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Won-Mok Son

Do-Yeon Kim

Ki-Dong Sung

Yi Sub Kwak

Yeong Ho Baek

See next page for additional authors

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Authors

Won-Mok Son, Do-Yeon Kim, Ki-Dong Sung, Yi Sub Kwak, Yeong Ho Baek, and Song-young Park

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Won-Mok Son¹, Do-Yeon Kim¹, Ki-Dong Sung¹, Yi-Sub Kwak², Yeong-Ho Baek¹ and Song-Young Park^{3*}

¹Department of Physical Education, Pusan National University, Busan 609-735, Korea

²Department of physical education, Dong-Eui University, Busan 614-714, Korea

³School of Medicine, Boston University, Boston, USA

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Although exercise training has been utilized to improve vascular function in animals and humans, the impact of moderate intensity exercise training on fibrinolytic activities and nitric oxide (NO) bioavailability has not been well documented. Therefore, the purpose of the current study was to examine the impact of moderate intensity aerobic exercise training on fat mass, blood lipid profiles, fibrinolytic activity, and NO levels in high-fat-diet induced rats. The body weight, fat mass, blood lipid profiles, fibrinolytic activity, and nitrite/nitrate were measured pre- and postexercise (10 weeks) training. The body weight and fat mass reduced significantly in the exercise (EX) group compared to the control (CON) group. Blood lipid profiles and low-density lipoprotein were unchanged in the EX group compared to the CON group. However, triglyceride and free fatty acid were significantly lower in the EX group compared to the CON group, and high-density lipoprotein was significantly greater in the EX group compared to the CON group. In addition, fibrinolytic activity and nitrite/nitrate were significantly greater in the EX compared to the CON group. These results suggest that 10 weeks of the moderated intensity aerobic exercise training improves blood lipid profiles, fibrinolytic activity, and the nitrite/nitrate ratio, which may improve vascular health and reduce obesity-related cardiovascular disease risks in high-fat-diet induced rats.

Key words : Blood lipid profiles, fat mass, fibrinolytic activity, nitric oxide

Introduction

Obesity has been known as a high risk factor of cardiovascular disease [27], and defined as disorder of energy imbalance which is highly dependent upon variations in both dietary energy intake and energy expenditure [3]. Research suggested that obesity is the major risk factor of metabolic syndrome such as the diabetes, hypertension and dyslipidemia and coronary arterial diseases [12].

A person with obesity has elevated total cholesterol (TC), triglyceride (TG), and low density lipoprotein cholesterol (LDL-C) but lower high density lipoprotein cholesterol (HDL-C) [35] and these altered lipid profiles are associated with increased risks of thrombosis and cardiovascular disease [18].

In human body, blood coagulation and anticoagulation is relatively well balanced but in general anticoagulation suppresses coagulation for normal circulation of blood [38]. However, damage on the endothelial layer in the blood vessels which is mainly derived from increased triglyceride and fibrinogen level results from high fat diet, stress, lack of exercise may cause a formation of thrombosis [47].

Endothelial dysfunction is a common phenomenon in obese individuals which is characterized by an imbalance between Nitric Oxide (NO) and Endothelin-1 (ET-1) [10, 15, 44].

NO is soluble gas has half-life of 3~6 sec, and consistently synthesized by a series of reaction in L-arginine, and NO synthase (NOS) dependent enzyme calcium - calmodulin endothelial cell. Vascular endothelial cell plays an important role in the regulation of vascular function, specifically, endothelial mediated vasodilation by producing vasoactive substances NO [36].

Exercise has been known as a non-pharmacological method for reducing and preventing cardiovascular risk factors. Additionally, regular exercise has positive effects on improving blood lipid profile and cardiovascular health [23, 33].

*Corresponding author

Tel : +82-10-2886-6819, Fax : +82-51-515-1991

E-mail : songpark@bu.edu

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Furthermore, it has been well documented that regular practices of planned exercise improves blood lipid profiles [43], fibrinolytic activities [42], and nitric oxide level [31].

Although the impact of exercise on blood lipid profiles has been studied in last decade, there are only limited data available for the effect of aerobic exercise on fibrinolytic activities and nitric oxide production. Therefore, the purpose of this study is to investigate the effects of aerobic exercise on blood fibrinolytic activities, nitric oxide levels and blood lipid profiles in rats.

Materials and Methods

Animals

All experiments were performed in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the Pusan National University (PNU-2012-0082). Sixteen male Sprague Dawley at 3 weeks of age were purchased from Hyochang Science Co, Daegu, Korea. Rats were housed at 22°C with a 12 hr light, 12 hr dark cycle. Rats were separated into two groups: control group (n=8) and exercise group (n=8). (Table 1). The animals were fed either standard diet or high fat diet for 10 weeks.

