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Improvement of thoracic aortic vasoreactivity by continuous and intermittent exercise in high-fat diet-induced obese rats

HONGPENG LIU[1,2*], ZHEN YANG[1*], JIAN HU[1], YAN LUO[1], LINGQIN ZHU[3], HUIFANG YANG[3] and GUANGHUA LI[1]

[1]Department of Physiology, School of Basic Medical Science; [2]Institute of Laboratory Medicine; [3]Public Health School, Ningxia Medical University, Yinchuan, Ningxia 750004, P.R. China

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Abstract. The aim of the present study was to explore the effects of continuous and intermittent exercise on the thoracic aortic vasoreactivity and free radical metabolism in rats fed with a high-fat diet (HD). Sprague-Dawley (SD) rats were randomly divided into four groups (n=8, each group): Conventional diet (CD), HD, HD with continuous exercise (HCE) and HD with intermittent exercise (HIE). HCE rats swam once/day for 90 min; HIE rats performed swimming exercises 3 times/day, 30 min each time with an interval of 4 h. In these two groups, the exercise was conducted 5 days a week for 8 weeks. Rats in the CD and HD groups were fed without swimming training. At the end of the exercise, all the rats were sacrificed and the blood, thoracic aorta and myocardium were collected immediately. The thoracic aortic vasoreactivity, the plasma total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), superoxide dismutase (SOD), malondialdehyde (MDA) and vascular endothelial nitric oxide synthase (eNOS) gene expression were measured. Compared to the control group, in the HD group the enhanced contractile response of the thoracic aortic rings to noradrenaline (NA) was observed ($P<0.01$). The levels of TC and LDL ($P<0.01$) were also increased in serum while the HDL level was reduced without statistical significance. In addition, the MDA content was upregulated in the myocardium, but the SOD level decreased ($P<0.01$). Furthermore, the expression of vascular eNOS mRNA was reduced ($P<0.01$). However, following the exercise the contraction of the thoracic aorta vascular rings to NA was reduced in the HCE and HIE groups ($P<0.01$), and the decreased contractile response was more evident in the HIE group compared to the HCE group ($P<0.01$). Additionally, in the HCE group the level of TG ($P<0.01$) was decreased, while the HDL ($P<0.01$) level was increased. Although the reduction of the TC and LDL level was also observed there was no significant difference compared to the HD group. In the HIE group, the TG, TC and LDL were downregulated while the HDL was enhanced ($P<0.01$). The TC and LDL levels were decreased more than those of the HCE group; however, there was no significant difference in the TG and HDL levels between these two groups; additionally, in these two exercise groups, the MDA level was decreased in the myocardium ($P<0.01$) while the SOD level was increased ($P<0.01$). Furthermore, the expression of eNOS was upregulated ($P<0.01$), but the increase was much more in the HIE group than that in the HCE group. In conclusion, exercise may attenuate the aggravated contraction induced by NA and improve the activity of the thoracic aorta in obese rats, which may be associated with enhanced antioxidant enzyme activity and reduced free radical generating. Additionally, intermittent exercise is better than the continuous exercise in improving the thoracic aorta vasoreactivity.

Introduction

Obesity is a serious public health problem worldwide (1), which is often accompanied by excessive visceral fat, dyslipidemia, hypertension, diabetes, coronary arteriosclerotic heart disease, cancer, non-alcoholic fatty liver disease, cardiovascular disease and other chronic diseases (2,3). Arterial elasticity is an important factor in predicting the cardiovascular risk. One of the most basic and direct indicators reflecting the functional status of artery blood vessels is vascular compliance (4). Studies have shown that a high-fat diet (HD) and lack of physical activity are the most important factors for the development of obesity. Long-term aerobic exercise markedly improved the abnormal hemorheological property and the oxidative stress in rats with hypercholesterolemia. It has been shown that aerobic exercise plays an important role in anti-free radicals, lipid peroxides, prevention and treatment of cardiovascular disease (5,6). However, regular aerobic exercise for a long period and short exhaustive exercise have different effects on vasoreactivity (7). Exercise

Correspondence to: Professor Guanghua Li, Department of Physiology, School of Basic Medical Science, Ningxia Medical University, 1160 Shengli Street, Yinchuan, Ningxia 750004, P.R. China
E-mail: ligh@nxmu.edu.cn

*Contributed equally

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intensity, frequency, duration and different diet have various effects on the metabolic activity (8). Therefore, sports as a non-drug therapy is an important method to control obesity and other complications, and this research has attracted increasing attention. The present study was designed to evaluate the effect of the continuous and intermittent exercise on the contraction of thoracic aortic vascular rings and metabolism of free radicals in rats fed with HD and to explore the different mechanisms of exercise on the cardiovascular function status.

