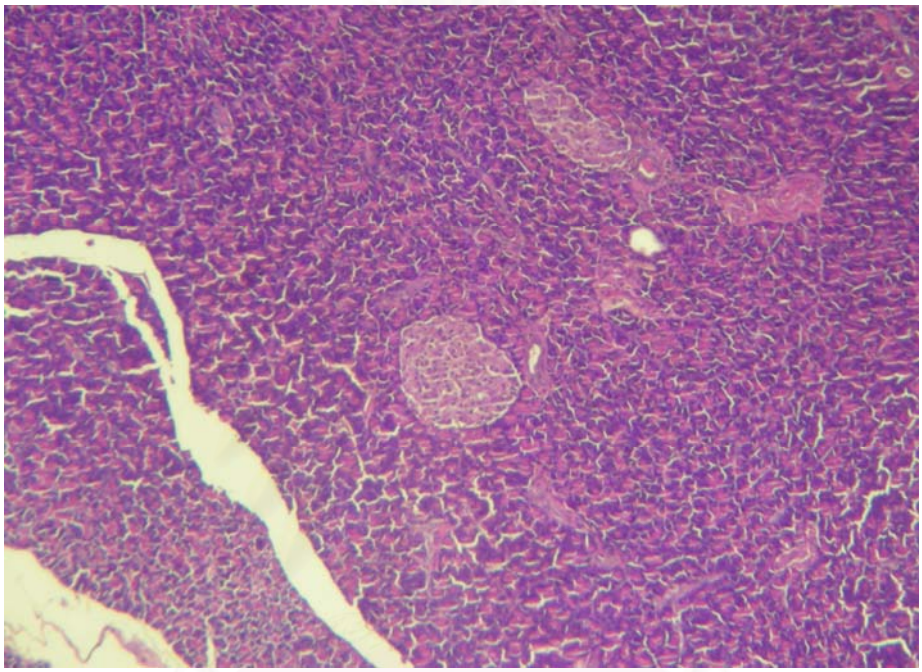
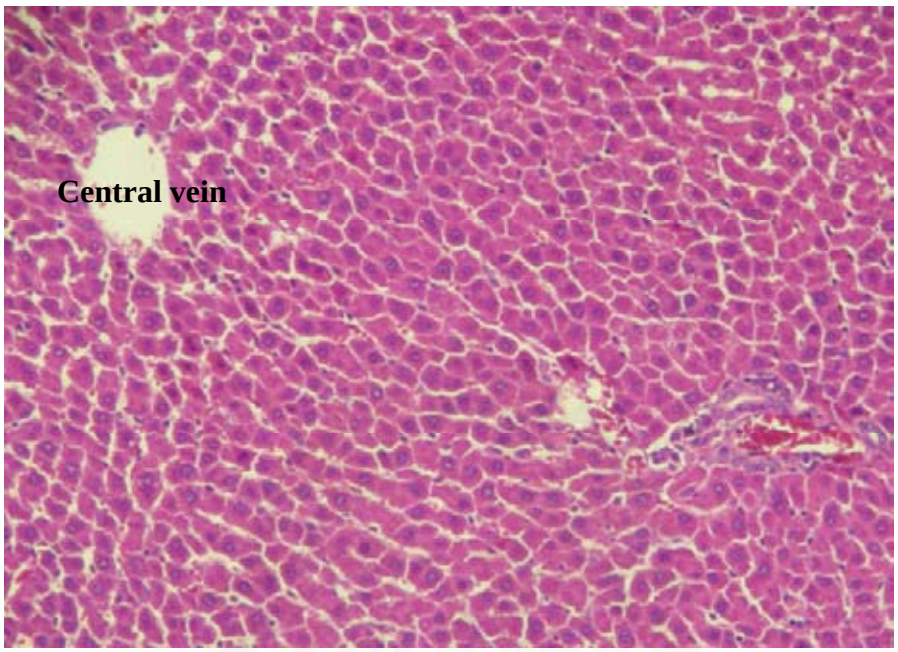
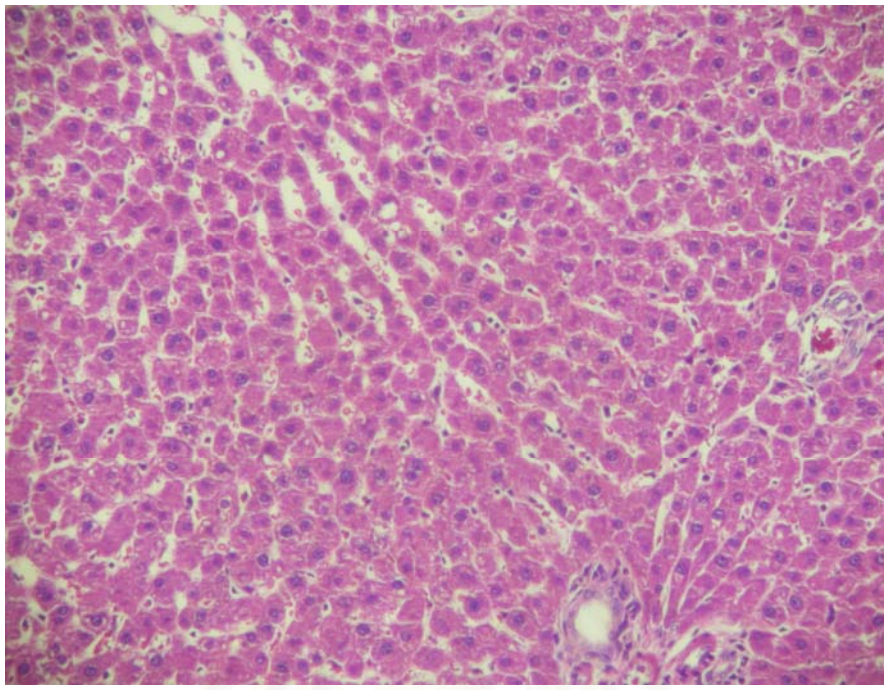


Figure 14. Pancreas section (H&E, 100X) in normal control group.



A

Figure 15. Example of liver in 75% VO_{2max} demonstrating mild congestion and edema ; mild inflammatory cells (A and B, H&E. 100X).



B

Figure 16. Example of liver in 75% VO_{2max} demonstrating mild congestion and edema ; mild inflammatory cells (A and B, H&E. 100X).