Aerobic training

Four week old rats were performed aerobic training as described previously [7]. Briefly, exercise group performed an exercise intensity at 55% of VO_{2max} (14-15m/min) for 30min for the first week. For week 2 and 3, an exercise intensity at 50-60% of VO_{2max} (15-16m/min) for 35min was utilized. In the week 4, a exercise intensity at 50-60% of VO_{2max} (16-17m/min) for 40min was performed. Control group was on the treadmill with only noise and shakes to mimic the same condition of the exercise group.

Fat weight

Fat mass was measured using epididymal fat after dis-

Table 1. Physical characteristics of experimental rats

Group	Age (weeks)	Body weight(g)		
		4 weeks	10 weeks	14 weeks
A(n=8)	14	79.06 ±5.86	265.25 ±33.14	302.04 ±44.67
B(n=8)	14	78.81 ±5.71	263.13 ±19.51	312.14 ±28.38

Values are M±SD

A: exercise group, B: control group

Table 2. Composition of the high fat diet

Ingredient	High fat diet (45cal%)
	g
Casein (from milk)	200
Corn starch	155.036
Sucrose	50
Dextrose	132
Cellulose	50
Soybean oil	25
Lard	175
Mineral mixture	35
Vitamin mixture	10
TBHQ	0.014
L-Cystine	3
Choline bitartrate	2.5
Total	837.6

sected and cleansed in the phosphate buffered saline (PBS).

Blood lipid profile

TC, TG, HDL-C and LDL-C were measured using spectrophotometric methods with chemistry analyzer (TBA-80FR, Toshiba, Japan).

Fibrinolytic activity

Fibrinolytic activity was determined using the fibrin plate method [4]. Briefly, in the petri dish, 10 ml of 0.6% plasminogen-rich fibrinogen (Sigma, St. Louis, MO, USA) solution in 10 mM phosphate buffered saline (pH 7.8) was mixed with an equal volume of 2% agarose solution and 0.1 ml of thrombin solution (100 NIH units/ml; Sigma). The petri dish was left to stand at room temperature for 30 min to allow a fibrin clot layer to be formed. 50 ul of ME of oil was dropped into a hole on the plate that was made with a Pasteur pipette. The plate was incubated at 37°C for 24 hr. Fibrinolytic activity of sample was calculated based on the plasmin activity. The size of the clear zone formed by the sample was compared with the area created by 0.5 U/ml of plasmin. The activity was expressed as gram.

Nitric oxide

NO has an extremely short half-life and is rapidly oxidized to nitrite and nitrate in the presence of oxygen. Hence, one of the most common methods used to assess NO production is to measure total nitrite/nitrate concentration using Griess reagent. In the present study, we utilized a Griess assay kit from Cayman Chemical (Ann Arbor, MI, USA).

Sodium nitroprusside (SNP) was utilized as a NO donor. Samples containing SNP (200 μ M) and various concentrations of MHY-794 were loaded into the wells of a 96-well transparent microplate. After adding the enzyme cofactor mixture and nitrate reductase mixture, the plate was incubated at room temperature for 1 hr, and then 50 μ l of a 1% solution of sulfanilamide in 5% phosphoric acid and 50 μ l of a 0.1% solution of N-(1-naphthyl) ethylene-diamine in H₂O were added to each well. The amount of nitrite/nitrate produced in the reaction mixture was determined spectrophotometrically at 540 nm (OD540) using a microplate reader [14].

Data analysis

Data are presented as mean \pm SD. Statistical analyses were performed with independent t-test by SPSS 20.0 version software (SPSS Science, Chicago, IL, USA) analysis. Data (mean \pm SD) was considered statistically significant at a value of $p < 0.05$.

Results and Discussion

Fat weight

Fat weight was lower in exercise group compared to control group ($p < 0.01$; Fig. 1).

Obesity is defined as a pre-disease condition which results from excessive body fat accumulation due to the energy intake that exceeds the energy requirement of the body, and it is a critical risk factor for various diseases. A large quantity of fat accumulation which refers to the increased number and size of adipocytes. Additionally, this increased size of adipocytes may cause dysfunction of adipocytes such as over production of inflammatory cytokines and adipokines [32]. However, aerobic exercise training reduces expression of genes related to lipogenesis, thus inhibiting lipid accumulation in tissues [8].

Previous studies suggested that obesity-induced rats were

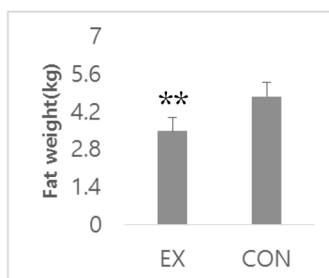


Fig. 1. Change in fat weight after 4 weeks treadmill exercise.

subjected to treadmill running, 30 min/day, 5 times a week, for 8 weeks, and the fat mass were decreased significantly [45]. In the present study, we could not measure the size of adipose tissues but EX had significantly lower fat mass than that of the control group; this maybe the consequence of the increased energy expenditure which results from the increased physical activity by the aerobic exercise training.

