Aerobic Training Prevents Atherosclerosis Through Increased Production and Bioavailability of Nitric Oxide

Tyas Putri Utami, Ermita Isfandiary Ibrahim Ilyas, Minarma Siagian, Ani Retno Prijanti

1Magister Program in Biomedical Science, Faculty of Medicine, Universitas Indonesia, Jakarta
2Department of Physiology, Faculty of Medicine, Universitas Indonesia, Jakarta
3Department of Biochemistry and Molecular Biology Faculty of Medicine, Universitas Indonesia, Jakarta

Abstract

Introduction: Atherosclerosis are related to endothelial dysfunction characterized by decreased nitric oxide (NO) production and bioavailability. Increased shear stress during aerobic exercise can increase endothelial nitric oxide synthase (eNOS) and antioxidant activity in the vascular wall. The aim of this study was to evaluate the effect of aerobic training on the production and bioavailability of nitric oxide in abdominal aorta tissues.

Methods: Ten 9 week old male Wistar rats (150-200 grams) were randomly allocated into 2 groups: training group and control group. Five times a week for 8 weeks, 20 minutes aerobic exercise with 90 seconds rest between 5 minutes run was conducted on a treadmill at a speed of 20 m/min. NO production, superoxide dismutase (SOD) specific activity, and malondialdehyde (MDA) in abdominal aorta tissues were measured using colorimetric (NO assay kit by BioVision™) and spectrophotometry (RANSOD kit by Randox Lab™) method.

Results: Nitric oxide production of training group (0.0099±0.0074 mmol/g protein) was significantly (p=0.0045) higher than control group (0.0036±0.001 mmol/g protein). SOD specific activity of training group (0.2721±0.1183 U/mg protein) also significantly (p=0.0195) higher than control group (0.1361±0.0335 U/mg protein), whereas malondialdehyde of training group (0.0073±0.0018 nmol/mg tissue) slightly less than control (0.0079±0.0012 nmol/mg tissue).

Conclusion: Aerobic training increased nitric oxide production and bioavailability by enhancing SOD specific activity and decreasing malondialdehyde in abdominal aorta tissues.

Key words: aerobic training, atherosclerosis, endothelial dysfunction, nitric oxide

Korespondensi: Tyas Putri Utami
Email: tyaspu@yahoo.com


**Aerobic Training Prevents Atherosclerosis**

Tyas Putri Utami,1 Ermita Isfandiary Ibrahim Illyas,2 Minartha Siagian,2 Ani Retno Prijanti3

1Program Magister Ilmu Biomedik, Fakultas Kedokteran, Universitas Indonesia, Jakarta
2Departemen Fisiologi, Fakultas Kedokteran, Universitas Indonesia, Jakarta
3Departemen Biokimia dan Biologi Molekuler, Fakultas Kedokteran, Universitas Indonesia, Jakarta

**Abstract**

**Pendahuluan:** Aterosklerosis berkaitan dengan disfungsi endotel yang ditandai oleh penurunan produksi dan bioavailabilitas oksida nitrit (ON). Peningkatan tegangan-geser selama latihan fisik aerobik dapat meningkatkan aktivitas sintase oksida nitrat endotel dan antioksidan di dinding vascula. Penelitian ini bertujuan untuk mengetahui manfaat latihan fisik aerobik terhadap produksi dan bioavailibiltas ON pada jaringan aorta abdominal.

**Metode:** Sepuluh tikus jantan Wistar berusia 9 minggu (150-200 gram) dibagi secara acak menjadi dua kelompok: latihan dan kontrol. Latihan aerobik menggunakan treadmill berdurasi 20 menit dengan interval istirahat 90 detik setiap 5 menit berlari 20 m/menit dilakukan sebanyak 5x seminggu selama 8 minggu. Kadar ON, malondialdehyde (MDA), dan aktivitas spefisik superoksida dismutase (SOD) jaringan aorta abdominal diukur menggunakan metode kolorimetri (NO assay kit dari BioVisionTM) dan spektrofotometri (RANSOD kit dari Randox LabTM).

**Hasil:** Produksi ON kelompok latihan (0,0099±0,0074 mmol/g protein) lebih tinggi secara bermakna (p=0,0045) dibandingkan dengan kelompok kontrol (0,0036±0,001 mmol/g protein). Aktivitas spesifik SOD kelompok latihan (0,2721±0,1183 U/mg protein) juga lebih tinggi secara bermakna (p=0,0195) dibandingkan dengan kelompok kontrol (0,1361±0,0335 U/mg protein), sedangkan kadar MDA kelompok latihan (0,0073±0,0018 nmol/mg jaringan) sedikit lebih rendah dibandingkan dengan kelompok kontrol (0,0079±0,0012 nmol/mg jaringan).