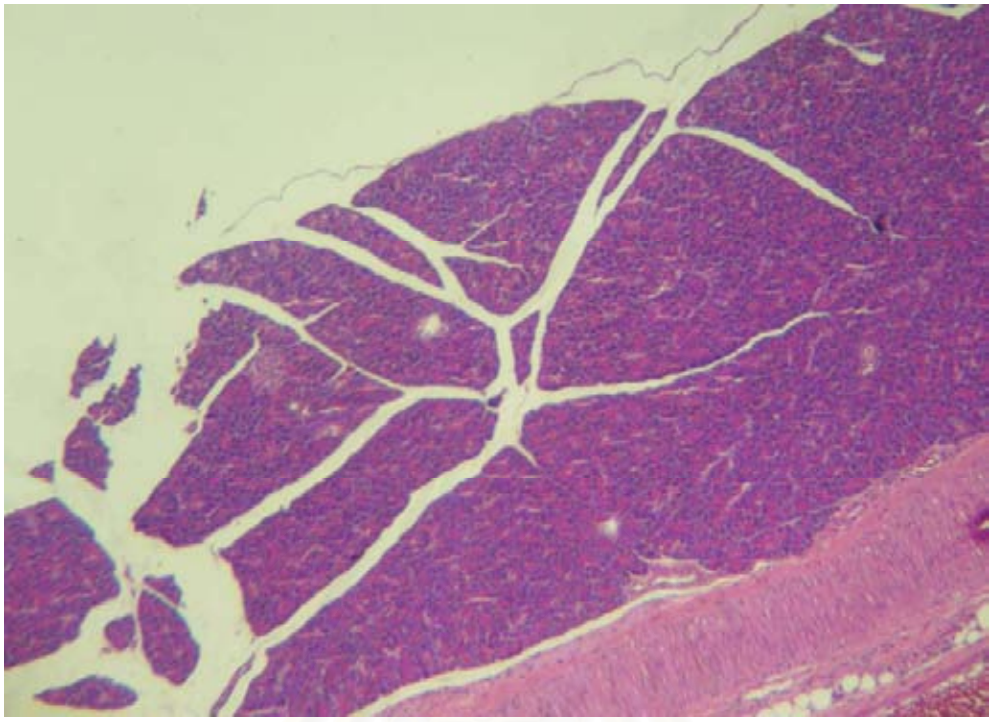
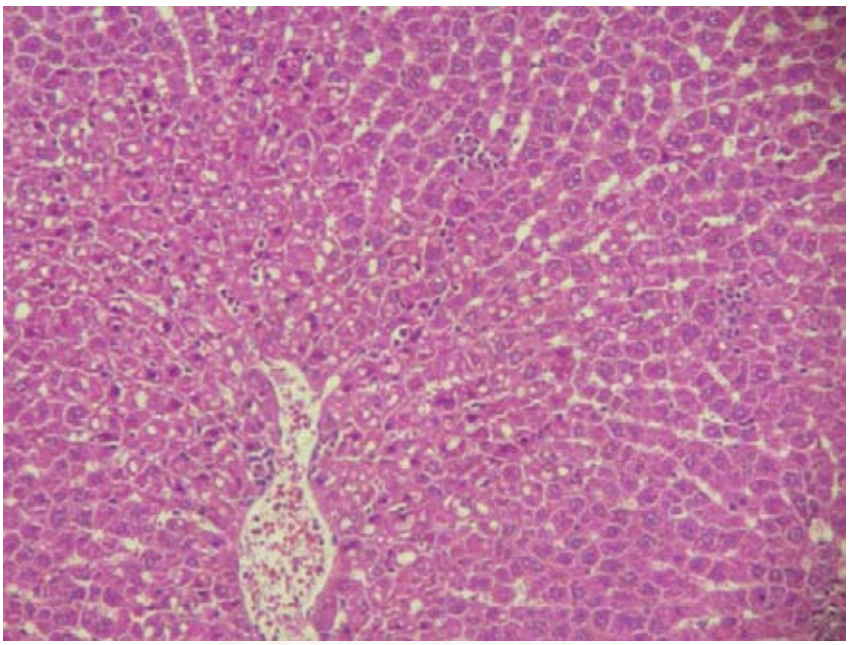
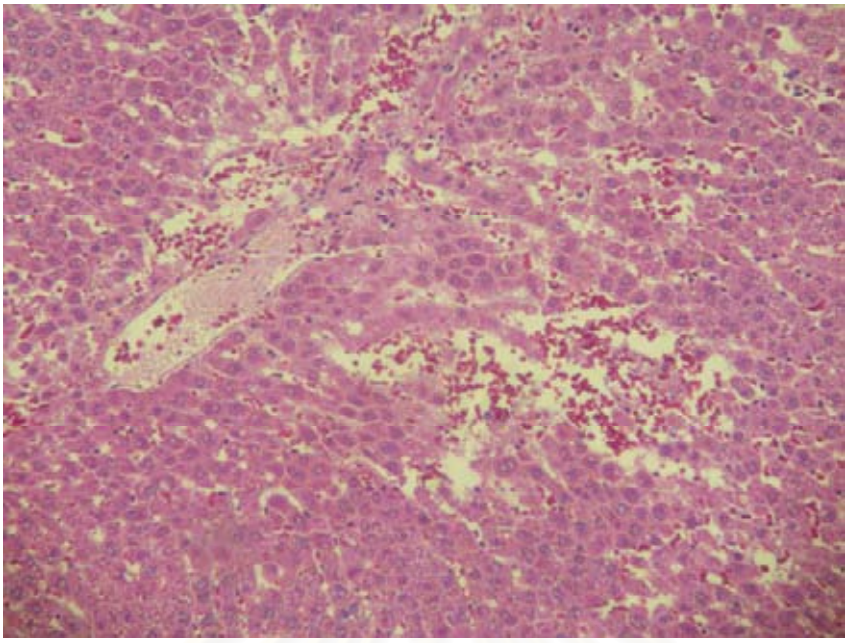


Figure 17. Example of the pancreas in 75% VO_{2max} exercise group demonstrating mild congestion and mild edema (H&E, 40X).



A

Figure 18. Example of liver in 75% VO_{2max} exercise +6 h group showing mild congestion and edema ; slightly inflammatory necrosis cells (A and B, H&E, 100X),.



B

Figure 19. Example of liver in 75% VO_{2max} exercise +6 h group showing mild congestion and edema ; slightly inflammatory necrosis cells (A and B, H&E, 100X),.

