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Intermittent exercise improves the vasoreactivity of the thoracic aorta in rats

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Abstract. Vasoreactivity is the most basic and direct indicator to reflect the artery vascular functional state in the body. The majority of previous studies have shown that a high-fat diet (HD) is often associated with a variety of cardiovascular diseases. However, the type of exercise that improves vasoreactivity, as is induced by a HD, remains to be elucidated. In the present study, the effects of aerobic moderate-intensity intermittent exercise through swimming were investigated on thoracic aorta vascular ring contraction and free radical metabolism using Sprague-Dawley rat models of common diet (CD; 23 g protein, 49 g carbohydrate, 4 g fat, 5 g fiber, 7 g bone meal and 6 g vitamins per 100 g), HD (peanuts, milk chocolate and sweet biscuits, in a weight ratio of 3:2:2:1), CD with intermittent exercise (CIE) and HD with intermittent exercise (HIE). The food utilization rate in the swimming group (CIE) decreased in comparison with the CD group. Lee's index in the CIE group decreased in comparison to that of CD after 8 weeks ($P<0.05$) and also HIE decreased compared to HD ($P<0.05$) after 8 weeks. Compared with the HD group, contractile response of the thoracic aortic rings to NA decreased in the HIE group, while high-density lipoprotein cholesterol content increased, total cholesterol, triglycerides and low-density lipoprotein cholesterol decreased ($P<0.05$); the malondialdehyde (MDA) concentration reduced in the myocardium, but the superoxide dismutase (SOD) level improved ($P<0.05$). In the HIE group, nitric oxide level was similar to the CD group. Compared with CD, contractile response of the thoracic aortic rings to NA increased in the CIE ($P<0.05$), the MDA concentration reduced in the myocardium, but the SOD level improved ($P<0.05$). Tunica media smooth muscle of the thoracic aortic rings in the CIE group arranged more regularly in comparison with the CD group (without swimming training). In conclusion, intermittent exercise improves the thoracic aorta vasoreactivity and function by enhanced antioxidant enzyme activity and reduced free radical generating.

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Introduction

As living standards improve, high-fat diet-induced obesity has become a common phenomenon (1). Obesity is often associated with a variety of diseases, such as atherosclerosis, hyperlipidemia, hypertension, diabetes and cardiovascular diseases (2-10). Vasoreactivity is the most basic and direct indicator of reflecting artery vascular function state, which has become an important indicator of cardiovascular disease (11). Studies have found that a high-fat diet (HD) and lack of exercise training are the most important factors in the development of obesity. Therefore, exercise training can control obesity and cardiovascular disease, and it has become a research focus. However, the exercise intensity, duration and different diets will produce different effects on metabolic activity (12). Long-term aerobic exercise could markedly improve the abnormal hemorheological property and the oxidative stress in rats with hypercholesterolemia (13). Inadequate nutrition may enhance oxidative stress, and intense chronic physical training may activate antioxidant defenses, possibly by hormesis (14). Therefore, aerobic exercise may reverse the effects induced by a poor diet. The majority of present studies focus on aerobic high-intensity intermittent exercise, which can reduce oxidative stress, arterial stiffness and body weight (15-18). While high-intensity intermittent exercises are not suitable for all subjects, studies on moderate-intensity intermittent exercise are performed rarely. The aim of the present study was to explore the mechanism of how the exercise improves cardiovascular function.

Materials and methods

Animals. Male Sprague-Dawley rats, weighing 180-190 g, were provided by the Experimental Animal Center of Ningxia Medical University (Yinchuan, Ningxia, China). The protocol was approved by the institutional animal care and use committee and the local experimental ethics committee. Rats were bred for 8 weeks with a free diet and water *ad libitum* at 32±1˚C and

12-h light/dark cycle. In total, 32 rats were randomly assigned to 4 groups: Common diet (CD), HD, CD with intermittent exercise (CIE) and HD with intermittent exercise (HIE).

Animal model preparation

Diet composition. The CD and CIE groups were fed with the following formula: 23 g protein, 49 g carbohydrate, 4 g fat, 5 g fiber, 7 g bone meal and 6 g vitamins per 100 g. The HD and HIE groups were fed with the following formula: Peanuts, milk chocolate and sweet biscuits, in a weight ratio of 3:2:2:1. The protein content accounted for 20%, fat content for 20%, sugar content for 48% and cellulose content for 4%. Calories in the HD group were 5.12 kcal/g (equal to 35% fat calories), while there were 4.07 kcal/g in the CD group.

