

Original article

# Effects of intense exercise on biochemical and histological changes in rat liver and pancreas

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**Background:** Exercise has various effects on hepatic and pancreatic function. There is little information available for effects of exercise on histopathological changes in the liver and pancreas.

**Objective:** Investigate the effect of intensive exercise on blood biochemical changes and histopathology in rat liver and pancreas.

**Materials and methods:** Male Sprague-Dawley rats were randomly divided into five groups: 1 (normal control): no exercise, 2 (exercise 75%  $VO_{2max}$ ): running on treadmill at 75%  $VO_{2max}$  and sacrificed immediately after exercise, 3 (exercise 75%  $VO_{2max}$  +6 hours): running on treadmill at 75%  $VO_{2max}$  and sacrificed at six hours after exercise, 4 (exercise 90%  $VO_{2max}$ ): running on treadmill at 90%  $VO_{2max}$  and immediately sacrificed after exercise, 5 (exercise 90%  $VO_{2max}$  +6 hours): running on treadmill at 90%  $VO_{2max}$  and sacrificed at six hours after exercise. Samples of blood, liver and pancreas were collected at the end of each experiment.

**Results:** The levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) increased significantly in group 2 and 4, compared with normal control. Serum total bilirubin (TB) and enzyme lipase increased significantly in group 4 compared with normal control. In all group of exercise, liver histopathology showed hepatocyte edema and necroinflammation, and pancreas showed congestion and edema.

**Conclusion:** High-intensity exercise at 75% and 90%  $VO_{2max}$  caused an increase of biochemical parameters in liver and pancreas. The levels of exercise also caused histopathology changes in the liver and pancreas.

**Keywords:** Intense exercise, lipase, liver function, pancreas

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Exercise has various effects on hepatic and pancreatic function. In rats, endurance training reduces hepatic accumulation of total fat and cholesterol [1, 2]. Training modulates antioxidant enzymes in the liver, reducing oxidative damage [3, 4]. Physical exercise promotes the oxidative metabolism of drugs. In humans, regular exercise has been found to increase hepatic processing of antipyrine and enhances clearance from the plasma [5, 6].

Pancreatic enzyme synthesis and secretion may change with physical exercise [7]. In dogs, postprandial pancreatic secretion decreases after acute exercise [8]. In rats, endurance running training increases pancreatic protein content, pancreatic

enzyme activity, and basal amylase secretion [9].

There has been no histological study of the effects of intensive and acute exercise on changes of function and pathology of liver and pancreas. This study objective was to investigate the effects of various exercise intensities on hepatic and pancreatic function. We examined biochemical changes and histopathology in rats immediately after exercise during ischemia-reperfusion.

## Materials and methods

### 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 before use in the experiment. The animals were kept in a room with a controlled temperature

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(25±3°C) under standard conditions (12-hour dark and 12-hour light cycle). They were fed on dry rat chow ad libitum, and had free access to drinking water.

All protocols and procedures were approved by the Committee on Animal Experimentation, Faculty of Medicine, Chulalongkorn University.

### *Exercise protocols*

The treadmill exercise intensity was determined by the  $VO_{2max}$  tests [10]. All rats were divided into five groups as follows:

*Group 1* (Control group): rats were kept sedentary without performing exercise (n = 6)

*Group 2* (Exercise 75%  $VO_{2max}$ ): rats were sacrificed immediately after 75%  $VO_{2max}$  exercise (n = 5).

*Group 3* (Exercise 75%  $VO_{2max}$ +6 hours): rats were sacrificed 6 hours after exercise 75%  $VO_{2max}$  (n = 4).

*Group 4* (Exercise 90%  $VO_{2max}$ ): rats were sacrificed immediately after 90%  $VO_{2max}$  exercise (n = 5).

*Group 5* (Exercise 90%  $VO_{2max}$ +6 hours): rats were sacrificed 6 hours after exercise 90%  $VO_{2max}$  (n = 4).

In Group 2, the initial exercise intensity speed was 8.2 m/min, 0 grade for 10 minutes. Exercise intensity was increased to 19.3 m/min, 10 grade and the running time was extended until a running time of 60 minutes. In Group 4, initial exercise intensity was 8.2 m/min, 0 grade for 10 minutes. Exercise intensity was increased to 26.8 m/min, 10 grade and the running time was extended until a running time of 60 minutes. In Group 2 and 4, rats were sacrificed immediately for blood collection and for histopathology.

In Group 3 and Group 5, each rat was placed in a separate cage following the completion of exercise, and given ad libitum access to food and water for six hours before being sacrificed.