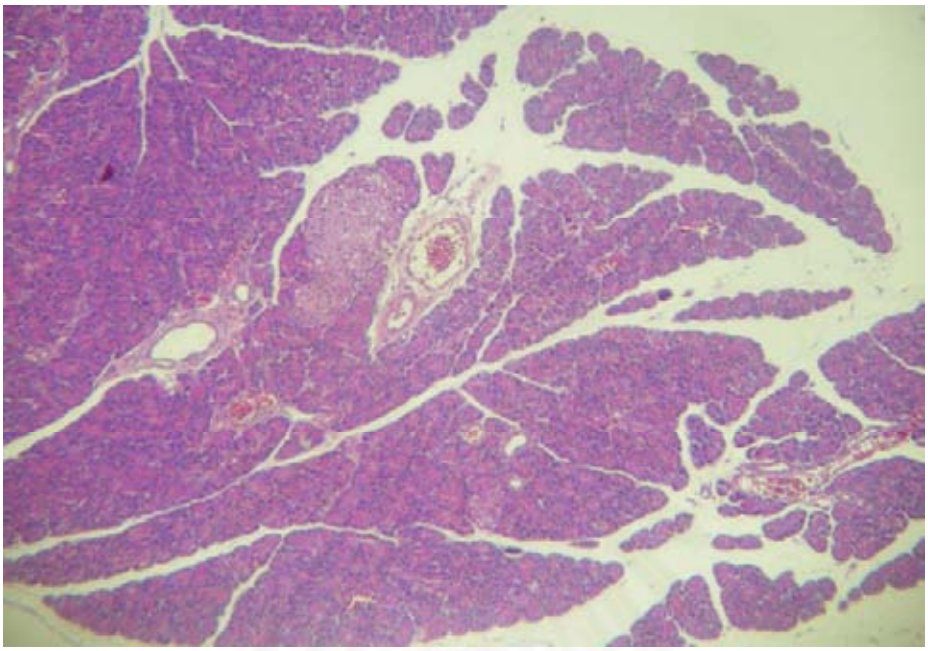
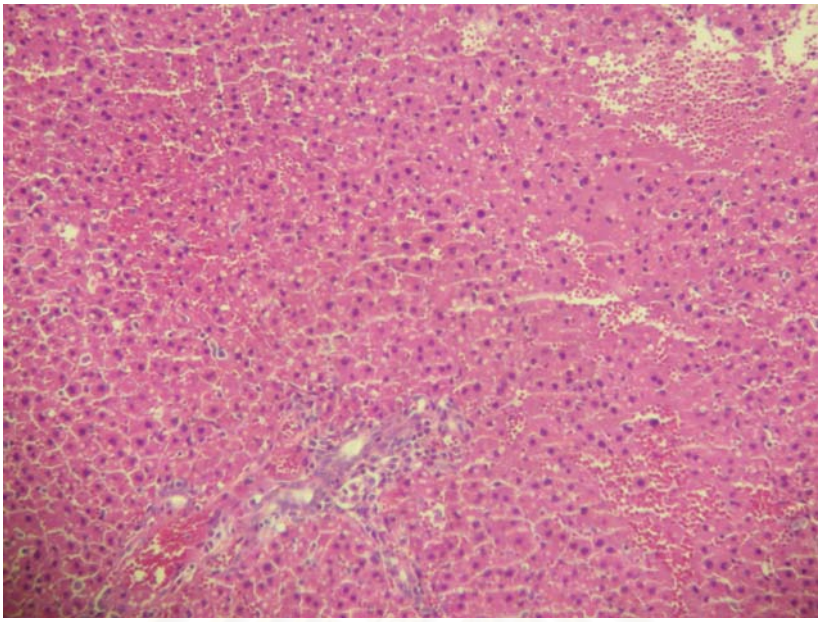
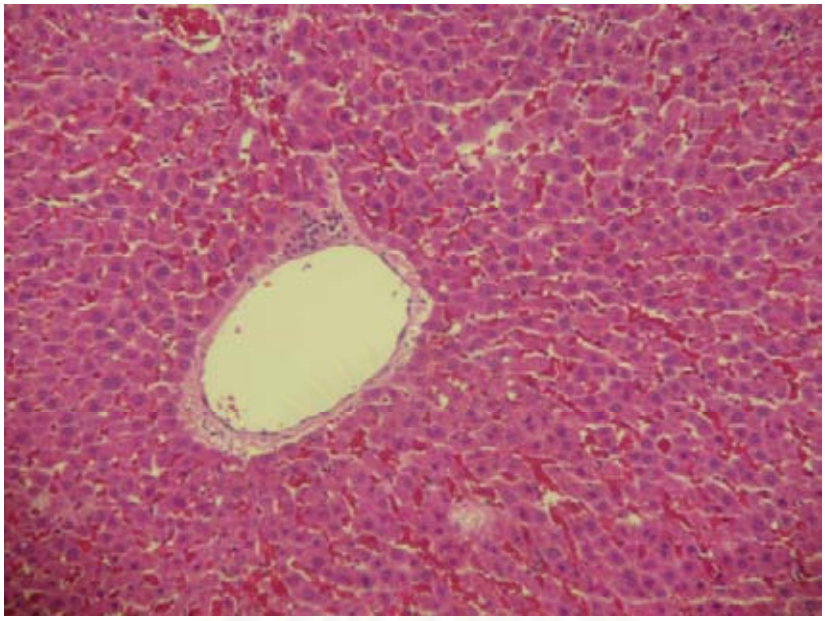


Figure 20. Example of the pancreas of 75% VO_{2max} exercise +6 h group showing mild congestion and edema (H&E, 40X).



A

Figure 21. Example of hepatic histopathology in 90% VO_{2max} exercise group showing moderate congestion and edema ; increased inflammatory cells and hemorrhagic necrosis (A and B, H&E, 100X).



B

Figure 22. Example of hepatic histopathology in 90% VO_{2max} exercise group showing moderate congestion and edema ; increased inflammatory cells and hemorrhagic necrosis (A and B, H&E, 100X).