Blood lipid profile

TC and TG were lower in exercise group compared to control group ($p < 0.05$). However, HDL-C was greater in exercise group compared to control group ($p < 0.05$; Fig. 2).

Obesity is accompanied with elevation of body fat, TG, and LDL-C, and reduction of lean mass and HDL-C, resulting in artery diseases, including atherosclerosis and hyperlipidemia [9, 15]. However, it was reported that medium intensity aerobic exercise regulates lipid metabolism by reducing levels of plasma triglycerides and free fatty acids and increasing HDL-C levels, thus having a preventative effect against cardiovascular disease [2, 40].

TC is a useful indicator for cardiovascular diseases because it exists in all cells and helps in maintaining cholesterol homeostasis [1]. Previous studies reported that obesity-induced rats were subjected to treadmill exercises 60 min per day, speed at 8-15 m/min, 5 times a week, for 6 weeks, fat mass significantly lower [34]. These previous studies are well aligned with our findings, the exercise group had significantly lower TC than that of the control group; which is considered to be due to the increased catabolism of cholesterol in the body by exercise training.

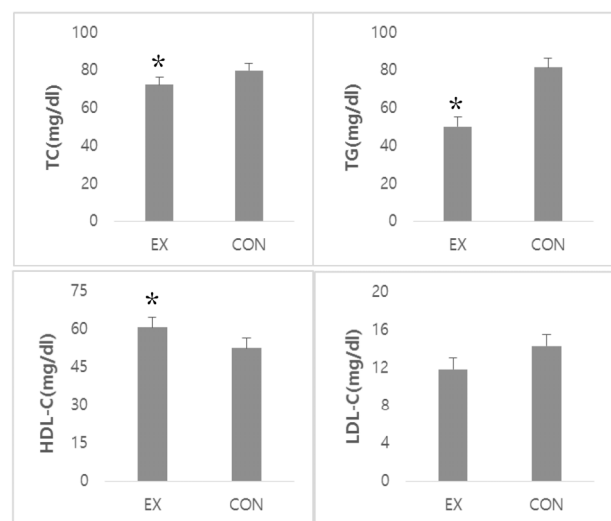


Fig. 2. Change in blood profile after 4 weeks treadmill exercise.

Although TG is mostly affected by diets, exercise training reduces it by 20-60% [41]. Previous studies reported that obesity-induced rats were trained for treadmill exercises 60 min per day, speed at 5-15 m/min, 6 times a week, for 6 weeks and TG significantly lower [29]. Our findings further confirm the previous findings, the EX had significantly lower TG than that of the control group. This finding can be explained by increased lipolysis during exercise. Additionally, we utilized moderate exercise intensity which has been known as a useful exercise intensity to increase lipolysis but does not occur any adverse effects on cardiovascular system [13].

HDL-C has been known as a good cholesterol which inhibits accumulation of body cholesterol and removes cholesterol buildup in arteries. Additionally, it is considered as a preventive factor for atherosclerotic disease or an anti-cholesterol factor [21]. Previous studies reported that SD rats were subjected to treadmill exercises 25-30 min/day, 5 times a week, for 2 weeks, HDL-C significantly higher [6] and Similar to these previous studies, the present study found that the EX had significantly higher HDL-C than that of the control group. This finding can be explained by increased lipoprotein lipase activity (LPLA) in the plasma activated by aerobic exercise, increasing the conversion ratio from cholesterol to chylomicron, and from VLDL and LDL to HDL; furthermore, total and hepatic triglyceride lipase activity (HTGLA) increased by exercise which reduces catabolism of HDL [16].

Increased LDL-C has been known as a risk factor for hyperlipidemia and atherosclerosis. Also, LDL-C is a transporter for cholesterol to peripheral tissues [5]. Many studies suggested that moderate exercise intensity training reduces LDL-C level in the blood [19]. Interestingly, the present study identified that LDL-C was not significantly difference between EX and CON which can be supported by Lee et al [30] which suggested that the moderate intensity of aerobic exercise may positively affect LDL-C but it does not always reduced, and further suggested that the LDL-C level is the least sensitive markers of exercise induced improved lipid metabolism in rats.

Fibrinolytic activity

Fibrinolytic activity was greater in exercise group compared to control group ($p < 0.01$; Fig. 3).

