Myocardial Pathological Changes in Overtraining Exercise

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Abstract. Excessive training practices without sufficient recovery period (overtraining) may be influential to promote myocardial injury. The aim of this study was to tested the hypothesis that overtraining exercise caused myocardial changes within histopathobiological analysis in rats. In this study, male wistar rats (n= 16) underwent ten weeks of overtraining weeks. Rats were divided into 2 groups: 1) controls (swam 15 minute/day, 5 days/week) and 2) overstrained rats that in 6 week swam twice a day for 1 hour. After sacrificing, the hearts excised for pathological preparation slides. There was significant difference in morphologic histopathological slides between groups. Compared to controlled groups, there was histopathological analysis showed increased chromatin activity fragmentation of myocardial structure in the overstrained group. Hypertrophic of left ventricle also higher in overstrained groups than control. Necrotic bodies spread in left ventricles myocardium of overstrained groups and there were not found in controlled group. The results of study gives an add it ion of evidence about negative effects of overtraining for myocardium. Furthermore, this study shows that avoiding overtraining should be an important rules in order to protect that myocardial injury and needed for an extended investigations.

1. Introduction
The benefits of exercise for health has been proven by numerous studies proving the main exercise has the benefit of which is able to prevent degenerative diseases, cardiovascular diseases, and also has the function of anti-aging [1,2,3]. The physiological changes that are beneficial from the exercise due to physical activity in exercise lead to a positive response from the multiple organs system and other physiological changes [4, 5]. World Health Organizations (WHO) and American Heart Association (AHA), American College of Sports Medicine (ACSM) and the United Kingdom Department of Health are established the recommended exercise to maintain good health state, carried out for 150 minutes per week in the form of aerobic exercise with moderate intensity, or in other words do during as much as 30 minutes per day 5 days a week [6,7].

Especially in sports, the beneficial and also achievement as professional are goals of training programmer for athlete. Athletes are defined as individuals whose activities associated with regular physical exercise and participate in sports competitions by focusing on excellence and achievement [8]. Overloading training is often given to the preparation of athletes facing competition. Overloading
exercise then not accompanied by an adequate recovery period that cause athlete's fall in a state of overtraining.

During the training period of overtraining, Reactive Oxygen Species (ROS) may be discharged exceed the protective capacity of anti-ROS system and cause deregulation of the inflammatory system, oxidative phosphorylation and neuroendocrine [9,10,11]. Increasing the intensity of exercise has been proven through several research can increase free radical production in the cell [2, 11, 12, 13]. Overtraining also cause muscle damage that usually occurs in people who rarely exercise are experiencing exercise overloading [14,15,16,17].

A phenomenon as the other side of the exercise, that has not been well documented is the cases of sudden cardiac death in athletes. Cases of sudden cardiac death in athletes become public attention as it is the case that, paradoxically with the public perception of the athlete's health status [18, 19]. Cases of sudden cardiac death is declared as the main cause of death due to medical conditions in case of death of athlete. Several studies have described the incidence of sudden cardiac death. Within the limitations of the study noted that the incidence of cases of sudden cardiac death in athletes in the world are not well documented due to the age group of the study sample in a very wide range (ages 8-40 years) and underestimated the actual cases because the source of the data obtained is limited to media reports, insurance claims and the report notes from the case of sudden cardiac death are had autopsied. In the United States, the incidence of sudden cardiac death has not led to proper value with accuracy. The best estimate of the incidence of sudden cardiac death in athletes in the United States varies greatly ranging from 1: 23,000 to 1: 300,000 athletes per year [19, 20, 21, 22, 23, and 24]. In other cases, there are some cases of athlete’s death without a formed of autopsy, that leads cause of death in athletes cannot be described exactly. Nowadays, refers to the trend of training, called overtraining, through some animal studies have shown changes in the cardiac myositis. Myocardial damage that occurs in overtraining assumed can deliver sudden cardiac death in exercise. Thus myocardial damage in overtraining investigate in this study by analyzing some histopathology changes of myocardium in rats with overtraining exercise.

2. Method
This research was true experimental with posttest only control group design. Samples are young adult male Wistar rats (n=16; m=180-225 gr) [25]. Rats were housed in room with constant temperature of 22±2 C with a 12/12 h light-dark cycle and fed a standard laboratory rat diet. Rats were classified into two groups, in which the control group is the group with exercise proportional and the group with the treatment of overtraining. Protocols of overtraining experiment are modified from Olah’s study protocols. Author created a new equipment pool and method for rats swam (. The control group was given treatment proportional which swam for 20 minutes per day, five times a week, for six weeks. While overtraining group swam for one and half hour, twice a day, every day, for six weeks [26,27,28]. Last day of treatment, the rats in both groups, were sacrificed and the heart to be examined. Two experts helped for examination of thus slides of myocardium. Experts were worked independently and without discussion between each other. Research parameters measured were hystopathological changes probably appeared. Assessment is based on the average observed in 5 visual fields using 100x magnification. [28, 29]. Histopathology data in form ordinal data were analyzed descriptively.

3. Results
After six weeks in vivo hemodynamic studies, rats of both groups sacrificed. The left ventricle was cut into 3 transverse sections: apex, middle ring, and base. From the middle ring, 5-μm sections were cut and stained with Hematoxicilin Eosin. Left ventricle hypertrophy appeared on slides of both groups. Compare with overtraining group, there was different thickness of the left ventricles wall, whereas larger hypertrophy appeared in overstrained rats. However, in overstrained group, observed that left
ventricular wall thickening greater than in the control group. On the measurement of the thickness of the control group were categorized in moderate LVH while overstrained group in which caused thickening of the left ventricular wall in the severe one.

