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The Effect of Physical Exercise to Level of Nuclear Factor Kappa B on Serum, Macrophages and Myocytes

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Abstract**—**Background: Physical exercise induces a pattern of hormonal and immunological responses that prevent endothelial dysfunction by maintaining the availability of nitric oxide (NO). Regular and moderate exercise stimulates NO release, that can be considered as protective factor of cardiovascular diseases, while strenuous exercise induces increased levels in a number of pro-inflammatory and anti-inflammatory cytokines. Pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) triggers endothelial activation which results in an increased vascular permeability. Nuclear gene factor kappa B (NF-κB) activates biological effect of TNF-α. Aim of Study: To determine the effect of physical exercise on the endothelial and skeletal muscle, we measured the level of NF-κB on rats' serum, macrophages, and myocytes after strenuous physical exercise. Methods: 30 male *Rattus norvegicus* in the age of eight weeks were randomly divided into five groups (each containing six), and there were treated groups (T) and control group (C). The treated groups obtain strenuous physical exercise by ran on treadmill at 32 m/minutes for 1 hour or until exhaustion. Blood samples, myocytes of gastrocnemius muscle, and intraperitoneal macrophages were collected sequentially. There were investigated immediately, 2 hours, 6 hours, and 24 hours (T1, T2, T3, and T4) after sacrifice. The levels of NF-κB were measured by ELISA methods. Results: From our study, we found that the levels of NF-κB on myocytes in treated group from which its specimen was taken immediately (T1), 2 hours after treadmill (T2), and 6 hours after treadmill (T3) were significantly higher than control group ($p<0.05$), while the group from which its specimen was taken 24 hours after treadmill, was no significantly different ($p>0.05$). Also on macrophages, NF-κB in treated groups T1, T2, and T3 was significantly higher than control group ($p<0.05$), but there was no difference between T4 and control group ($p>0.05$). The level of serum NF-κB was not significantly different between treatment group as well as compared to control group ($p>0.05$). Serum NF-κB was significantly higher than the level on macrophages and myocytes ($p<0.05$). Conclusion: This study demonstrated that strenuous physical exercise stimulates the activation of NF-κB that plays a role in vascular inflammation and muscular damage, and may be recovered after resting period.

Keywords**—**Endothelial function, inflammation, NF-κB, physical exercise.

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I. Introduction

PHYSICAL activity is defined as any bodily movement produced by skeletal muscles, that requires energy consumption. Regular and adequate levels of physical activity in adults can reduce the risk of coronary heart disease, hypertension, stroke, diabetes, breast and colon cancer, and depression, can improve bone and functional health, and can be a key to energy balance and weight control [1], [2]. During the last century, the population of developed and developing countries has become less physically active due to rapid economic, social development, urbanization, and industrialization. This alteration has led to remarkable increases in the incidence of chronic diseases such as cardiovascular diseases and type 2 diabetes, obesity, and musculoskeletal disorders [3], [4].

Physical activity that is planned, structured, repetitive, with the purpose of improvement or maintenance of one or more components of physical fitness is referred as physical exercise [1]. Physical exercise can be regarded as a prototype of physical stress that induces a pattern of hormonal and can have both positive and negative effects on immune function [5]. Regular moderate intensity of physical exercise may enhance immune function to protect an invasion of intracellular microorganisms, through activation of Th1 cells. While high-intensity exercise may impair immune function due to increase the concentrations of anti-inflammatory cytokines, that causes an increasing the risk of infectious diseases and damage a muscular tissue resulting from inflammation [6]-[8]. Pro-inflammatory cytokines such as IL-6 and IL-8, which are produced as a result of tissue damage, inducing signaling pathways that activate NADPH-oxidase, cause the release of reactive oxygen species [9], [10].