Blood fibrinogen level can be elevated by stress, lack of exercise, and an increased in blood cholesterol or triglycerides as a result of increased fat intake [24]. Once blood

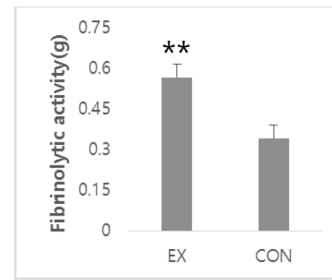


Fig. 3. Change in fibrinolytic activity after 4 weeks treadmill exercise.

fibrinogens promote to thrombosis, the thrombi flow in the blood and block capillaries, and then results in the various arterial occlusive diseases, including myocardial infarction and ischemia [20].

It has been suggested that exercise training reduces levels of platelets and fibrinogens for blood coagulation, and positively affects elevation of anticoagulation components [47]. Elevated levels of fibrinolysis, degradation of fibrins and anticoagulation processes, was elevated in people with high aerobic fitness levels compared to people without regular exercise [11]. The exact mechanism responsible for this elevated level of fibrinolysis results from exercise training has not been well understood, but the increased shear stress during exercise stimulates mechanoreceptors on the endothelial cell and produces NO which may trigger the activity of fibrinolytic activity [26].

Previous human studies reported post-menopausal women were subjected to aerobic exercises of intensity 50-70% HRR and resistance training intensity of 1RM 40-60%, 90 min/day, 4 times a week, for 12 weeks, fibrinolytic activity significantly increased [26]. Similar to these previous human studies, the present study also identified that the exercise group had significantly greater fibrinolytic activity than that of the control group. This finding confirmed that moderated intensity of aerobic exercise training increases fibrinolytic activity.

Nitric oxide

Nitric oxide was greater in exercise group compared to control group ($p < 0.01$; Fig. 4)

Nitric oxide (NO) is a soluble gas with a 3-6 sec half-life [36] and is a potent vasodilation compound that is isolated in the conversion process from L-arginine to citrulline by activation of NOS (nitric oxide synthase) in a Ca^{2+} dependent manner with in vascular endothelial cells [37]. It plays important role in functional and structural development of an-

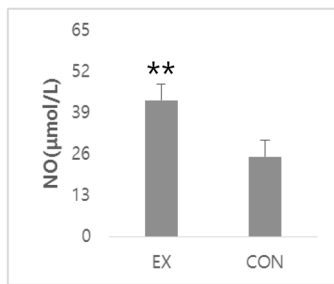


Fig. 4. Change in NO after 4 weeks treadmill exercise.

ti-inflammation and anti-thrombosis [28].

It has been reported that vascular endothelial dysfunction is an initial stage of atherosclerotic vascular changes and is mostly affected by reduction of NO bioavailability [48] and eNOS was remarkably decreased in rats fed a high fat diet [25]. However, aerobic exercise increases cardiac output and blood flow, which escalated the shear stress load on vascular walls, resulting in increased NO concentration.

Our previous study revealed that fisher rats were subjected to treadmill exercises 30~60 min/day, speed at 8~20 m/min (grade 10%), for 8 weeks, and the level of nitric oxide was significantly higher [22]. In the present study, the exercise group showed significantly greater NO markers than that of the control group. This might be due to the increased frequency to expose to increased shear stress induced by exercise training. This increased shear stress during exercise may increase NO production in the endothelial cells which is mediated by exercise induced elevated calcium handling capacity in the vasculature includes both endothelial and smooth muscle cells. This explanation has not been fully understood and warrants further investigations.

This present study demonstrated that the impact of moderate intensity of aerobic exercise on fat mass, blood fibrinolytic activity, lipid profiles, and NO levels and we confirmed that exercise training improves these cardiovascular risk factors which likely suggest that exercise training is a useful non-pharmacological method to improve cardiovascular health.

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초록 : 유산소 운동이 고지방 식이 흰쥐의 지방량, 혈중지질, 혈전용해능 및 산화질소에 미치는 영향

손원목¹ · 김도연¹ · 성기동¹ · 광이섭² · 백영호¹ · 박송영^{3*}

(¹부산대학교 체육교육과, ²동의대학교 체육학과, ³보스톤 의과대학)

본 연구는 생후 3주령 Sprague-Dawley계 수컷 흰쥐를 16마리로 6주간 고지방식이를 통해 비만을 유도 후 운동군(8마리), 대조군(8마리)로 구분하였다. 운동기간 중 운동군과 대조군 모두 고지방식이를 섭취시켰다. 1주차는 14-15 m/min의 속도로 1일 30분, 2, 3주차는 15-16 m/min의 속도로 1일 35분, 4주차는 16-17 m/min의 속도로 1일 40분으로 주 6회 실시한 후 다음과 같은 결론을 얻었다. TC, TG는 운동군이 대조군 보다 유의하게 낮았으며, HDL-C는 운동군이 대조군 보다 유의하게 높았다. 혈전용해능, 산화질소는 운동군이 대조군 보다 유의하게 높았다. 이상을 종합하여 볼 때 유산소 운동이 혈관기능개선에 도움을 주는 것으로 나타났다.