Materials and methods

Experimental animals. A total of 32 male Sprague-Dawley (SD) rats (180±10 g) were bred (provided by the Experimental Animal Center of Ningxia Medical University, Yinchuan, Ningxia, China) for 8 weeks with a free diet and water *ad libitum*, and a temperature of 32±1˚C with 12-h light. Rats were randomly divided into four groups: Conventional diet (CD), HD, HD with continuous exercise (HCE) and HD with intermittent exercise (HIE). There were 8/cage and rats with a poor ability for swimming were eliminated. The experimental procedures were approved by the Animal Ethics Committee of the Ningxia Medical University and Use Committee in accordance with the guidelines of the Council of the Physiological Society of China.

Preparation of the experimental animal models. In the control group, rats were fed with CD: 23 g protein, 49 g carbohydrate, 4 g fat, 5 g fiber, 7 g bone meal and 6 g vitamins/100 g. In the HD group, rats were fed with the high-fat along with the standard diet: Peanuts, milk chocolate and sweet biscuits in a ratio of 3:2:2:1. Protein accounted for 20%, fat for 20%, sugar for 48% and cellulose for 4%. Calories in the fat diet were 5.12 kcal/g (equivalent to 35% fat calories), while there was 4.07 kcal/g in the standard diet.

Exercise training protocol. The rats in the CD and HD groups were fed at room temperature without swimming training. The rats in the exercise groups swam in a plastic pool with a diameter of 60 cm, the water depth was 55 cm and temperature was 28-32˚C. For the continuous exercise, rats swam for 30, 60 and 90 min in the first, second and third day, respectively, then the rats swam one time for 90 min/day. For the intermittent exercise, rats performed intermittent swimming for 10, 20 and 30 min in the first, second and third day, respectively, and the total swimming time throughout the experiment was 90 min/day, which was divided into three time periods and maintained at a 4-h interval for the following time schedule: The rats swam at 7:00 a.m., 11:30 a.m. and 3:30 p.m., respectively, for 30 min each time. Rats with intermittent exercise were reared in the respective cages. All the rats swam with a load of 5% of their body weight strapped to their tail. The exercise was conducted for 5 days a week for 8 weeks with moderate intensity.

Sample preparation. After 8 weeks of training, rats were rested and fasted for 24 h. Subsequently the rats were anesthetized with 20% urethane (0.5 ml/kg) and the blood was taken from the heart. The serum was separated and stored at -80˚C for further use. The heart was obtained and the residual blood was washed with saline. Tissue homogenate (10%) was prepared and the supernatant was stored at -80˚C.

Recording the tension of the aortic vascular rings. At the same time, the thoracic aorta was isolated immediately and the connective tissue surrounding the blood vessels was carefully removed. The aorta was cut into 3-4 mm sections for measuring the tension. The vascular rings were hung in the isolated organ tissue bath with 10 ml K-H solution (10 mM NaCl, 4.6 mM KCl, 2.5 mM $CaCl_2$, 24.8 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 1.2 mM $MgSO_4$ and 5.6 mM glucose) at 37˚C (pH 7.4) and continuously perfused with 95% O_2 and 5% CO_2. The resting tension was adjusted to 1 g, after equilibration for 1 h with flushing every 15 min. The maximum contraction tension was induced with 60 mmol/l KCl, and subsequently the cumulative dose-response curve for noradrenaline (NA) (10^{-10}-10^{-5} M) was examined. The KCl-induced maximum contraction was regarded as 100%, and the values of the NA-induced contraction were expressed as a percentage of the maximal contraction induced by KCl (10^{-10}-10^{-5} M).