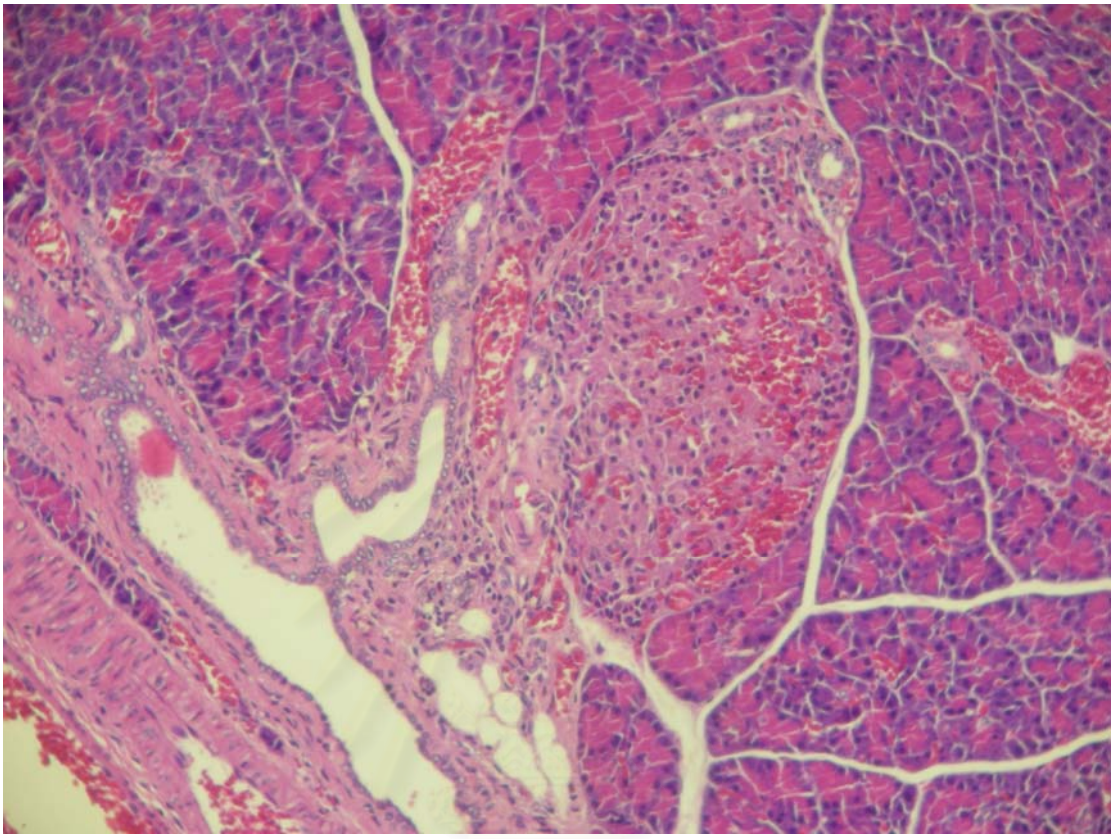


Figure 23. Example of the pancreas in 90% VO_{2max} exercise group showing marked congestion and edema (H&E, 100X).

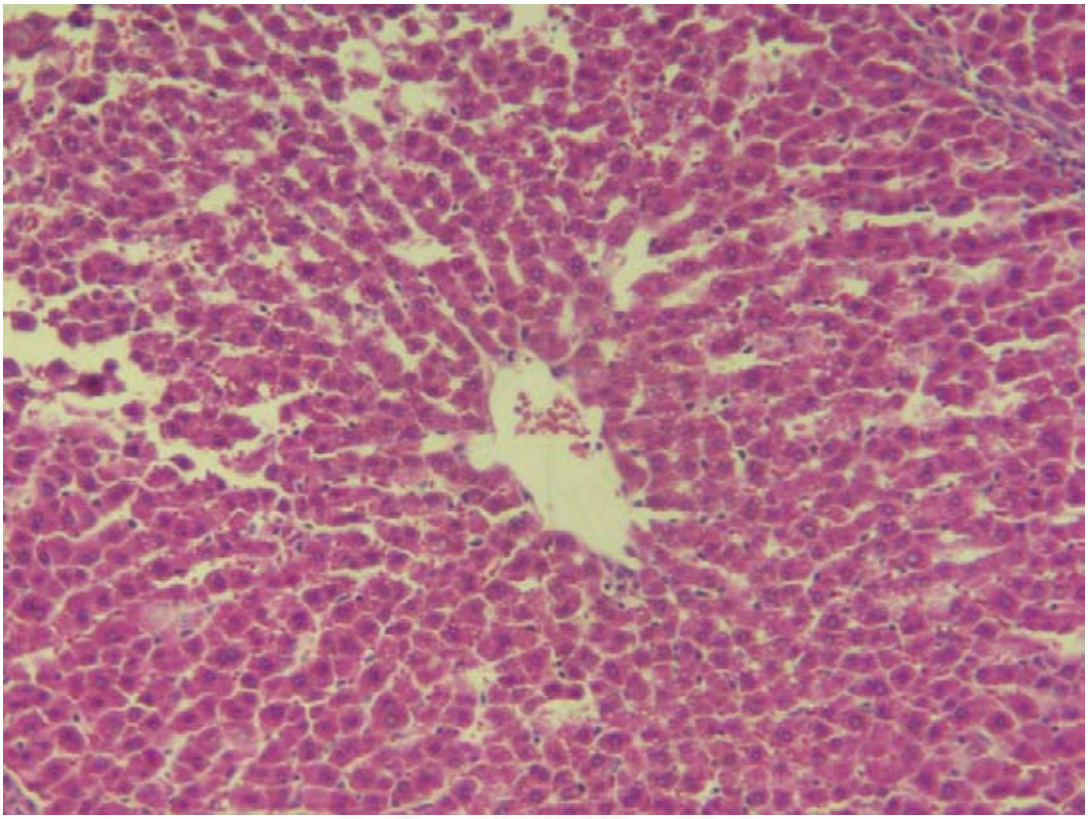


Figure 24. Example of hepatic histopathology of 90% VO_{2max} exercise + 6 h group showing marked congestion and edema ; increased inflammation necrosis cells (H&E, 100X).