Exercise training protocol. The rats in the CD and HD groups were fed at room temperature without swimming training. The rats in the CIE and HIE groups were swimming in a plastic container with a diameter of 60 and 55 cm water depth at 28-32˚C. Rats in the intermittent swimming groups swam for 10, 20 and 30 min on days 1, 2 and 3, respectively, performed 3 times/day. Following this the rats were subjected to swimming for 30 min each time, 3 times/day, maintained at 4 h intervals and the time schedule for swimming was: 7:00 a.m., 11:30 a.m and 3:30 p.m., respectively. All the rats were subjected to swimming exercise with loads of 5% of their body weight attached to their tails. The swimming exercise was performed for 8 weeks, 5 days a week. The exercise intensity was moderate.

Food utilization rate and Lee's index determination. Rats and food consumption were weighed at the last day of every week after 12 h of fasting, and the food utilization rates were calculated from the equation: Food utilization rate = weight gain/food consumption x 100%. The body lengths of the rats were measured from nose to anus, and Lee's index was calculated from the equation: Lee's index = [body weight x 10^3/body length (cm)]$^{1/3}$.

Sample preparation. After 8 weeks of swim training, rats were rested and fasted for 24 h. Following this, the rats received intraperitoneal anesthesia with 20% urethane (0.5 ml/kg), the blood was taken from the heart. Subsequently, the serum was separated and stored at -80˚C for determination. The heart was removed rapidly and the residual blood was removed with saline. Tissue homogenate (10%) was made and the supernatant was stored at -80˚C.

Tension of the aortic vascular rings. The thoracic aorta was isolated and the surrounding connective tissue was removed. Subsequently, the aorta was separated into 3 mm segments for measuring the tension. The vascular rings were suspended in a tissue bath (37˚C) with 10 ml K-H solution containing 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 (pH 7.4), and ventilated with mixed gas of 95% O_2 and 5% CO_2 continuously. The vascular resting tension was adjusted to 1 g. After equilibration for 1 h, K-H solution was replaced once every 15 min, the maximum contraction tension was determined with 60 mM KCl, subsequently the cumulative dose-response curve for noradrenaline (NA) (10^{-10}-10^{-5} M) was examined. The rates of vascular tension range induced by NA (10^{-10}-10^{-5} M) were expressed as percentages of the maximum contraction tension range (100%) induced by KCl (60 mM).

Superoxide dismutase (SOD), malondialdehyde (MDA) and nitric oxide (NO) levels in the myocardium. Oxidative stress parameters were detected to explore whether the intermittent exercises could reduce high-diet induced oxidative stress or improve the common diet. SOD was determined by thiobarbituric acid (TBA) levels in the myocardium, and MDA was measured by the WST-1 method. NO was determined by the NO reductase method.

High-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), triglycerides (TG) and total cholesterol (TC) levels in the serum. The levels of high HDL, low LDL, TG and serum TC were measured by microplate reader and ultraviolet spectrophotometry at 546 nm.

Hematoxylin and eosin (H&E) staining. Thoracic aorta in all 4 groups was fixed in 10% formalin for 12 h, embedded in paraffin and subsequently serial sections were performed. The sections were stained with H&E.

Drugs and reagents. Norepinephrine (also known as NA) was purchased from Shanghai Hefeng Pharmaceutical Co., Ltd. (Shanghai, China). The kits for MDA and SOD and NO assays were purchased from Nanjing Jian Cheng Bioengineering Institute (Nanjing, China); and the TC, TG, HDL and LDL kits were provided by the First Japanese Chemical Company (Tokyo, Japan).

Statistical analysis. All the data are presented as mean ± standard error. Two-way analysis of variance (ANOVA) was used to evaluate any difference between the groups. One-way ANOVA was used to analyze the remaining data. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Effects of aerobic intermittent exercises on food utilization rate. The food utilization rate in the CIE group was lower compared to that of the CD group at all weeks, while it was significantly lower for the weeks 3, 5 and 7 ($P<0.05$). The food utilization rate in the HIE groups was lower compared to that of the HD group, except for weeks 3 and 5, while it was significantly lower for weeks 4, 6 and 7 ($P<0.05$). The food utilization rate in the HD group significantly increased compared to that of the CD group ($P<0.01$) (Table I).

Effects of aerobic intermittent exercises on Lee's index. Lee's index in the HD group significantly increased compared to that of the CD group, except for week 1, while the Lee's index in the CIE group was lower in all weeks, except for week 2, and was significantly lower at weeks 6 and 8. Lee's index in the HIE group was significantly lower compared to that of HD only for the week 8 group ($P<0.05$) (Table II).