**Kesimpulan:** Latihan fisik aerobik meningkatkan produksi dan bioavailibiltas ON melalui peningkatan aktivitas spesifik SOD dan penurunan MDA pada jaringan aorta abdominal.

Kata kunci: latihan aerobik, aterosklerosis, disfungsi endotel, oksida nitrat

Introduction

WHO predicted that mortality caused by cardiovascular diseases will increase from 17% in 2008 to almost 24% in 2030.1 Cardiovascular diseases such as hypertension and atherosclerosis are related to endothelial dysfunction characterized by decreased nitric oxide production, increased endothelin-1, and decreased nitric oxide bioavailability.2,3 Endothelial dysfunction is an early marker for atherosclerosis development before vascular wall structural changes occurred.4

Nitric oxide has many important functions in homeostasis of vascular regulation. Nitric oxide also has atheroprotective effect in blood vessels through several pathways.5 Decreased nitric oxide bioavailability can occur due to high level of reactive oxygen species (ROS) that cause peroxynitrite formation.5

Abdominal aorta has higher inflammatory gene expression and immune-cell infiltration compared to thoracic aorta.6 Cardiovascular risk factors can activate several pro-oxidative genes in the vascular wall enhancing radical oxygen species production, as well as adhesion molecules and pro-inflammatory cytokines transcription.7

Physical inactivity is one of the risk factors for cardiovascular diseases development.8 Therefore, regular aerobic exercise can help prevent cardiovascular diseases.9 Increased shear stress during aerobic exercise can increase endothelial nitric oxide synthase (eNOS) activity in vascular wall.8 Furthermore, aerobic exercise has also been known to increase endogen antioxidant in vascular tissue.10

The aim of this study was to evaluate the effect of aerobic exercise in preventing atherosclerosis by measuring nitric oxide (NO) production, malondialdehyde (MDA), and superoxide dismutase (SOD) specific activity in abdominal aorta tissue.

**Methods**

**Animals**

Protocol of this study has been approved by the Ethical Committee of the Faculty of Medicine, Universitas Indonesia.
Aerobic Training Prevents Atherosclerosis

Nine week-old male Wistar rats weighing 150-200 gram were randomly divided into two groups: 1) training group and 2) control group. Prior to and during intervention, the rats were maintained properly according to the ethics of experimental animal usage. They were housed in an environmentally controlled room (temperature 23±1°C; lights on at 0600 h and off at 1800 h) in groups of five per cage with rat food and water ad libitum.

Aerobic Exercise Protocol

At the beginning, the rats were adapted to the laboratory environment without any treatment in one week period of acclimatization. The rats were then adapted to animal treadmill for another one week. In this period, training group ran at a low speed of 9 m/min for 10 minutes each day to get familiar with the running treadmill and control group were placed on animal treadmill without running for 10 minutes.

Aerobic exercise program started at 11 week-old. In this period, training group ran on animal treadmill with speed 20 m/min. Exercise was conducted 5 days a week (Monday-Friday), 20 minutes duration with 90 seconds rest between 5 minutes running to avoid fatigue. Control group was placed on animal treadmill without running for 20 minute.

In order to avoid the acute effects of exercise, euthanasia was done by cervical dislocation without anesthesia on day-3 after treatment. Euthanasia was performed by trained experienced personnel. Abdominal aorta were harvested by dissection, weighed, and stored at -80°C until used. Homogenate (10% w/v) of abdominal aorta was prepared in medium containing 10 mM Tris-HCL, 0.1 mM EDTA-2Na, 10 mM sucrose, 0.8% NaCl and pH 7.4. A supernatant was obtained from the tissue homogenate after 10 minutes centrifugation at 5000 rpm 4°C. The supernatant was used to measure NO production, MDA, and SOD specific activity.

Nitric Oxide Measurement

NO level in abdominal aorta tissue was measured with colorimetric method using NO colorimetric assay kit (BioVision™, USA). The amount of NO in abdominal aorta reflected by the amount of azochromophore that formed by nitrite conversion using Griess Reagent. Nitrate also measured by coverted it to nitrite using nitrate reductase. Therefore, this measurement measured total NO in abdominal aorta tissue.

Malondialdehyde Measurement

The level of lipid peroxide was measured through ROS product of lipid peroxidation. The level of MDA was measured by spectrophotometer using absorbance 530 nm. In this measurement, trichloroacetic acid was used for protein precipitation and thiobarbituric acid was used as chromogen to detect lipid peroxide.