Detection of superoxide dismutase (SOD) and malondialdehyde (MDA) levels in the myocardium. Oxidative stress indices were measured to study whether the continuous and intermittent exercises reduced HD-induced oxidative stress. SOD was measured by thiobarbituric acid in the myocardium; MDA was determined using the WST-1 method.

Detection of high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG) and total cholesterol (TC) levels in plasma. The levels of serum Lipid (TC, HDL, LDL and TG; Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan) were measured using a microplate reader and ultraviolet spectrophotometry.

*Expression of endothelial nitric oxide synthase (*eNOS*)*. Total RNA was extracted from the thoracic aorta according to the manufacturer's instructions for the Axygen RNA extraction kit (Axygen Biosciences, Union City, CA, USA). The concentration of the total RNA was determined by spectrophotometry, and quality was assessed using the A260/A280 nm ratio within 1.8-2.0. cDNA was synthesized using a reverse transcription kit (Beijing TransGen Biotech Co., Ltd., Beijing, China). Quantitative polymerase chain reaction (qPCR) was performed using a QuantiTect SYBR-Green PCR kit (Beijing TransGen Biotech Co., Ltd.) as follows: 40 cycles of denaturation at 94˚C for 30 sec, annealing at 60˚C for 30 sec and extension at 72˚C for 30 sec. Primers designed for eNOS and β-actin are shown in Table I. The relative levels of the gene expression are shown by the $2^{-\Delta\Delta Ct}$ method: $\Delta Ct = Ct_{(target\ gene)} - Ct_{(action\ gene)}$; $\Delta\Delta Ct = \Delta Ct_{(sample)} - \Delta Ct_{(control)}$.

Statistical analysis. Statistical analysis was performed using SPSS 18.0 software (IBM Corp., Armonk, NY, USA). All the data are expressed as mean ± standard deviation. Two-way analysis of variance (ANOVA) was used to evaluate any differences among groups; one-way ANOVA was used to analyze the remaining data. P<0.05 was considered to indicate a statistically significant difference.

Table I. GenBank accession code, primer sequences and predicted size of the amplified product.

Gene	Primer sequences	GenBank	bp
eNOS	Forward: 5'-CACACTGCTAGAGGTGCTGGAA-3' Reverse: 5'-TGCTGAGCTGACAGAGTAGTAC-3'	NM_021838	109
β-actin	Forward: 5'-TCATGAAGTGTGACGTTGACATCCGT-3' Reverse: 5'-CCTAGAAGCATTTGCGGTGCAGGATG-3'		285

bp, base pairs; eNOS, endothelial nitric oxide synthase.

Table II. Influences of HCE and HIE on body weight of HD-induced fat rats.

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
CD	181.89±3.14	204.25±4.92	228.56±7.33	248.11±5.21	265.78±4.66	283.89±3.76	310.33±5.77	336.22±6.78
HD	184.33±4.27	224.89±5.23[c]	268.56±4.25[c]	303.30±6.83[c]	340.22±6.55[c]	377.30±5.29[c]	415.50±10.53[c]	449.30±12.00[c]
HCE	183.53±2.61	222.89±3.98	266.11±4.23	299.54±6.53	335.56±4.19	370.44±3.21[a]	399.78±4.00[b]	425.78±7.10[b]
HIE	184.00±4.50	217.87±3.83	259.12±4.61	295.00±6.86	333.50±7.33	365.50±4.29[a]	392.87±5.81[b]	411.37±4.28[b,d]

[a]P<0.05 and [b]P<0.01 vs. HD; [c]P<0.01 vs. CD; [d]P<0.05 vs. HCE. Values are expressed as mean ± standard deviation (n=8). HD, high-fat diet; CD, conventional diet; HCE, HD with continuous exercise; HIE, HD with intermittent exercise.

Drugs and reagents. NA was provided by Shanghai Hefeng Pharmaceutical Co., Ltd., (Shanghai, China) batch no. H12020621; the SOD and MDA assay kits were provided by Nanjing Jian Cheng Bioengineering Institute (Nanjing, China); and lipid (TC, TG, HDL and LDL) kits were purchased from the Daiichi Pure Chemicals Co., Ltd.