Immunity is divided into innate and adaptive immunity. Innate immunity includes first line defense mechanism such as skin and second line defense mechanism such as macrophages and NO. Adaptive immunity involves T lymphocytes B, cytokines, and antibodies. Many stimuli including exercise can trigger immune response [11]-[13]. The differentiation of T lymphocytes into activated T lymphocytes is stimulated from IL-12 produced by macrophages and dendritic cells, while IL-4 stimulates activated B cells [14]. Physical exercise induces steroids release, production of neuroendocrine mediators, cytokines, and oxi-reduction, that are associated to the production of free radicals [15].

Physical exercise which has been demonstrated to improve endothelial function reduces the severity of inflammation and

the risk of cardiovascular events. But, strenuous physical activity invokes a variety of different stress responses in the body such as inflammatory response and induce micro lesion in target organs. Once the inflammatory process has been initiated within the skeletal muscle, fluid, plasma proteins, and inflammatory cell populations such as neutrophils and monocytes or macrophages infiltrate the affected skeletal muscle tissue [16], [17]. Muscle contractions in physical exercise stimulate sarcoplasmic calcium release, increase ROS, and mitogen-activated protein kinase (MAPK) activation to activate nuclear factor kappa B (NF-κB) [18], [19]. NF-κB, also known as stress sensor because of its activity, was induced by various stimuli including TNF-α that induced inflammatory responses [20].

II. Material and Methods

This study was carried out on 30 male *Rattus norvegicus* Strain Wistar at the age of eight weeks. They were acclimatized for one week in a room temperature, then randomly divided into five groups. The first group was control group (C), while the other four groups (T) were treated with strenuous physical exercise by running on the treadmill at 32 m/min for 1 hour or until exhaustion. The treated groups were killed by cervical dislocation sequentially, that is performed either immediately (T1) or in 2 hours (T2), 6 hours (T3) and 24 hours (T4) after treadmill. Blood was collected intracardially, myocytes were obtained from gastrocnemius muscle, and macrophages were obtained from intraperitoneal fluid. Serum NF-κB was measured from blood, while NF-κB on macrophage was measured from the isolation of macrophages, and on muscle was measured from isolation of myocytes. Serum NF-κB was measured by ELISA methods. This study was approved by Research Ethics Committee of Faculty of Medicine Andalas University.

A. Animals and Exercise Protocol

This experimental study was designed to analyze the post-treatment group. All experimental procedures were performed according to the guidelines of the Helsinki declaration and approved by Research Ethics Committee of Faculty of Medicine Andalas University. 30 male *Rattus norvegicus* Wistar rats, weighing 150-200 g at eight weeks of age were obtained from animal breeding of Medical Faculty, Gajah Mada University. They were acclimatized for one week in room temperature and were fed with rat chow and water ad libitum throughout the study period. They were randomly divided into three groups (six rats per group); one group was control, while the other four groups (T) were treated groups. Initially, all animals were familiarized by walking on a modified treadmill for 10 min of five consecutive days. 48 hours after the last adaptation session, rats were submitted to a strenuous physical exercise on the treadmill at 32 m/min, for 1 hour or until exhaustion [21]. The treated groups were killed by cervical dislocation in sequentially resting time, there were immediately (T1), 2 hours (T2), 6 hours (T3) and 24 hours (T4) after treadmill.

B. Specimen Collection

All trained animals were anesthetized with ketamine and before being killed, and blood samples were collected. Macrophages were collected by injecting RPMI intraperitoneal, then peritoneal fluid was aspirated, and after centrifugation, macrophages were washed and prepared for measurement of NF-κB. Gastocnemius muscles were quickly removed immediately after being killed, and rinsed in ice-cold physiological saline solution. The myocytes were incubated with collagenase for 24 hours. After centrifugation, myocytes were washed and prepared for measurement of NF-κB. Blood samples were collected into heparinized tubes and centrifuged immediately to separate plasma. Plasma, macrophages, and muscle samples were stored at -80 °C until the time for assay. NF-κB (Abcam NF-κB p65) was measured by double antibody sandwich ELISA. All measurements were performed in duplicate.