Blood was stored frozen at -80°C until analysis. Blood was analyzed for total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase, and lipase. The liver and pancreas were dissected and fixed in 10% formalin solution at room temperature. An experienced pathologist evaluated all samples blinded. All fields in each section were examined and graded for necroinflammation [11].

The hepatic injury/inflammation 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/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 [11].

The pancreas congestion/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*

The biochemical data were expressed as mean±SD using the SPSS version 13 for the windows program. Statistical comparisons between groups were analyzed by one-way analysis of variance (ANOVA) and post hoc comparisons were done with Tukey correction. Values of  $p < 0.05$  were considered significant. The analysis for necroinflammation, hepatic congestion, and pancreatic congestion/edema were shown in ranking order data.

## **Results**

### *Blood biochemical parameters*

Levels of serum TB, AST, and ALT measured in all groups are shown in **Table 1**. The serum TB level increased significantly in Group 4, compared with the normal group, but decreased significantly in Group 5, compared with Group 4. The serum AST level increased significantly in Group 2 and 4, compared with the normal group. The serum ALT level increased significantly in Group 2 and 4, compared with the control group.

**Table 2** shows levels of serum enzyme amylase and enzyme lipase measured in all groups. The serum enzyme amylase did not changed significant in any groups. The serum enzyme lipase increased significantly in Group 4 compared with normal control. The serum lipase level increased significantly in Group 4, compared with Group 2 with Group 5 decreased significantly as compared to Group 4.

### *Histopathological examination*

The histologic appearance of the liver and pancreas in the control group was normal. All exercise

group samples revealed liver congestion, necroinflammation and pancreas congestion and edema. These are summarized in **Table 3** and **4**, and **Fig. 1** and **2**.

**Table 1.** Serum total bilirubin (TB), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels in Group 1, 2, 3, 4 and 5.

Group	TB (mg/dL)	AST(U/L)	ALT(U/L)
Group 1 (Normal control)	0.08±0.02	131.16±16.44	40.50±5.57
Group 2 (Exercise 75% VO <sub>2max</sub> )	0.13±0.54	221.20±51.84*	81.60±28.64*
Group 3 (Exercise 75% VO <sub>2max</sub> +6 h)	0.13±0.31	180.25±37.65	62.50±15.26
Group 4 (Exercise 90% VO <sub>2max</sub> )	0.15±0.35*	267.40±60.12*	76.00±22.54*
Group 5 (Exercise 90% VO <sub>2max</sub> +6 h)	0.08±0.01 <sup>#</sup>	134.75±19.95 <sup>+</sup>	46.75±11.05

Values are expressed as mean±SD, \*Significant difference (p<0.05) compared with normal control. <sup>#</sup>Significant difference (p<0.05) compared with Group 4. <sup>+</sup>Significant difference (p<0.01) compared with Group 4.

**Table 2.** Levels of serum enzyme amylase and enzyme lipase measured in Group 1, 2, 3, 4, and 5.

Group	Serum amylase (U/L)	Serum lipase (U/L)
Group 1 (Normal control)	2991.16±280.31	9.83±0.75
Group 2 (Exercise 75% VO <sub>2max</sub> )	2872.80±346.19	11.60±0.89
Group 3 (Exercise 75% VO <sub>2max</sub> +6 h)	2418.00±173.37	12.75±3.40 <sup>+</sup>
Group 4 (Exercise 90% VO <sub>2max</sub> )	2397.20±698.66	28.80±16.75*
Group 5 (Exercise 90% VO <sub>2max</sub> +6 h)	2563.25±128.47	10.25±0.50 <sup>#</sup>

Values are expressed as mean±SD. \*Significant difference (p<0.05) compared with normal control. Significant difference (p<0.05) compared with Group 2. <sup>#</sup>Significant difference (p<0.05) compared with Group 4.