Results

Influences of continuous and intermittent exercise on the body weight of obese rats. As shown in Table II, the body weight of the rats in each group increased every week. In the late stage of the swimming exercise, the weight increased more in the HD group than that in the control group (P<0.01); continuous and intermittent exercise (HCE and HIE groups, respectively) decreased the gain in body weight compared to that of the HD group (P<0.01), and additionally, the intermittent exercise was more effective than the continuous exercise (P<0.05).

Effects of continuous and intermittent exercise on the contraction of thoracic aortic vascular ring in HD rats. As shown in Fig. 1, contractile reactivity of thoracic aortic rings to NA increased with its increasing concentration in each group. The contractive response of thoracic aorta from rats fed with HD was significantly increased compared to that of the control group (P<0.01). The increased contraction induced by NA was attenuated by the continuous and intermittent exercise (P<0.01). Compared to the HCE group, this effect induced by the intermittent exercise in the HIE group was more evident (P<0.01).

Effects of continuous and intermittent exercise on the metabolism of free radicals in myocardium from rats fed with HD. As shown in Fig. 2, the SOD activity of the myocardium significantly decreased in the HD group compared to that in the control group (P<0.01), and the continuous and intermittent exercise elevated the SOD activity significantly compared to that in the HD group (P<0.01). Furthermore, the myocardial SOD activity in the HIE group was higher than that in the HCE group, although there was no significant difference. The MDA level in the myocardium was also significantly increased in the HD group compared to that in the control group (P<0.01), but

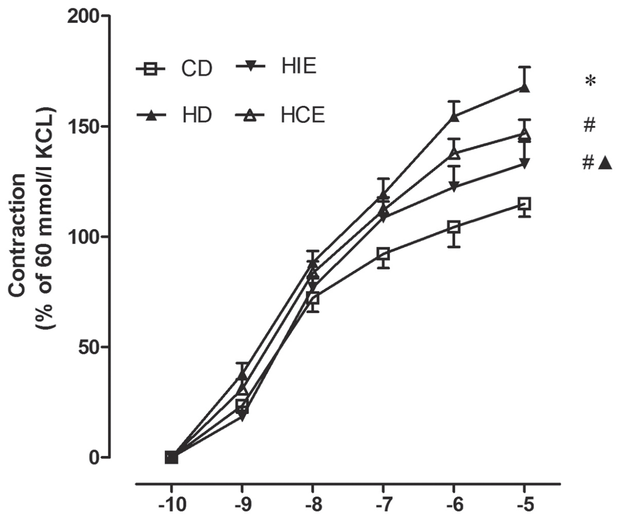

Figure 1. Contractile response of vascular ring to noradrenaline (NA). Dose-dependence of NA on the contraction of the thoracic aorta rings separated from rats in the conventional diet (CD), high-fat diet (HD), HD with continuous exercise (HCE) or HD with intermittent exercise (HIE) groups. The contraction induced by 60 mmol/l KCl was taken as 100%. Data are expressed as mean ± standard deviation (n=8). #P<0.01 vs. HD; *P<0.01 vs. CD; ▲P<0.01 vs. HCE.

Table III. Effects of HCE and HIE on blood lipids in HD fed rats.

Group	TG	TC	HDL	LDL
CD	1.43±0.92	1.61±0.24	0.85±0.17	0.33±0.10
HD	1.58±0.87	7.95±1.17[b]	0.78±0.17	6.45±1.64[b]
HCE	0.75±0.30[a]	6.90±1.85	1.03±0.15[a]	6.15±1.76
HIE	0.40±0.18[a]	2.75±1.09[a,c]	1.04±0.08[a]	2.00±0.82[a,c]

[a]P<0.01 vs. HD; [b]P<0.01 vs. CD; [c]P<0.01 vs. HCE. Values are expressed as mean ± standard deviation (n=8). HD, high-fat diet; CD, conventional diet; HCE, HD with continuous exercise; HIE, HD with intermittent exercise; TG, triglyceride; TC, total cholesterol; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