C. Statistical Analysis

All data were expressed as the means ± standard deviation (means±SD). The statistical significance among experimental groups was evaluated by two-way analysis of variance. Student's t test was used to examine differences between control and training groups. The level of significance was set at $p < 0.05$.

III. Results

In this study, we subjected *Rattus Norvegicus* rats to voluntary running and performed comprehensive NF-κB analysis to determine the effects of strenuous exercise on endothelial and skeletal muscle. We confirmed the homeostatic and remodeling of vascular and skeletal muscle tissue of rats following strenuous exercise with sequentially resting time. The level of NF-κB level on control and treated groups is shown in Table I.

TABLE I
MEANS VALUE OF NF-KB LEVEL (HG/MG CELLS) ON MYOCYTES, MACROPHAGES, AND SERUM OF CONTROL GROUP (C), AND TREATED GROUP WITH DIFFERENT TIME OF THE RAT SPECIMEN COLLECTION (T1, T2, T3, AND T4) AFTER TREADMILL

Specimen	Control group	Treated Group			
		T1 (0 hour)	T2 (2 hours)	T3 (6 hours)	T4 (24hours)
Myocytes	0.57±0.002	0.68±0.002	0.98±0.005	0.78±0.002	0.58±0.001
Macrophages	0.53±0.000	0.64±0.003	0.93±0.001	0.73±0.001	0.53±0.001
Blood serum	1.23±0.002	1.31±0.002	1.58±0.002	1.44±0.003	1.23±0.001

$p<0.05$: significantly different

Table I showed that the means of NF-κB level on myocytes of control groups (C) (0.57±0.002), were significantly different from the treated groups which specimen collected immediately (T1) (0.68±0.002), 2 hours (T2) (0.98±0.005), and 6 hours after treadmill (T3) (0.78±0.002), ($p<0.05$). There was no significant difference between the control group and the treated group of the specimen collected 24 hours after treadmill (T4) (0.58±0.001). NF-κB level on macrophages of control group (C) (0.53±0.000) was significantly different from the treated groups (T1) (0.64±0.003), (T2) (0.93±0.001) and (T3) (0.73±0.001), ($p<0.05$), but was not significantly different from T4 (0.53±0.001). NF-κB level on blood serum of control groups (C) (1.23±0.002) was significantly different from T1 (1.31±0.002), T2 (1.58±0.002), and T3 (1.44±0.003) ($p<0.05$), but was not significantly different from T4 (1.23± 0.001).

Differences between the five groups of different levels of NF-κB were compared by analysis of variance (ANOVA). The results were $p=0.0005$ ($p<5$), and the means were significantly different between the control group and the treated groups. Then, statistical analysis is followed by Post Hoc Benferoni to analyze the differentiation of NF-κB level between groups. The Post Hoc Benferoni test showed that NF-κB level on myocytes between groups was significantly different ($p<0.05$). NF-κB on macrophages and on blood serum was significantly different between the groups except with T4.

IV. Discussion

Physical exercise has an implication to the health and benefits in preventing and reducing the risk of some diseases, because physical exercise is able to recover and improve endothelial function. Low physical activity and decrease of oxidative fibers intake play a role in chronic disorders such as coronary heart disease, obesity, and diabetes mellitus type 2 [18], [22]. Our study was to determine the effect of strenuous exercise.