**Table 3.** Effects of intensive exercise on liver histopathology (scores of congestion, edema and necroinflammation).

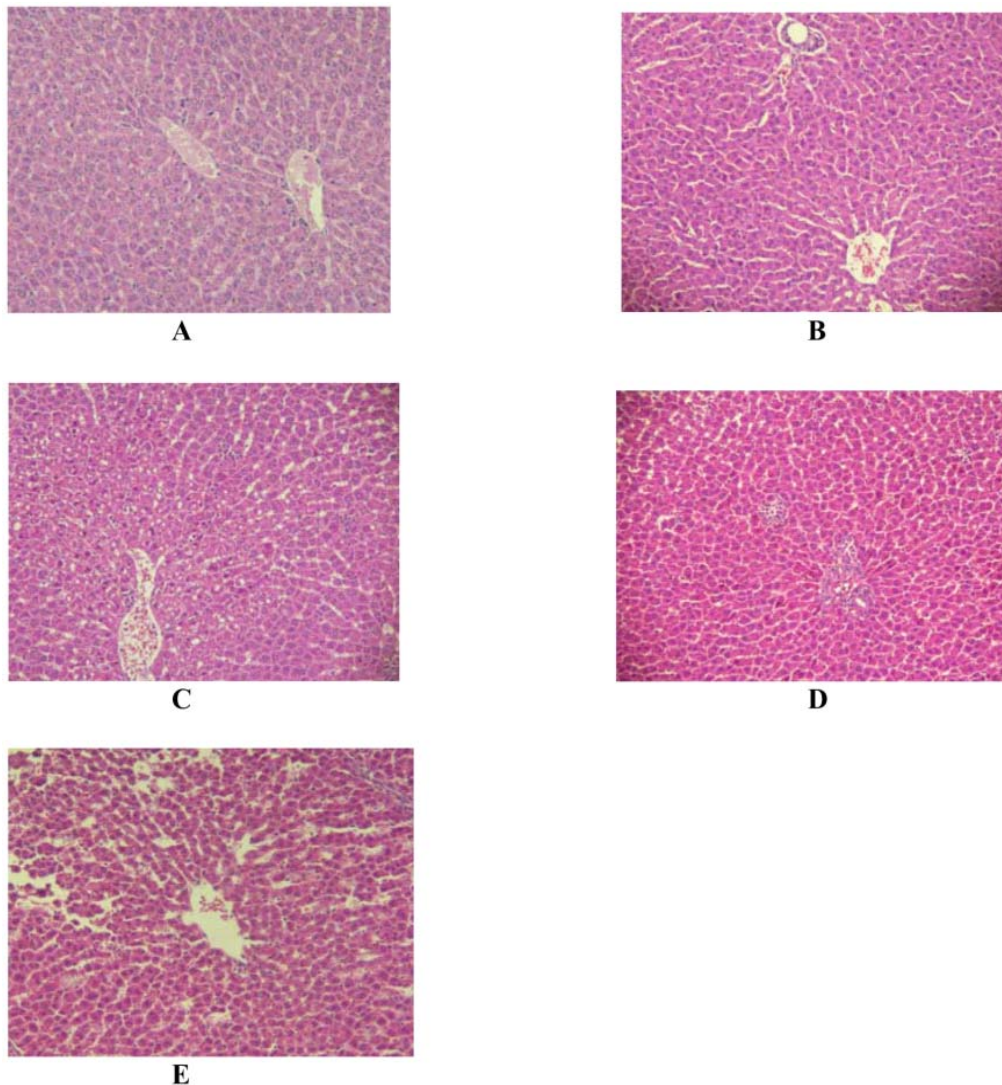
Group	Number	Level of congestion and edema				Level of necroinflammation			
		0	1	2	3	0	1	2	3
Group 1 (Normal control)	6	6	-	-	-	6	-	-	-
Group 2 (Exercise 75% VO <sub>2max</sub> )	5	-	5	-	-	-	4	1	-
Group 3 (Exercise 75% VO <sub>2max</sub> +6 h)	4	-	4	-	-	-	2	-	2
Group 4 (Exercise 90% VO <sub>2max</sub> )	5	-	5	-	-	-	3	-	1
Group 5 (Exercise 90% VO <sub>2max</sub> +6 h)	4	-	2	-	2	-	2	2	-

The severity of congestion and edema is graded as follows. 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 graded as follows. 0 = no hepatocyte injury/inflammation, 1 = sparse or mild hepatocyte injury/inflammation, 2 = noticeable hepatocyte injury/inflammation, 3 = severe hepatocyte injury/inflammation.