From experimental rats in overstrained group, then 14 slides, thus all left heart ventricle describe necrosis. The nucleus changes are loss of chromatin and nucleus becomes wrinkled. Nucleus seems more solid, color turned to more darken (psychosis), divided into fragments, and shredded (caryorexis) [30, 31]. Necrosis area presented with following figure 2.

Increased activity of chromatin is obtained from 6 slides were observed from the overstrained group. Experts also conclude that there are increase of chromatin activity area in slides of overstrained rats. Increase of chromatin activity signed that there are some activities of nucleus. Increase of chromatin activity presented with following figure 3.

3.1. Left Ventricle Hypertrophy (LVH)

3.1.1. Differences of LVH measurement between two groups

- Left ventricle myocardium after proportional exercise, differ from standard myocardium of rats.
- Myocardium thickness length: 1488, 19μm. This is a physiological changes called in few studies with athlete’s heart.

![Figure 1.a. Left Ventricle of control group](image1)

- Myocardium looked hypertrophic formed with normal shape of cardiomyocite but its size is larger than controls.
- Myocardium thickness length: 1836, 39 μm. This probably pathological.

![Figure 1.b. Left ventricle of overstrained group](image2)
3.1.2. **Histopathological changes of Myocardium**

- Area without normal cardiomyocyte
- Appearance of nuclei didn’t clearly shown
- Psychosis and caryorecsis shown

![Figure 2. Necrosis Cardiomyocyte of Overstrained Group](image)

**Figure 2.** Necrosis Cardiomyocyte of Overstrained Group

![Figure 3. Increased chromatin activity of Overstrained Group](image)

**Figure 3.** Increased chromatin activity of Overstrained Group

Nucleus with the amount chromatin fragment increase. Chromatin in circle surrounding the wall of nucleus.

Refers to all the results of slides examination by experts, author conclude that overstrained rats have pathological sign of myocardial damage.

4. **Discussion**

In exercise activity, systolic ejection and VO2 max become ones of hearts persist myocardial function responsibilities filling which are important in cardiorespiratory performance [32,33]. However, chronic training of exercise influence antioxidant system, which resulted in an imbalance between the formation of free radicals and antioxidant response. This situation causes oxidative stress [9, 11]. Free radicals easily defect membrane lipids, proteins and nucleic acids, organelles of cell and causing cellular damage and disturb normal function and reproduction of cells [9, 11, 12, 13, 33, and 35]. Free radicals such as anion oxygen superoxide, would reacts with lipid membranes, proteins and nucleic acids, causing cumulative and irreversible cell damage of tissues and organs [33, 34, and 35].
Overstrained induced damage in several organs by some experiments in rats, which is overstrained delivered changes for kidney and skeletal muscles [36,37,38]. Previous study established that myocardium of overstrained rats gave an apoptotic cardiomyocytes [39, 40, 41].

From research by Benito, through research using rats that two groups, one sedentary group and the other group is intensive training. Intensive exercise group was given treatment 60 minute treadmill run at a speed of 60 cm / sec for 5 days a week. After 8 weeks of intensive training found a histopathological changes of concentric left ventricular hypertrophy, and at the end of the 16th week of the eccentric left ventricular hypertrophy and diastolic [42,43]. Thus in line with this research that in both groups, appearance of left ventricle hypertrophy as a compensated structural of myocardium. Although in physiological condition of exercise that LVH known as normal compensation, but excessive wall thickness are worst to the myocardial contraction. In overstrained group that was pathological hypertrophy of left ventricle. Function of myocardium could be decrease in contractions because of that thickness and deliver to become rigid structure. In systolic phase and also diastolic phase of heart contraction, there will results some impairment of contractions and all decrease of function follow.

This findings also in line with other study conducted by Flora, which was investigate the effects of anaerobic and aerobic exercise in myocardium of rats. From that study we collected data that histology of cardiac muscles on day 1 did not show any signs of hypertrophy, ischemia, or infarction. On day 3, there were signs of cardiac muscle cell hypertrophy. Further histological examination revealed signs of cardiac muscle cell ischemia on day 7 and of infarction on day 10 in both groups [43].

This findings not corroborate with the conclusion of that study. The examination of blood, had not a significant measurement of oxidative stress in overstrained rats. We differ our research by using method of protocol analyzing and we examined the tissue organ. We limited our research only in histopathological changes only without investigate the pat mechanism of all changes that founded.

In this study result, we also describe a novel pathological sign of overstrained rats. There were necrosis and increased activity of chromatin. Necrosis of cardiomyocytes assumed that death areas of myocardium. Necrosis is an ischemic stage without compensated. Necrosis cardiomyocyte probably found in end stage of Ischemic Cardiac Injury conditions. Massive necrosis of cardiomyocytes which is manifested as worsening clinical condition rapidly and suddenly could cause death. Increased of chromatin activity couldn’t explained certainly. There are several process could describe with that chromatin condensed. Future researches are needed to elaborate the bio molecular and histopathological aspects in order to elucidate path mechanism of overtraining exercise.

5. Conclusion
Exercise’s benefits in preventing cardiovascular morbidity and mortality is well established. However, there is a hypothesis assumed circumstantial evidence has suggested that excessive exercise in overtraining exercise might have harmful effects on cardiac health, sometimes leading to rare but remarkable sudden cardiac events. Overtraining results some pathological changes of excessive left ventricle hypertrophy, necrosis and increased chromatin activity in myocardium.

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