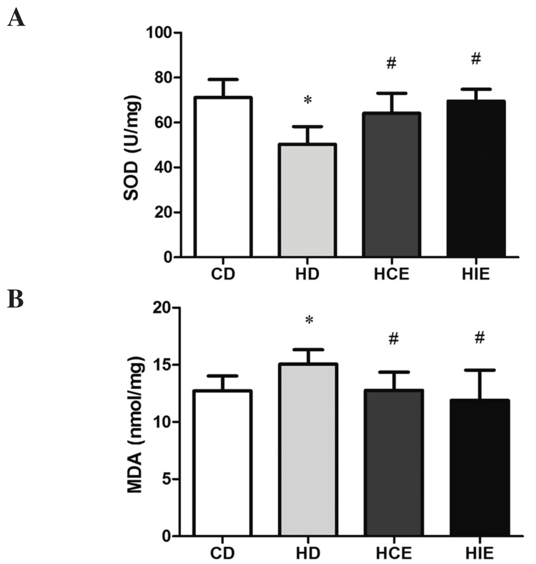

Figure 2. Effects of high-fat diet (HD) with continuous exercise (HCE) and HD with intermittent exercise (HIE) on (A) superoxide dismutase (SOD) and (B) malondialdehyde (MDA) metabolism in rats fed with HD. The values are expressed as mean ± standard deviation (n=8). #P<0.01 vs. HD; *P<0.01 vs. conventional diet (CD).

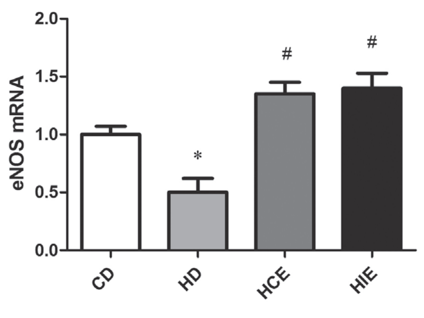

Figure 3. Effects of high-fat diet (HD) with continuous exercise (HCE) and HD with intermittent exercise (HIE) on the expression of endothelial nitric oxide synthase (eNOS) mRNA in the rats fed with HD. Data are expressed as mean ± standard deviation (n=8). #P<0.01 vs. HD; *P<0.01 vs. conventional diet (CD).

following the exercise, the MDA content was reduced (P<0.01). The MDA level was also lower in the HIE group compared to the HCE group.

Effects of continuous and intermittent exercise on plasma lipid metabolism in rats fed with HD. As shown in Table III, the lipid metabolism level could be affected by continuous and intermittent exercise. TC and LDL levels significantly increased in the HD group (P<0.01). Compared to the HD group, the level of TG (P<0.01) decreased in the HCE group, while the HDL (P<0.01) level increased, the reduction of the TC and LDL levels were also observed but there was no statistical significance. In the HIE group, the TG, TC and LDL decreased and the HDL increased (P<0.01). Of note, the TC and LDL levels decreased more in the HIE group than that in the HCE group.

Expression of eNOS mRNA. As shown in Fig. 3, the expression level of vascular eNOS mRNA in the HD group (P<0.01) was reduced compared to that in the normal diet group. However, its expression level in the HCE and HIE groups was upregulated compared to that of the HD group (P<0.01), but there was no statistical significance between the HCE and HIE groups (P>0.05).

Discussion

In the present study, the contractive response of the thoracic aortic rings to NA was significantly increased in the HD group, but following exercise, this increased contraction induced by NA was attenuated. In addition, the effect of intermittent exercise on thoracic aortic vasoreactivity was more evident than that of the continuous exercise. These findings were consistent with a previous study (9). With the improvement of living conditions, HD has become one of the important factors that lead to obesity. Obesity is well-known as a risk factor for cardiovascular events. Studies have demonstrated that damaged aortic endothelium and abnormal lipid metabolism appear in rats fed with HD (10). Furthermore, HD reduces the antioxidant enzyme activity and increases the lipid peroxidation *in vivo* (11).

Arterial compliance, also known as vascular compliance, is a prognostic indicator of arterial health (12). The impairment of the aortal function enhances vasoconstriction and weakens vasodilation (13). Usually, the response of the thoracic aorta vascular rings to NA reflects the arterial contractive function. Under the condition of the HD and aerobic exercise, the vascular elasticity and vasodilation are decreased by the excitation of the body's sympathetic nervous system (14,15). In addition, the contractile response of the thoracic aorta to KCl is enhanced in rats fed with HD (16) and vascular compliance reduced (17). The present results showed that the contractile response of the aorta was increased in rats fed with HD. However, the continuous and intermittent exercises attenuated the increase of the contraction induced by NA. Furthermore, the effect induced by the intermittent exercise was stronger compared to the continuous exercise, which indicated that the intermittent exercise was more effective in protecting the aorta.