**Table 4.** Effects of intensive exercise on pancreatic histopathology (scores of congestion and edema).

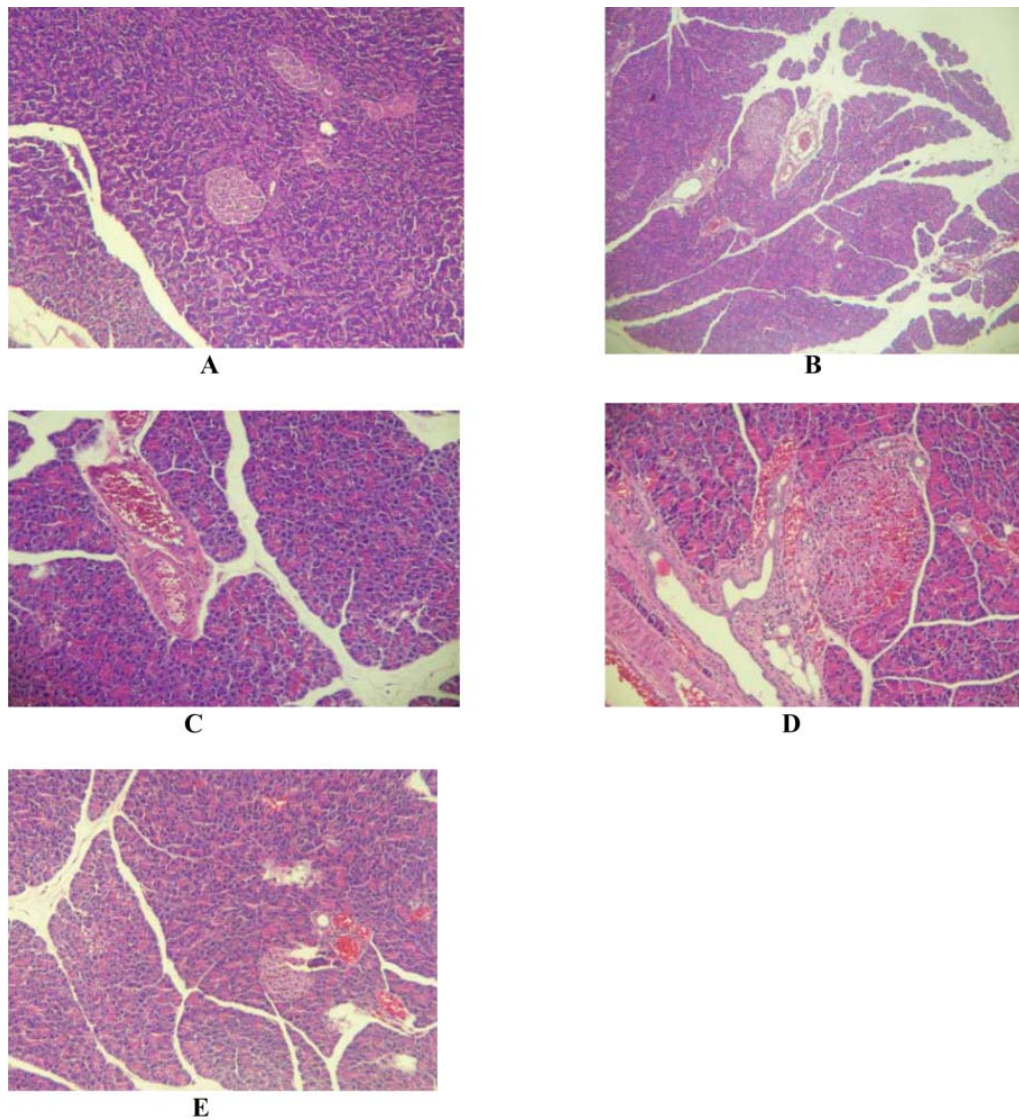
Group	Number	Level of congestion and edema			
		0	1	2	3
Group 1 (Normal control)	6	6	-	-	-
Group 2 (Exercise 75% VO <sub>2max</sub> )	5	4	1	-	-
Group 3 (Exercise 75% VO <sub>2max</sub> +6 h)	4	-	3	1	-
Group 4 (Exercise 90% VO <sub>2max</sub> )	5	1	4	-	-
Group 5 (Exercise 90% VO <sub>2max</sub> +6 h)	4	4	-	-	-

The severity of congestion and edema is graded as follows. 0 = no congestion and edema, 1 = mild congestion and edema, 2 = moderate congestion and edema, 3 = mark congestion and edema.



**Fig. 1** Liver sections in Group 1 (normal control) (A). Liver in Group 2 (exercise 75% VO<sub>2max</sub>): mild congestion, slightly increased inflammatory cells (B). Liver in Group 3 (exercise 75% VO<sub>2max</sub>+6 h): mild congestion, slightly increased inflammatory and cells showing hemorrhagic necrosis (C). Liver in Group 4 (exercise 90% VO<sub>2max</sub>): mild congestion, increased inflammatory cells and hemorrhagic necrosis (D). Liver in Group 5 (exercise 90% VO<sub>2max</sub>+6 h): marked congestion and edema and increased inflammatory cells (E). (H & E staining, 100x)





**Fig. 2** Pancreas sections from Group 1 (A). Pancreas in Group 2: mild congestion and mild edema (B). Pancreas in Group 3: mild congestion, and edema (C). Pancreas in Group 4: most severe histo-pathological changes with marked congestion and hemorrhage (D). Pancreas in Group 5: mild congestion and edema (E). (H & E staining, 100x).