Effects of intermittent exercise on thoracic aortic vascular rings contraction. The contractile responses of NA to the thoracic aortic vascular rings in all the groups were

Table I. Food utilization rate in different groups.

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
CD	16±0.21	22±1.50	17±1.20	12±0.59	21±0.39	14±0.96	23±1.25
HD	34±1.34[a]	36±0.58[a]	31±2.34[a]	36±3.34[a]	33±1.58[a]	32±2.64[a]	28±0.26[a]
CIE	14±0.74	16±0.86	14±1.17[b]	10±1.66	13±1.29[a]	12±0.56	13±0.75[a]
HIE	33±1.80	34±2.80	31±1.28	32±1.18[c]	33±1.66	26±1.34[c]	22±1.49[d]

Data are expressed as mean ± standard error (n=8). [a]P<0.01 and [b]P<0.05 vs. CD; [c]P<0.05 and [d]P<0.01 vs. HD. CD, common diet group; HD, high-fat diet group; CIE, common diet with intermittent exercise group; HIE, high-fat diet with intermittent exercise group.

Table II. Lee's index in different group.

Group	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
CD	409.60±31.24	395.58±19.70	425.80±14.59	451.64±11.69	471.07±18.48	491.21±8.54	518.83±14.82	566.50±15.74
HD	421.63±36.40	429.68±29.12[a]	494.64±26.57[a]	539.11±30.93[a]	601.32±26.49[a]	649.91±21.61[a]	698.12±29.50[a]	768.47±27.95[a]
CIE	401.41±23.76	413.10±19.80	419.84±20.35	448.08±13.43	458.63±17.09	476.49±18.51[a]	492.28±16.39	532.25±10.83[b]
HIE	391.43±19.88	436.33±12.08	489.72±15.33	544.06±19.27	607.28±20.11	616.04±16.85	688.06±14.28	706.32±13.83[c]

Data are expressed as mean ± standard error (n=8). [a]P<0.01 and [b]P<0.05 vs. CD; [c]P<0.05 vs. HD. CD, common diet group; HD, high-fat diet group; CIE, common diet with intermittent exercise group; HIE, high-fat diet with intermittent exercise group.

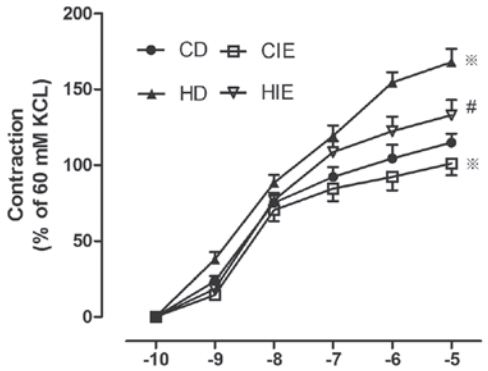

Figure 1. Contractile response of the vascular ring to NA. Dose-dependence of NA on contraction of the thoracic aorta rings separated from rats in the CD, HD, CIE or HIE groups. The contraction induced by 60 mM KCl was set as 100%. Data are expressed as mean ± SE (n=8). *P<0.05 vs. CD; #P<0.05 vs. HD. NA, noradrenaline; CD, common diet group; HD, high-fat diet group; CIE, CD with intermittent exercise group; HIE, HD with intermittent exercise group.

concentration-dependent increased. Compared with the CD group, the contractile response in the HD group was evidently increased (P<0.05), while in the CIE groups it was significantly decreased (P<0.05). The contractile response was markedly suppressed in the HIE group compared with the HD group (P<0.05) (Fig. 1).

Effects of intermittent exercise on myocardial radicals (SOD, MDA and NO). The myocardial MDA level in the HD group was increased significantly compared with the CD group (P<0.05), while in the CIE group it was significantly decreased (P<0.05). The myocardial MDA level in the HIE group was reduced significantly compared with the HD group, and was even lower compared to the CD group. The SOD level in the CIE group was increased compared with the CD group, but decreased significantly in the HD group (P<0.05). Compared with the HD group, the SOD level in the HIE group was increased significantly, similar to the level in the CD group. The NO level in the CIE group was higher, but not significantly different, compared to the CD group, and decreased significantly in the HD group (P<0.05). However, compared with the HD group, the NO levels increased without a significant difference, similar to the CD group (Fig. 2).

Table III. Intermittent exercise effects on blood lipid metabolism.