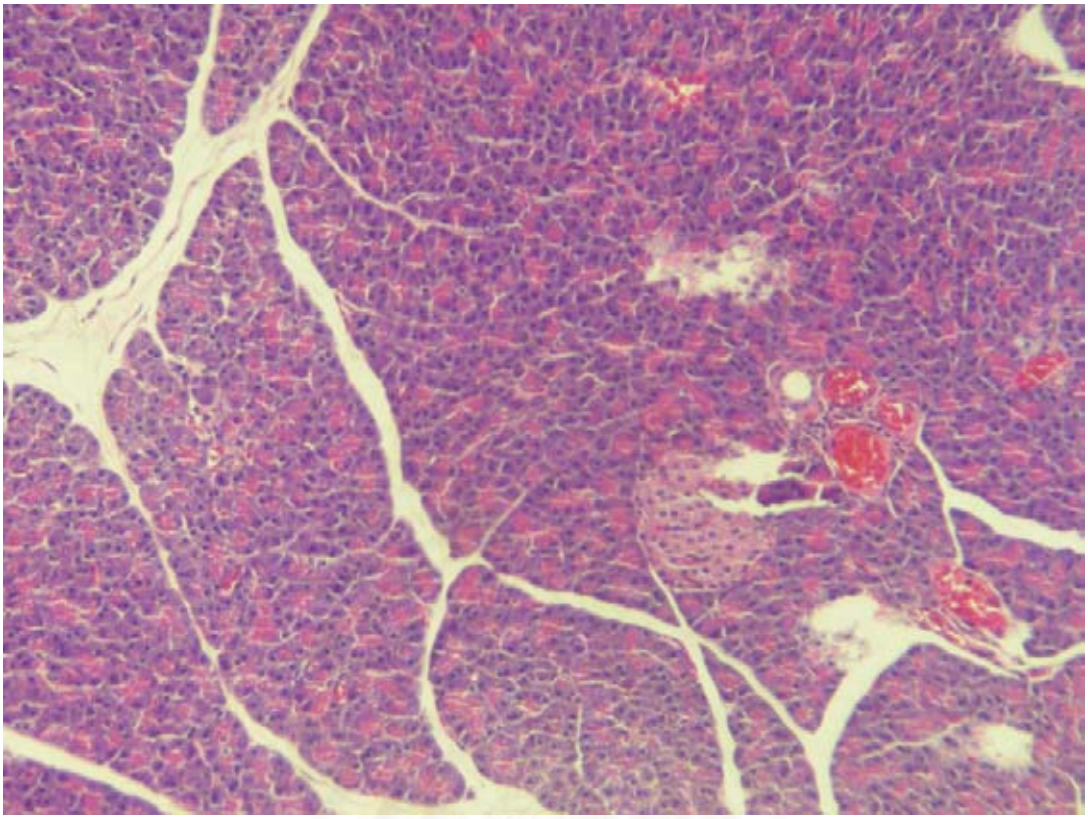


Figure 25. Example of the pancreas in 90% VO_{2max} exercise +6 h group showing mild congestion and edema (H&E, 100X).

CHAPTER V

DISCUSSION AND CONCLUSION

In the present study, the experiments were conducted to investigate the effects of intense exercise on change of function and pathology of liver and pancreas in rats. From the results, serum total bilirubin (TB), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and enzyme lipase and enzyme amylase as well as histopathology of the liver and pancreas were analyzed. Discussion is expressed in relation to the topic heading as follows.

Effects of intense exercise on change of function and histopathology of liver and pancreas in rats

The present experiment was designed to investigate exercise intensity level on function and pathology of liver and pancreas in rats. The experimental protocol was conducted by having the rats running at 60 minutes of acute exercise of two different high intensities. The rats used in the present study were Sprague-Dawley because their VO_{2max} during different velocities and elevations of treadmill running have been determined (Bedford et al., 1979).

Other researchers reported liver cell injury after exhaustive exercise (Fojt et al. 1976). Acute physical exercise in rats resulted in a shrinkage of the liver cells, while exercised liver displayed a 15% decrease in the hepatocellular hydration level compared with normal rest conditions (Latour et al., 1999). In the present study, we found liver cell injury after high intensity exercise both in 75% VO_{2max} group and 90% VO_{2max} group. Moreover, the higher intensity level of exercise induced more liver cell injury. The hepatic blood flow significantly decreased in the high intensity exercise group compared with sedentary control and moderate intensity exercise groups (Yano

et al., 1996). In addition, it was thought that the hepatic blood flow was responsible for reperfusion in the high intensity exercise group. It was well known that high intensity exercise greatly reduced hepatic blood flow (Rowell et al., 1964). The reduction of blood flow in the liver caused hypoxia of hepatocytes and eventually induced their necrosis, thus causing cells damage. Our results showed hepatocyte inflammation after high intensity exercise. Latour et al.,(1999) reported an absence of post-exercise hepatic function deterioration under their experimental conditions.

The ischemia-reperfusion could be induced by inflammation (Macord., 1987) and the ischemia-reperfusion which was also known to cause an excessive production of free radical. The high intensity exercise especially exhaustive exercise led to hepatic hypoxia-reperfusion and promoted the influence of free radicals and lipid peroxidation (Caraceni et al.1994). Additionally, we could not predict whether oxidative stress would occur or not in groups 6 hours waiting after high intensity exercise. In this study, a recovery period of 6 hours after both 75% VO_2max and 90% VO_2max exercise might have caused similar reflects to hypoxia-reperfusion in the liver. As a result, we found that some rats in the groups that rested 6 hours after finishing exercise at 75% VO_2max and 90% VO_2max died with in the period of 6 hours after completing 60 minutes of exercise. The live cells of rats waiting 6 hours after high intensity exercise period showed cell damage possibly induced by hepatic reperfusion. However, there have been a few studies clarifying the relationship between exercise and ischemic reperfusion-induced injury, particularly in the liver (Caraceni et al.1994).

Moreover, there were significant increased in the serum ALT activities in group of exercise 75% and 90% VO_{2max} ,AST and lipase activities in group of exercise 90% VO_{2max} compared to normal control group. In 6 hours after exercise, serum AST, ALT and lipase were decreased to the same level as the normal controls. However, the TB levels in 6 hours 75% VO_{2max} exercise after exercise remained stable compared with immediately after exercise in the 75% VO_{2max} group. Also, serum lipase levels did not change in 6 hours after exercise in the 75% VO_{2max} group. Jaeschke et al (1990), showed that time course of plasma ALT activities in two phases of liver injury after hepatic ischemia: an initial injury during the first hour of perfusion, and later progression phase. As a result of

increased serum enzymes by exercise, it can be explained that circulation changes and internal secretions in the liver caused metabolic substrates to be depleted, thus altering the membrane permeability of hepatocytes. The removal of oxygen to liver is caused by the reduction of a blood flow, and production of ATP also decreased (Yano et al., 2003). Accordingly, hypoxia should be more severe following high intensity exercise than moderate intensity exercise. Exhaustive exercise induced anoxia and promoted permeability of a cell membrane, resulting in the release of various enzymes into the blood. The exhaustion of ATP, glycolysis and cell expansion by the rise of osmotic pressure caused the enzyme deviation in the liver cells.

Pancreatic enzyme synthesis and secretion have been reported to change in response to various physical and dietary conditions, including physical exercise. Konturek et al., (1973) reported decreased postprandial pancreatic secretion with a decrease in splanchnic blood flow during acute exercise in dogs. In contrast, increases pancreatic enzymes activity and secretion induced by chronic swimming (Zsinka et al., 1983) or voluntary running (Kugino et al., 1991) have been reported in rats. The present data are the first to our knowledge to confirm the hypothesis of an acute high intensity exercise on change of function and pathology in rat pancreas. In the present study, the results suggested that after high intensity exercise enzyme lipase was increased significant in exercise 90% VO_{2max} compared with normal controls group. Additionally, the enzyme lipase was decreased in 6 hours after 90% VO_{2max} exercise. The results of Minato et al., 2000 enzyme lipase and amylase activity were increased significant compared with normal control in chronic exercise. The exocrine pancreas controls the synthesis, storage, and secretion of pancreatic enzymes and is regulated by both gastrointestinal hormones and the vagus nerve. Exercise is known to increase serum gastrointestinal hormones levels (Sullivan et al., 1984). Ohta et al., (1994) reported that chronic exercise increased cholecystokinin (CCK) content of the intestine. CCK may play an important role in the hypertrophy of acinar cells. The histopathological of pancreas in this study showed mild to moderate edema and congestion of acinar cell in all group of exercise groups and this might have caused by reduction of blood flow and hypoxia.

