



The Influence of Aerobic Exercise on Nitric Oxide Signal Transduction and Related Cytokines in ApoE^{-/-} Mice and the Underlying Mechanisms

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Abstract: Objective: To investigate the impact of aerobic exercise on nitric oxide (NO) signal transduction and related cytokines in ApoE^{-/-} mice and its underlying mechanisms. Methods: ApoE^{-/-} mice were divided into a control group and an aerobic exercise group. An 8-week aerobic exercise intervention using a treadmill training model was conducted to determine the expression levels of NO signal transduction-related cytokines. Results: Aerobic exercise significantly increased the expression of NO and related cytokines in the plasma of ApoE^{-/-} mice and reduced the level of inflammatory factors. Conclusion: Aerobic exercise can enhance NO signal transduction, reduce inflammatory responses, and improve the cardiovascular function of ApoE^{-/-} mice.

Keywords: aerobic exercise; ApoE^{-/-}; NO signal; cytokines; mechanism

1. Introduction

ApoE^{-/-} mice are a commonly used animal model for studying atherosclerosis. Due to certain genetic defects, they are prone to lipid metabolism disorders and spontaneous atherosclerosis. Studies have shown that there are significant abnormalities in the NO signal transduction pathway of ApoE^{-/-} mice, including reduced NO synthesis, decreased activity of nitric oxide synthase (NOS), and increased levels of inflammatory factors. Therefore, controlling NO signal transduction research through ApoE^{-/-} mice has also become a major form of related scientific research. In this study, aerobic exercise intervention was mainly conducted on ApoE^{-/-} mice to systematically evaluate its impact on NO signal transduction-related cytokines and explore its possible mechanisms of action.

2. Materials and Methods

2.1 General Information

Eight-week-old male ApoE^{-/-} mice, weighing 18-22g, were provided by a certain animal center. All mice were freely fed a standard rodent diet in a constant temperature (22±2°C) and humidity (55±5%) environment. Before the experiment, mice were adapted to the laboratory environment for one week to ensure the reliability of the experimental results. At the time of the experiment, mice were evenly divided into an aerobic exercise group and a control group, each with 10 mice. The subgroups were selected according to the inclusion criteria without significant differences.

2.2 Inclusion and Exclusion Criteria

Inclusion Criteria: (1) Mice confirmed to have the ApoE^{-/-} genotype; (2) No obvious signs of disease or infection; (3) Active, with body weight meeting experimental requirements (18-22g).

Exclusion Criteria: (1) Occurrence of disease, infection, or death during the experiment; (2) More than 20% change in body weight during the experiment; (3) Abnormal behavior during the adaptation period before the experiment.

2.3 Methods

The control group mice did not undergo any intervention, were freely active, and fed. The aerobic exercise group mice underwent 1 hour of treadmill training every day, with a speed set at 10m/min for 8 weeks. To ensure the appropriate exercise intensity, the exercise group mice underwent a one-week treadmill adaptation training before the official training, gradually increasing the exercise time and speed. After the exercise intervention, the experimental mice were fasted within 24 hours but allowed free drinking water. Subsequently, blood was taken through the abdominal aorta, and plasma and serum were separated. All samples were stored in a -80°C refrigerator, awaiting analysis.

2.4 Observational Indicators

Plasma NO level: After the exercise intervention, the NO level in mouse plasma was determined using the NO

colorimetric method.

NOS activity: The activity of NOS in plasma, including inducible NOS (iNOS) and endothelial NOS (eNOS), was detected using the chemiluminescence method.

Inflammatory factor level: The concentrations of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in plasma were determined by the enzyme-linked immunosorbent assay (ELISA) method.

Lipid level: Serum total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were measured.

2.5 Statistical Methods

All data were analyzed using SPSS 22.0 software, and data were represented as mean \pm standard deviation. The comparison between the two groups used the independent samples t-test, and $P < 0.05$ was considered statistically significant.

3. Results

3.1 Plasma NO Level and NOS Activity

The plasma NO level and NOS activity in the aerobic exercise group mice were significantly higher than those in the control group, as shown in Table 1.