Group	TG, mmol/l	TC, mmol/l	HDL, mmol/l	LDL, mmol/l
CD	1.43±0.36	1.61±0.24	0.85±0.17	0.33±0.10
HD	1.57±0.87	7.95±1.17[a]	0.78±0.17	6.45±1.64[a]
CIE	1.26±0.30	1.12±0.16	0.98±0.11	0.40±0.02
HIE	0.40±0.18[b]	2.75±1.09[b]	1.04±0.08[b]	2.00±0.82[b]

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

Effects of intermittent exercise on blood lipid metabolism. Intermittent exercise caused changes in the blood lipid of rats. Compared with the CD group, TC and LDL levels in HD group were higher (P<0.05), but the TG level was higher without a statistical significance, and HDL level was lower without statistical significance. TG and TC levels in CIE were lower than that of the CD group (without statistical significance), but the LDL and HDL levels were higher compared to that of the CD group (without statistical significance). Compared with the HD group, the TG, TC and LDL levels in the HIE group were

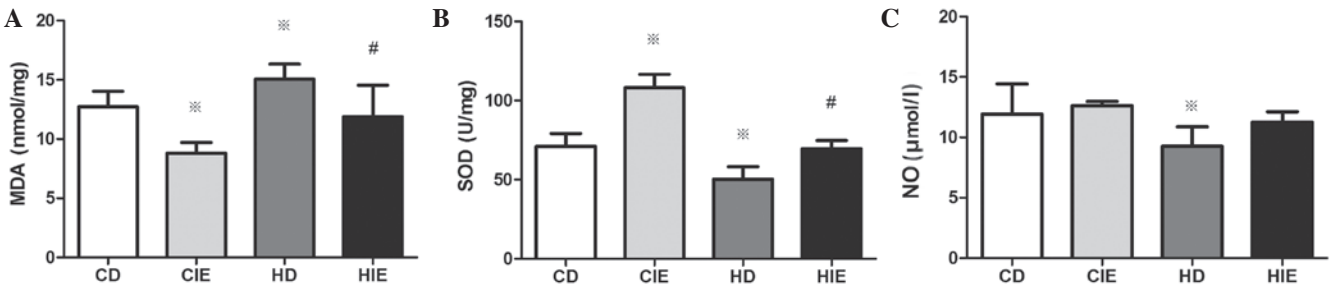

Figure 2. Effects of intermittent exercise on (A) MDA, (B) SOD and (C) NO in rats. Data are expressed as mean ± standard error (n=8). *P<0.05 vs. CD; #P<0.05 vs. HD. MDA, malondialdehyde; SOD, superoxide dismutase; NO, nitric oxide; CD, common diet group; HD, high-fat diet group; CIE, CD with intermittent exercise group; HIE, HD with intermittent exercise group.