SOD Activity Measurement

SOD activity in abdominal aorta tissue was measured with spectrophotometry method using RANSOD kit by Randox Lab™, UK. SOD activity reflected by the amount of red formazan dye that showed the degree of inhibition of reaction between superoxide radical, formed by xanthine and xanthine oxidase, with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride.

Statistical Analysis

All values were given as means±SD. The data were analyzed using the SPSS version 16.0. All comparisons were performed using t-test independent and Mann-Whitney test for parametric and nonparametric data and 1-sided p values of < 0.025 were considered statistically significant.

Results

Nitric Oxide

Nitric oxide production was presented in Figure 1. The level of NO in the training group (0.0099±0.0074 mmol/g protein) was higher than in the control group (0.0036±0.001 mmol/g protein).

Figure 1 Mann-Whitney test showed that NO level of training group was statistically significant higher compared to control group (p=0.0045).

Malondialdehyde

The level of MDA was presented in Figure 2. The level of MDA in the training group (0.0073±0.0018 nmol/mg tissue) was higher than in the control group (0.0079±0.0012 nmol/mg tissue).

Figure 2 showed that MDA level of the training group tended to increase compared to the control, but the difference was not statistically significant (p=0.271).

SOD Specific Activity

The level of nitric oxide SOD specific activity was presented in Figure 3. The level of SOD specific activity in the training group (0.2721±0.1183 U/mg protein) was higher than in the control group (0.1361±0.0335 U/mg protein).
Aerobic Training Prevents Atherosclerosis

Discussions

Wistar rats are active and can be expected to follow the exercise protocol. At the start of training program, the rats were 11 weeks old, approximately equivalent to 20 years in human.12 Aerobic training for 8 weeks in rats can be categorized as long-term aerobic training that can give chronic adaptation effects.13,14 A study by Sari et al15 showed that treadmill exercise for 20 minutes with 90-seconds rest between 5 minutes run 20 m/min is the threshold for aerobic exercise based on the rise in blood lactate concentration.

The result of this study showed that long-term aerobic exercise can increase basal NO production. The increase in NO production can also be caused by increased eNOS mRNA expression in aortic tissues post-long term exercise. Tanabe et al16 study showed increased eNOS mRNA and proteins in aortic tissue of young adult rats after 8 weeks swimming program compared to control. However, our study did not evaluate eNOS expression.

In endothelial dysfunction, LDL can easily cross endothelial barrier in the vascular tissues. LDL will be oxidized by high levels of ROS in vascular tissues, that will also react with NO producing peroxynitrite.17 Peroxynitrite is a strong oxidizing species. Accumulation of oxidized-LDL in subendothelial space will induce secretion of inflammatory factors which will in turn cause macrophages to enter subendothelial space and reduce the oxidized-LDL and become foam cells. Lipid and foam cells deposition inside vascular tissue will induce cell proliferation in the arterial wall and form a plaque, which will affect the diameter of blood vessel and obstructs blood flow.5

Aerobic exercise has been known to increase laminar shear stress on vascular walls.18 In addition to activation and increase in eNOS expression, laminar shear stress can also increase the production of superoxide and its derivates.19 However, exercise training can also decreases ROS by increasing antioxidant expression and activity.10 The result of this study showed that MDA level in the training group was lower than in control group. This can occurred because of the significant increase of SOD specific activity in the training group compared to control, as a compensation to the increase of ROS during acute exercise. Nitric oxide has also been known to induces SOD expression via the eNOS/cGMP/PKG pathway and the p38MAP kinase-dependent pathway.20,21

The increased of SOD specific activity not only decreased MDA level, but also peroxynitrite formation. It will followed by depletion of oxidized-LDL level inside the abdominal aorta tissue. Therefore, aerobic training that increased SOD activity could prevent and decrease foam cell and plaque formation. In addition, increased production of NO can regulate the diameter of abdominal aorta tissue which can increase blood flow.

Based on this study, aerobic exercise was shown to increase NO production and bioavailability by increasing SOD specific activity and decreasing MDA in abdominal aorta tissues. Therefore, regular aerobic exercise can helps prevent endothelial dysfunction, as well as atherosclerosis.

The limitation of this study was the parameter used to evaluate vascular function was obtained only from biomarkers in the blood vessels and not directly based on vascular function itself, such as aortic vaso-reactivity or blood pressure. This study also did not measure aortic histopathology to identify foam cell formation.

Conclusions

Aerobic training increased nitric oxide production and bioavailability by enhancing SOD specific activity and decreasing MAD in abdominal aorta tissue.

Acknowledgement

The authors wished to thank all who cooperatively participated in this study.

References

Aerobic Training Prevents Atherosclerosis