As this study, could not predict whether oxidative stress would occur or not at 6 hours after the high intensity exercise as described above. Further studies should be conducted to explore the indicator of oxidative stress, which is known to cause an excessive production of free radicals.

In conclusion, this study demonstrated the acute high intensity exercise caused changes of function and pathology of liver and pancreas in rats. The elevation ALT, AST and lipase were correlated with intensity of exercise and resoluted after resting for 6 hours. Even though there was no significant change in the serum amylase, we postulated the increase permeability of hepatocyte and pancreatic cell membrane damage by the prolonged insufficient blood flow.

REFERENCES

นทีทิพย์ กฤษณามระ. 2538. ฮอร์โมน: กลไลและการออกฤทธิ์ร่วม กรุงเทพ:ไทยวัฒนาพานิช.

ปิยา บูรณศิริ. 2525. การย่อยและการดูดซึมอาหาร พิมพ์ครั้งที่1. กรุงเทพ:จุฬาลงกรณ์มหาวิทยาลัย.

สัญญา ร้อยสมมติ. 2535. ตับอ่อนและการควบคุมกลูโคส พิมพ์ครั้งที่1. ขอนแก่น:ภาควิชาสรีรวิทยา คณะแพทยศาสตร์ มหาวิทยาลัยขอนแก่น.

Aoi, W., et al. 2004. Effect of exercise on hepatic gene expression in rats: amicroarray analysis. Life Sciences 75: 3117-3128.

Brunt, E.M., et al. 1999. Nonalcoholic steatohepatitis: A proposal for grading and staging the histological lessions. Am J Gastroenterol 94 (9): 2467-2474.

Blyakhamn, FA., et al. 1999. Quantal length changes in single contraction sarcomeres. J Muscle Res Cell Motil 20: 529.

Blomquist, CG., et al. 1982. Similarity of the hemodynamic responses to static and dynamic exercise of small muscle groups. Circ Res 48: 87.

Bedford, T. G., C. M. Tipton, N. C. Wilson, R. A. Oppliger, and C. V. Gisolfi. 1979. Maximum Oxygen Consumption of rats and its changes with various experimental procedures. Journal of Applied Physiology 47: 1278-1283.

Boni-Schnetzler M., et al. 1991. Insulin regulates insulin-like growth factor-1 mRNA in rat hepatocytes. Am J Physiol 260:846–51.

Cartee, G.D., and Farrar, R.P. 1988. Exercise training induces glycogen sparing during exercise by old rats. Journal of Applied Physiology 64 (1): 259.

Cappon J, Braesel JA, Mohan S., and Cooper DM. 1994. Effect of a brief exercise on circulating insulin-like growth factor–1. J Appl Physiol 76: 2490–6.

Caraceni, P., Rosenblum, E.R., Van Thiel, D.H., and Borle, A.B. 1994. Reoxygenation injury in isolated rat hepatocytes: relation to oxygen free radicals and lipid peroxidation. AM J Physiol 266: G799-G806.

Dyke, T.M., Sams, R.A., and Hinchcliff, K.W. 1998. Exercise-training-induced alterations in hepatic function in mares. Journal of Applied Physiology 85 (4): 1442–1447.

Donovan, C.M., and Sumida, K.D. 1997. Training enhanced hepatic gluconeogenesis: the importance for glucose homeostasis during exercise. Medicine and Science in Sports and Exercise 29 (5): 628–634.

Frenkl, R., Gyore, A., and Szeberenyi, S. 1980. The effect of muscular exercise on the microsomal enzyme system of the rat liver. European Journal of Applied Physiology 44 (2): 135-140.

Fiebig, R.G., Hollander, J.M., Ney, D., Boileau, R., Jeffery, E., and Ji, L.L. 2002. Training down-regulates fatty acid synthase and body fat in obese Zucker rats. Medicine and Science in Sports and Exercise 34 (7): 1106-1114.

Fojt, E., L.-G. Ekelund., and E.Hultman. 1976. Enzyme activities in hepatic venous blood under strenous physical exercise. Pflugers Arch 361: 287 – 296.

Fedele, M.J., Hernandez, J.M., Lang, C.H., Vary, T.C., Kimball, S.R., Jefferson, L.S., and Farrel, P.A. 2000. Severe diabetes prohibits elevation in muscle protein synthesis after acute resistance exercise in rats. Journal of Applied Physiology 88: 102-108.

Gorski, J., Oscai, L.B., and Palmer, W.K. 1990. Hepatic lipid metabolism in exercise and training. Medicine and Science in Sports and Exercise 22 (2): 213–221.

Gulati M, Pandey DK, Arnsdorf MF, Lauderdale DS, Thisted Ra., and Wicklund RH. 2003. Exercise capacity and the risk of death in women. The St.James women take heart project. Circulation 108: 1554-9.

Gleeson TT, Mullin WJ., and Baldwin KM. 1983. Cardiovascular responses to treadmill exercise in rats: effects of training. J Appl Physiol 54: 789–793.

Guo, Z., Xu, R.B., and Dong, R.C. 1986. Determination of half-life and characterization of the glucocorticoid receptor of isolated splenic cells in mice. Acta Biologiae Experimentalis Sinica (China) 19: 321–325.

Hagberg, JM., et al. 1978. Comparison of three procedures for measuring VO_{2max} in competitive cyclists. Eur J Appl Physiol 39: 47.

Henriksen EJ, Louters LL, Stump CS., and Tipton CM. 1992. Effects of prior exercise on the action of insulin-like growth factor-1 in skeletal muscle. Am J Physiol 263: 340–4.

Hepple, R.T., Hagen, J.L., Krause, D.J., and Jackson, C.C. 2003. Aerobic power declines with aging in rat skeletal muscles perfused at matched convective O_2 delivery. Journal Applied Physiology 94: 744-751.

Jaeschke, H., Farhood, A., and Smith, C.A. 1990. Neutrophils contribute to schema/referfusion injury in rat liver in vivo. FASEB J 4: 3355-3359.