A previous study has shown that HD can increase the formation of endogenous free radicals and enhance the response to lipid peroxidation, which results in the impairment of the cell membrane (18). MDA is an oxidative product of free oxygen radicals with polyunsaturated fatty acids on the cell membrane, and it indicates the degree of lipid peroxidation (19). In addition, SOD is an important antioxidant enzyme that specifically removes superoxide anion radicals and protects cells, and its activity can indirectly reflect the body's ability to eliminate oxygen free radicals. Aerobic exercise can reverse the effect of the superoxide anion inside and outside the body (20). The effects of exercise on the activity of SOD are not consistent. Certain studies reported that exercise training improved the activity of SOD (21), and it is proposed that during exercise, the oxygen consumption and production of free radicals increase, which leads to the increase of intracellular antioxidant enzymes to produce acute adaptive changes (18). This process causes the body to produce antioxidants gradually. The effect of exercise on the activity of SOD is well-accepted as different due to the difference of the type intensity and durative time of exercise. In the present study, the MDA level was higher in the HD group, while the activity of SOD was decreased, indicating that HD enhanced lipid peroxidation induced by the endogenous oxygen free radicals. The present results showed that aerobic exercise significantly increased SOD activity and inhibited the myocardial MDA level in obese rats. In addition, the response of vascular rings in the thoracic aorta to NA is weakened in the HCE and HIE groups, suggesting that aerobic exercise can improve arterial function and vascular elasticity via its antioxidant effects. This is consistent with one study that showed the improvement of vascular elasticity by reducing oxidative stress (22).

Studies often use animal models of hyperlipidemia induced by HD for prevention research. In order to establish the hyperlipidemia model, SD rats are fed with HD recipes for 8 weeks and HD leads to lipid metabolism disorders involving the increase in serum TC, TG and LDL, as well as the decrease in HDL. These changes are major risk factors for cardiovascular disease, such as arterial injury (10,23,24). A previous study has shown that the high cholesterol-facilitated vasoconstriction response and the vascular reactivity to NA in various studies were different (25). The effects of exercise on cholesterol metabolism have been studied for years, although there were certain differences in the results due to the difference in exercise types, exercise intensity, research objective and the methods (26). A large number of epidemiological and experimental studies found that long-term regular exercise improved their poor lipid structures effectively, so that the risk of cardiovascular disease was significantly reduced (27). The present study also showed that the continuous and intermittent exercises affected the plasma lipid metabolism, decreasing the levels of LDL, TC and TG, while increasing the HDL level, which may be associated with increased lipoprotein lipase activity to release more fatty acids from lipoproteins (28).

A number of studies have been performed to investigate the effects of different intensities of exercise on eNOS in rat cardiomyocytes. Previous studies have shown that eNOS activity of the thoracic aorta was decreased in rats fed with HD and following long-term exercise training, the activity and mRNA expression of eNOS were upregulated (29-31). In addition, the elevation of eNOS gene expression and activity was observed in aortic endothelial cells, coronary blood vessels and cardiac capillaries in dogs that exercised persistently for 10 days. Wang *et al* (32) also found that eNOS activity of the thoracic aorta significantly increased in rats after 90 or 150 min of swimming training. Furthermore, the nitric oxide level in plasma was also significantly increased. The increase of eNOS activity in the 150 min training group was smaller than that in the 90 min training group (33,34). In the present study, eNOS expression was significantly decreased in the HD group. Following the exercise, the expression of eNOS was significantly increased compared to that in the HD group and its increase was higher in the HIE group compared to the HCE group. These data indicate that aerobic training can enhance eNOS activity in myocardial cells, improve endothelial function and also delay the development of atherosclerotic plaques.

In conclusion, continuous and intermittent exercise can improve vasoreactivity of obese rats, which may be associated with improved exercise capacity, enhanced antioxidant enzyme activity and reduced free radicals and lipid peroxides, as well as increased gene expression of eNOS. In addition, the intermittent exercise had a better effect than the continuous exercise, but the detailed mechanisms of the regulation require further study.

Acknowledgements

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