Table 1. Plasma NO Level and NOS Activity

Group	NO Level	iNOS Activity	eNOS Activity
Aerobic Exercise Group	48.7 \pm 6.2	18.5 \pm 3.4	22.7 \pm 4.1
Control Group	35.2 \pm 4.5	12.4 \pm 2.3	15.6 \pm 3.1
t	4.13	5.64	4.52
P	0.001	0.001	0.001

3.2 Inflammatory Factors and Lipid Levels

The plasma levels of TNF- α and IL-6, total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) in the aerobic exercise group mice were significantly lower than those in the control group, while high-density lipoprotein cholesterol (HDL-C) was significantly increased, as shown in Table 2.

Table 2. Inflammatory Factors and Lipid Levels

Group	TNF- α	IL-6	TC	LDL-C	HDL-C
Aerobic Exercise Group	54.7 \pm 7.8	68.4 \pm 9.7	180.3 \pm 20.4	120.6 \pm 15.3	45.8 \pm 5.2
Control Group	78.5 \pm 10.2	102.3 \pm 15.6	220.5 \pm 25.6	150.4 \pm 18.2	35.7 \pm 4.3
t	7.61	12.6	6.52	5.63	4.26
P	0.001	0.001	0.001	0.001	0.001

4. Discussion

Atherosclerosis is the main cause of cardiovascular diseases, and the deposition of lipids on the inner walls of arteries can lead to inflammatory reactions and vascular endothelial dysfunction, resulting in severe cardiovascular problems. ApoE-/- mice serve as an animal model for studying atherosclerosis and are commonly used to analyze the role of nitric oxide (NO) in vascular function. Aerobic exercise has been proven to improve cardiovascular health, but research on its impact on NO signaling and cytokines in ApoE-/- mice and the underlying mechanisms is relatively scarce.

This study found that aerobic exercise significantly increased the levels of NO and NOS activity in the plasma of ApoE-/- mice, indicating that aerobic exercise improved NO signaling function by enhancing NO synthesis and release. NO, by activating guanylate cyclase, increases the production of cyclic guanosine monophosphate (cGMP), which relaxes vascular smooth muscle cells, thereby regulating vascular tone, and inhibits platelet aggregation and leukocyte adhesion, reducing thrombosis and inflammatory reactions. In addition, aerobic exercise reduced the levels of pro-inflammatory cytokines TNF- α and IL-6, effectively suppressing inflammatory responses and the occurrence of atherosclerosis.

The mechanisms of action of aerobic exercise include increasing shear stress on vascular endothelial cells, activating eNOS activity, and promoting NO synthesis and release; regulating the cellular redox state, reducing the generation of reactive oxygen species (ROS), and protecting NOS function; and modulating the function of immune cells through various

pathways, inhibiting the generation of inflammatory factors, accelerating the generation of regulatory T cells (Treg), and balancing immune effects.

The study results indicate that aerobic exercise improves cardiovascular function in ApoE^{-/-} mice by enhancing NO signaling and reducing inflammatory responses, providing a theoretical basis for the prevention and treatment of cardiovascular diseases. Future research should further explore the impact of exercise intensity and duration on NO signaling and more deeply clarify the specific mechanisms of NO in exercise intervention, fully revealing the protective effect of aerobic exercise on cardiovascular health.

In summary, this study systematically evaluated the effects of aerobic exercise on NO signal transduction-related cytokines and explored the underlying mechanisms by conducting aerobic exercise intervention in ApoE^{-/-} mice. The results showed that aerobic exercise significantly increased the plasma NO levels and NOS activity, reduced the levels of inflammatory factors TNF- α and IL-6, and improved blood lipid levels in ApoE^{-/-} mice. These findings suggest that aerobic exercise can improve the cardiovascular function of ApoE^{-/-} mice by enhancing the NO signaling pathway and reducing inflammatory responses.

This study provides a new perspective for understanding the protective mechanism of aerobic exercise on cardiovascular health and offers a theoretical basis for the prevention and treatment of cardiovascular diseases. Future research should further explore the impact of different exercise intensities and durations on NO signal transduction, and more deeply clarify the specific mechanisms of NO in exercise intervention through methods such as gene knockout or overexpression. At the same time, attention should also be paid to other potential signal transduction pathways and molecular mechanisms to fully reveal the protective role of aerobic exercise on cardiovascular health

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