Kasch, FW., et al. 1966. A comparison of maximal oxygen uptake by treadmill and step test procedures. J Appl Physiol 21: 1387.

Konturek, Sj., Tasker, J., and Obtulowicz, W. 1973. Effect of exercise on gastrointestinal secretion. J Appl Physiol 34: 324-328.

Kirwan, J.P., and Aguila, L.F. 2003. Insulin signalling, exercise and cellular integrity. Biochemical Society Transacions 31 (6): 1281-1285.

Kinoshita, S., Yano, H., and Tsuji, E. 2003. An increase in damaged hepatocytes in rats after high intensity exercise. Acta Physiol Scand 178: 225-230.

Koziris LP, Hickson RC, Chatterton RT, Groseth RT, Christie JM, Goldflies DG., and Unterman TG. 1999. Serum levels of total and free IGF-1 and IGFBP-3 are increased and maintained in long-term training. J Appl Physiol 86:1436–42.

Kemi, O.J., Haram, P.M., Leonnechen, J.P., Osnes, J.B., Skomedal, T., Wisloff, U., and Ellingsen, O. 2005. Moderate vs. high exercise intensity: Differential effects on aerobic fitness, cardiomyocyte, and endothelial function. Cardiovascular Research 67: 161-172.

Lemaster, J.J., Stemkowski, C.J., Ji, S. and Thurman, R.G. 1983. Cell surface changes and enzyme release during hypoxia and reoxygenation in the isolated, perfused rat liver. J Cell Biol 97: 778-786.

Latour, M.G., Brault, A., Huet, P.M., and Lavoie, J.M. 1999. Effects of acute physical exercise on hepatocyte volume and function in rat. American Journal of Physiology 276 (5): R1258- R1264.

Li, Z., Murakami, T., Nakai, N., Nagasaki, M., Obayashi, M., Xu, M., Sato, J., Oshida, Y., Sato, Y., and Shimomura, Y. 2001. Modification by exercise training of activity and enzyme expression of hepatic branched-chain alpha-ketoacid

dehydrogenase complex in streptozotocin-induced diabetic rats. Journal of Nutritional Science and Vitaminology (Tokyo) 47 (5): 345–350

Lee IM, seeso HD, Oguma Y, and Paffenbarger Jr RS. 2003. Relative intensity of physical activity and risk of coronary heart disease. Circulation 107: 1110-6.

Lewis, SF., et al. 1983. Cardiovascular responses to exercise as functions of absolute and relative work load. J Appl Physiol 54: 1314.

Laughlin, MH., et al. 1994. Physical activity and the microcirculation in cardiac and skeletal muscle. In: bouchard C, et al, eds. Physical activity, Fitness, and health. Campaign, IL: Human kinetics.

Lieber, RL., 1992. Skeletal muscle structure and function. Baltimore: Williams & Wilkins.

Lieber, Rl., et al. 1994. In vivo measurement of human wrist extensor muscle sarcomere length changes. J Neurophysiol 71: 874.

MacDougall, JD., et al. 1979. Mitochondrial volume density in human skeletal muscle following heavy resistance training. Med Sci Sports 11: 164.

Mitchell, JH., and Raven, PB. 1994. Cardiovascular adaptation to physical activity. In: Bouchard C, et al., eds. Physical activity, Fitness, and health. Champaign. IL: Human Kinetics.

Menier, DR. 1968. Body composition and aerobic capacity in young and middle-aged endurance-trained men. Med Sci Sports Exerc 19: 557.

McArdle, WD., et al. 1971. Metabolic and cardiorespiratory response during free swimming and treadmill walking. J Appl Physiol 30: 733.

McArdle, WD., et al. 1978. Specificity of run training on VO_{2max} and heart rate changes during running and swimming. Med Sci Sport 10: 16.

Magel, JR., and Faulkner, JA. 1967. Maximum oxygen uptake of college swimmers. J Appl 22: 929.

Minato, K., 1997. The Effect of endurance training on pancreatic enzyme activity in rats. Eur J Appl Physiol 76: 491-474.

McCord, J.M. 1987. Oxygen-derived radicals: a link between reperfusion injury and inflammation. Fed Proc 46: 2402-2406.

Musch TI, Moore RL, Smaldone PG, Riedy M., and Zelis R. 1989. Cardiac adaptations to endurance training in rats with a chronic myocardial infarction. J Appl Physiol 66: 712-719,

Moore RL, Musch TI, Yelamarty RV, Scaduto-RC J, Semanchick AM, Elensky M., and Cheung JY. 1993. Chronic exercise alters contractility and morphology of isolated rat cardiac myocytes. Am J Physiol Cell Physiol 264: C1180–C1189,

Mokelke EA, Palmer BM, Cheung JY., and Moore RL. 1997. Endurance training does not affect intrinsic calcium current characteristics in rat myocardium. Am J Physiol Heart Circ Physiol 273: H1193–H1197.

Mazzeo, R.S., Brooks, G.A., and Horvath, S.M. 1984. Effects of age on metabolic responses to endurance training in rats. Journal Applied Physiology 57: 1369-1374.

Mauriz, J.L., Tabernero, B., Garcia-Lopez, J., Jorquera, F., Villa, J.G., and Gonzalez-Gallego, J. 2000. Physical exercise and Improvement of liver oxidative metabolism in the elderly. European Journal of Applied Physiology 81 (1–2): 62–66.

Minato, K. 2000. The effect of chronic exercise on the rat pancreas. International Journal of Pancreatol 27 (2): 151-156.

Nagel, D., D. Seiler, Franz, H and Jung, K. 1990. Ultra-long distance running and the liver. Int J Sports Med 11: 441-445,

Nguyen, U.N., Mougin, F., Simon-Rigaud, M.L., Rouillon, J.D., Marguet, P., and Regnard, J., 1998. Influence of exercise duration on serum insulin-like growth factor and its blinding protein in athletes. European Journal Applied Physiology 78: 533-537.

Nielsen, OB., and Clausen, T. 2000. The Na^+/K^+ –pump protects muscle excitability and contractility during exercise. Exerc Sport Sci Rev 28: 159.

Ohta, M., Ichikawa, M., Sazaki, N., Okubu, K., Miyasaka, K., Fujita, Y., Masumoto, and M., Funakoski, A. 1994. Effect of long term exercise under restricted-feeding on intestinal content of cholecystokinin and on the pancreas in aging rats. Arch Gerontol Geriatr 18: 43-51.

Ohnishi K., et al. 1985. Portal venous hemodynamics in chronic liver disease: effects of posture change and exercise. Radiology 155: 757–61.

Pawlik W., et al. 1976. Dopamine effects on the intesitinal circulation. Am Heart J 91: 325–31.