### Discussion

The liver plays a role in the physiology of exercise. Exercise has various effects on liver function, enhancing both nutrient metabolism and antioxidant capacity. Physical exercise increases the blood flow in working skeletal muscles, while it decreases blood flow in the liver [12] and the portal vein [13]. During high intensity exercise, hepatic microcirculation is regulated by endothelin-1. Then, the decrease in blood to the liver often causes liver damage. In fact, swelling of the mitochondria is observed in pericentral hepatocytes after exhaustive exercise [14]. Acute physical exercise in rats decreases the hepatocyte

volume, and this volume change is not entirely linked to a decrease in hepatic glycogen level [15]. The reduction of blood flow in the liver causes hypoxia of hepatocytes, and eventually induces their necrosis. This suggests that strenuous exercise might cause hepatic necrosis or ischemic reperfusion.

According to Yano et al. [16], the hepatic blood flow significantly decreases in the high intensity exercise group compared with sedentary controls or moderate intensity exercise groups. Tissue involved in hepatic blood change may be responsible for reperfusion under high intensity exercise. Latour et al. [15] suggested an absence of post-exercise hepatic

function deterioration under their experimental conditions.

Liver cell injury after exhaustive exercise was first described by Foit et al. [17]. The present experiment showed that high intensity exercise caused changes in liver function and in the number of damaged hepatic parenchymal cells. Ischemia-reperfusion could induce inflammation [18], and could cause an excessive production of free radicals. High intensity exhaustive exercise leads to hepatic hypoxia-reperfusion and promotes an adverse effect from free radicals and lipid peroxidation [19]. Acute physical exercise in rats results in shrinkage of liver cells. Latour et al. [15] reported that liver in exercised rats displayed a 15% decrease in the hepatocellular hydration level compared with normal rest conditions. In the present experiment, the histopathologic changes of liver correlated with the intensity of exercise, and deteriorations of histopathology occurred after rest for six hours. In fact, in Group 2 (exercise 75%  $VO_{2max}$ ), the liver showed mild congestion and a slight increase of inflammatory cells. In Group 3 (exercise 75%  $VO_{2max}$ +6 h), the liver showed mild congestion, and a slight increase of inflammatory and hemorrhagic necrosis. In Group 4 (exercise 90%  $VO_{2max}$ ), the liver showed mild congestion, and an increase in inflammatory cells and hemorrhagic necrosis. In Group 5 (exercise 90%  $VO_{2max}$ +6 h), the liver showed marked congestion and edema, and an increase in inflammation cells.

Studies of effects of exercise on the pancreas are limited except pancreatic enzyme synthesis and secretion [7], postprandial pancreatic secretion [8], and so on. The exocrine pancreas controls the synthesis, storage, and secretion of pancreatic enzymes. It is regulated by gastrointestinal hormones and the vagus nerve. Pancreatic enzyme synthesis and secretion have been reported to change in response to various physical and dietary conditions including physical exercise. Konturek et al. [8] reported decreased postprandial pancreatic secretion with a decrease in splanchnic blood flow during acute exercise in dogs. In rats, increases in pancreatic enzymes activity and secretion induced by vigorous swimming [20].

The present study confirmed the hypothesis of acute high-intensity exercise on changes of function and pathology in rat pancreas. After high intensity exercise, enzyme lipase increased significantly in exercise in the 90%  $VO_{2max}$  group compared with

normal controls and enzyme lipase decreased after rest for six hours. Our result agrees with the study by Minato et al. [7] where enzyme lipase activity increased significantly with exercise. However, we did not find any significant change in the amylase in acute high intensity exercise.

Our study demonstrated that the histopathological changes of pancreas from mild to moderate edema and congestion, correlated with intensity of exercise. In the exercise 75%  $VO_{2max}$  group, the pancreas showed mild congestion and mild edema. In the exercise 90%  $VO_{2max}$  group, pancreas showed most severe histo-pathological changes such as marked congestion and hemorrhage.

In conclusion, acute high intensity exercise caused changes in function and pathology of liver and pancreas in rats. The elevation of TB, ALT, AST, and lipase correlated with intensity of exercise and resolved after rest for six hours. There was no significant change in serum amylase. We postulated that there was an increase in the permeability of hepatocytes and pancreatic cell membrane damage by prolonged reduction in blood flow.

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