In this study, we found that the highest level of NF-κB on myocytes was in the group of the specimen collected 2 hours after treadmill. These results provide that strenuous exercise may lead to activation of NF-kB. NF-κB appears to be lower in the groups with longer resting period, indicating transient activation of NF-kB. Demand of ATP that is produced by mitochondrial electron transport chain (ETC) through oxidative phosphorylation is increased during muscle contraction. Increase in exercises intensity increases oxygen flux through the ETC, while some other ROS-generating pathways may be activated during heavy exercise [23], [24]. Rigorous muscle contraction stimulates immune response that induced cytokines, chemokines, and ROS. The most relevant redox signaling pathways that have a significant impact on exercise physiology are NF-κB, MAPKs, and peroxisome proliferator-activated receptor (PPAR)-γ coactivator 1α (PGC-1α) [25], [26]. The study in acute heavy of exercise revealed that NF-κB pathway was activated by increased phosphorylation of IKK, increased phosphorylated IκB-to-IκB ratio, and increased of nuclear p65 concentration, while the study in session of exercise showed that IKK activation and IκB phosphorylation occur immediately after an acute bout of exercise, and the peak of NF-κB-DNA binding happens 2 hours after exercise [27].

This study showed that the highest levels of NF-κB on macrophages were seen in the group of the specimens collected 2 hours after treadmill. It could be because muscular contractions during exercise activate NF-κB in response to mechanical stress which still continues within 2 hours after strenuous exercise. When physical exercises are performed regularly, muscular contraction produced IL-6, namely myocine or myokines, that will act as signal transmission to stimulate anti-inflammatory activity, while during prolonged exercise, higher level of IL-6 was released into the circulation [10], [28]. However, strenuous exercise can cause muscular injuries leading to acute inflammation. Inflammation in response to physical, physiological, and/or oxidative stress is associated with activation of the canonical NF-κB signaling pathway, which is conserved in all multicellular animals. NF-κB represents a central factor in inflammation, stress response, cell differentiation, or proliferation as well as cell death [29], [30]. In our study, 24 hours after treadmill, NF-κB level was equal to control group. It was because, after 24 hours, the muscles did not contract and affect the decrease of production of pro-inflammatory cytokines such as TNF-α and IL-6; otherwise, anti-inflammatory cytokines will be increased, which leads to reduce stimulation of NF-κB production. Keller et al. [31] reported that the high level of TNF-α returned to normal after 1 hour of acute swimming exercise in mice.

Similar to the results NF-κB levels on myocytes and macrophages, NF-κB levels in blood serum were also highest in the group of the specimens collected 2 hours after treadmill. The physical exercise-induced shear stress provides potent physiological stimulus for adaptation in endothelial function and vascular remodeling. Physical exercise training improves NO-mediated endothelial function and may be related to direct hemodynamic effects. An increase in NO production enhances blood flow due to NO-induced vasodilation and indirectly enhances muscle antioxidant defense during exercise [32].

Increasing of iNOS, ROS and inflammatory cytokines has been shown in heavy exercise. NO can react with superoxide to form peroxynitrite, one of the most reactive ROS, and can cause damage of myocytes and endothelial cells. Although activation of NF-κB and MAPK is important in response to oxidative stress to induce antioxidant adaptation, prolonged activity of these signaling pathways also promotes pro-inflammatory cytokines and chemokines, leading to potential ROS generation, inhibition of PGC-1α function, and degradation processes [33], [34]. The equal level of NF-κB on blood serum 24 hours after treadmill can be assumed that if the activities of physical exercise does not happen in a long time, the blood flow returns to normal as the process of homeostasis. Bernecker et al. [35] determined that regular physical activity of moderate intensity improves cardiovascular risk factors including low-grade inflammation. However, acute vigorous exercise increases the circulating

pro-inflammatory markers such as Il-6 and TNF-α, that stimulate NF-κB.

V. Conclusion

Regular physical activity of moderate intensity improves endothelial function including low-grade inflammation. However, strenuous physical exercise can induce muscle damage and it may be associated with a local inflammation involving leukocyte accumulation in damaged muscle tissue. Inflammation and muscular contraction can activate NF-κB. NF-κB may induce an important pro-inflammatory response for muscle regeneration after exercise. Resting periods are required for recovery from the effects of strenuous physical exercise in relation to the inflammatory processes and homeostasis

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