Podolin, D.A., Pagliassotti, M.J., Gleeson, T.T., and Mazzeo, R.S. 1994. Influence of endurance training on the age-related decline in hepatic glyconeogenesis. Mechanisms of Ageing and Development 75 (1): 81–93.

Phillips LS., et al. 1998. Molecular regulation of insulin-like growth factor-I and its principal binding protein, IGFBP-3. Prog Nucleic Acid Res 60: 195–265.

Poehlman ET, Rosen CJ, and Copeland KC. 1994. The influence of endurance training on insulin-like growth factor-1 in older individuals. Metab Clin Exp 43: 1401–5.

Porch JV, Jain K, Reilly A, Valdez C, Mazariegos M, Ziegler TR, Solomons N, and Smith RJ. 1997. Aging, physical activity, insulin-like growth factor-1, and body composition in Guatemalan women. Am J Clin Nutr 66: 874–9.

Peijie, C., Zicai, D., Haowen, X., and Renbao, X., 2003. Effects of chronic and acute training on glucocorticoid receptors concentrations in rats. Life Sciences 75: 1303-1311.

Peter, A. 2007. Human Physiology: From cells to systems, 603-610. 6th eds. The United States: Thomson Brooks & Cole. Higher Education.

Rappaport, A.M. 1956. Anatomic considerations, 1-49. 4th eds. The United States:Philadelphia.

Reddy Avula, C.P., and Fernandes, G. 1999. Modulation of antioxidant enzymes and lipid peroxidation in salivary gland and other tissues in mice by moderate treadmill exercise. Aging (Milano) 11 (4): 246–252.

Roelen CA, de Vries WR, Koppeschaas HP, Vervoorn C, Thijssen JH, and Blankeinstein MA. 1997. Plasma insulin like growth factor-1 and high affinity growth hormone-binding protein levels increase after two weeks of strenuous physical training. Int J Sports Med 18: 238–41.

Ross, M. H., Kaye G. I., and Pawlina W. 2003. Histology: A text and atlas, 539-540. 4th eds. The United States: Lippincott Williams & Wilkins.

Roth, S.M., Martel, G.F., Farrell, R.E., Metter, E.J., Hurley, B.F., and Rogers, M.A. 2003. Myostatin gene expression is reduced in human with heavy-resistance strength training: a brief communication. Experimental Biology and Medicines 228 (6): 706-709.

Rowell LB, Blackmon JR, and Bruce RA. 1964. Indocyanine green clearance and estimated hepatic blood flow during mild to maximal exercise in upright man. J Clin Invest 43: 1677–90.

Stromme, SB., et al. 1977. Assessment of maximal aerobic power in specially trained athletes. J Appl Physiol 42: 833.

Sullivan, Sn., Champion, MC., Christofides ND., Adrian, TE., Bloom, Sr., Binns, Smith, M., and Worobetz, L. 1984. The gastrointestinal regulatory peptide changes of long distance running. Phys sports Med 12: 77-82.

Svec, F. 1985. Glucocorticoid receptor regulation. Life Sciences 36 (25): 2539–2545.

Schwarz AJ, Brasel JA, Hintz RL, Mohan S, and Cooper DM. 1996. Acute effect of brief low- and high-intensity exercise on circulating insulin-like growth factor (IGF) I, II, and IGF binding protein-3 and its proteolysis in young heathy men. J Clin Endocrinol Metab 81: 3492–3497.

Simonelli, C., and Eaton, R.P. 1978. Reduced triglyceride secretion: a metabolic consequence of chronic exercise. American Journal of Physiology 234 (3): E221–E227.

Tortora, G.J., and Grabowski, S. R. 2000. Priciples of anatomy and physiology, 843-845. 9th eds. The United States: John Witey & Sons.

Toner, MN., et al. 1983. Cardiorespiratory responses to exercise distributed between the upper and lower body. J Appl Physiol 54: 1403.

Venditti, P., and Di Meo, S., 1996. Antioxidants, tissue damage, and endurance in trained and untrained young male rats. Archives of Biochemistry and Biophysics 331 (1): 63–68.

Vitiello MV, Wilkinson CV, Merriam GR, Moe KE, Prinz PN, Ralph DD, Colasurdo EA, and Schwartz RS. 1997. Successful 6-month endurance training does not alter

insulin-like growth factor-1 in healthy older men and women. J Gerontol 52: M149–54.

Wideman, L., Weltman, J.Y., Hartman, M.L., Veldhuis, J.D., and Weltman, A. 2002. Growth hormone release during acute and chronic aerobic and resistance exercise: recent findings. Sports medicine 32 (15): 987-1004.

Wells, T.A. 1964. The rat. New York: Dover publication: 38.

Wisloff, U., Helgerud, J., Keml, O.J., and Ellingsen, O., 2000. Intensity-controlled t readmill running in rats: Vo2max and cardiac hypertrophy. Am J Physiol Heart Circ Physiol 280: H1301-H1310.

Yano, L., Yano, H., and Taketa, K., 1998. Portal blood flow during and after high-intensity physical exercise in rats: response of plasma endothelin-1 and catecholamine. International Hepatology Communications 11: 1-11.

Yano, H., Kinoshita, S., and Yano L. 1997. Acute exercise induces mitochondria swelling of hepatocytes surrounding the terminal hepatic venule in rat liver acinus. Jpn J Phys Fitness Sports Med 46: 49-54.

Yano, L., Yano, H., and Takeda, K. 1996. Electromagnetic determination of portal venous flow in rats during exercise. Inter Hepato Commun 5: 184-190.

Yano, H., and Nagao, N. 1995. Decline in maximal oxygen uptake on work performance in rats during the developmental phase. Japanese Journal of Physiology 45: 889-893.

Yamamoto, K.R. 1985. Steroid receptor regulated transcription of specific genes and gene networks. Annual Review of Genetics 19: 209–252.

Zanconato, S., Moromisato, D.Y., Moromisato, M.Y., Woods, J., Brasel, J.A., Leroith, D., Roberts Jr., C.T., and Cooper, D.M. 1994. Effect of training and growth hormone suppression on insulin-like growth factor I mRNA in young rats. Journal of Applied Physiology 76 (5): 2204–2209.

Zanconato S, Moromisato DY, Moromisato MY, Woods J, Brasel JA, Leroith D, Roberts CT Jr, and Cooper DM. 1994. Effect of training and growth hormone supression on insulin-like growth factor-1 mRNA in young rats. J Appl Physiol 76: 2204.

Zsinka, AJn., and Frenkl, R. 1983. Exocrine function of the pancreas in regularly swimming rats. Acta Physiol Hung 62: 123-129.

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