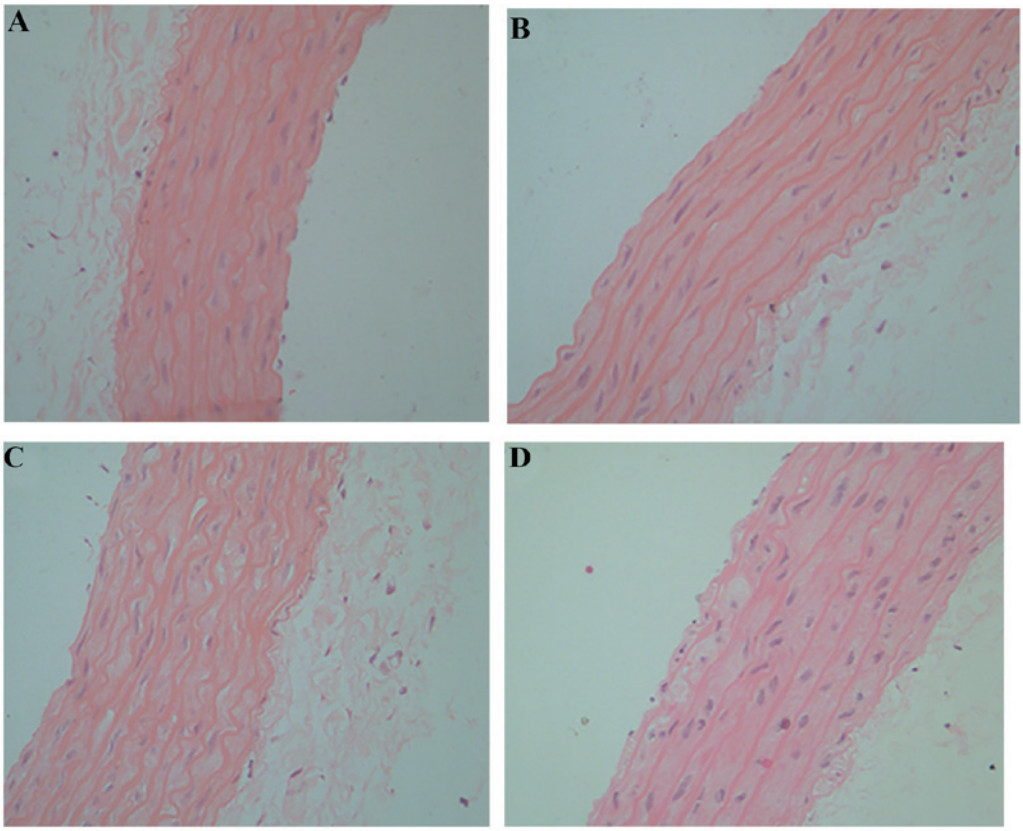

Figure 3. Effects of intermittent exercise on the thoracic aorta. After 8 weeks, rats were sacrificed and the thoracic aorta was sectioned and stained with H&E (magnification, x200). Representative pathology from one rat per group. Thoracic aorta sections from the (A) CD, (B) CIE, (C) HD and (D) HIE groups. CD, common diet group; HD, high-fat diet group; CIE, CD with intermittent exercise group; HIE, HD with intermittent exercise group; H&E, hematoxylin and eosin.

significantly decreased (P<0.05), while the HDL level was increased (P<0.05) (Table III).

Effects of intermittent exercise on the thoracic aorta. Compared with the CD group, tunica media smooth muscle and endomembrane cells in the CIE group were arranged more regularly, while the membrane smooth muscle was arranged more regularly and atherosclerosis evidently improved in the HIE group, compared with the HD group (Fig. 3).

Discussion

The results of the present study demonstrated a significant increase in the food utilization rate of Sprague-Dawley rats fed a HD, and this effect could be suppressed by intermittent exercise; while it was increased in the common diet, the intermittent exercise reversed the effect. These studies were consistent with the studies of Ebal *et al* (19) and Elj *et al* (20). Haghshenas *et al* (18) reported that aerobic exercise could improve the incidence of obesity. Lee's index is generally believed to only apply to the obesity model of neonatal rats induced by monosodium glutamate, an index reflecting obese degree in rats. In week 8, this index was increased significantly in the HD group, and rats were with evidently of a different size (short and stout), while intermittent exercise mitigated the effects. In the CD, its counterpart exercise had the same results. The food utilization rate and body weight were decreased, induced by changing the composition of the fat content and

increased energy expenditure. Together, these two parameters were observed in the exercised rats and indicated that the intermittent exercise groups achieved a good level of fitness.

Obesity is a significant risk factor for cardiovascular events (2,3,6-8), it not only causes aortic injury in rats fed with a HD, but it also lowers the antioxidant enzyme activity in the body and leads to lipid metabolism disorder (21-23).

Vasoreactivity refers to the occurrence of acute changes in vascular function, and increased vasoreactivity is an important mechanism for the development of cardiovascular diseases, as it is also a prognostic indicator of arterial health (24). Damage to aortic function mainly shows as enhanced vasoconstriction and decreased diastolic function, and this experiment was determined by the effects of thoracic aortic rings to NA in order to reflect artery systolic function. On the dual factors of HD and aerobic exercise condition, vasoreactivity can be decreased due to the sympathetic nervous excitement *in vivo* (25,26). The experimental results demonstrated the following: Compared with the CD group, the HD group increased the contractile response of the aorta, and the CIE group reduced the contractile response; while HIE attenuated the NA-induced contractile response in comparison with HD, suggesting that aerobic intermittent exercise can improve the function of arteries to a certain extent. NA is a strong vasoconstrictor promoting smooth muscle cell proliferation. It is important in the development of atherosclerosis, hypertension and other diseases. The endovascular NA level was increased in vascular diseases, such as atherosclerosis and hypertension, and vasoreactivity to NA was enhanced. While aerobic exercise could reduce the reactivity and peripheral vascular resistance, it is important to protect the body and adapt to the environment. Therefore intermittent exercise can improve vasoreactivity and improve vascular elasticity.

Studies have shown that a HD can improve the content of endogenous oxygen free radical, and enhance the lipid peroxidation. A HD is harmful to the cell membrane system (13,22,27). MDA is an oxidative product of reactive oxygen species against polyunsaturated fatty acids on the biological membrane and the last product of lipid breakdown attributed to oxidative stress (28). It can affect the key enzyme activity of mitochondria *in vitro*, thereby affecting mitochondrial function. Its content reflects the degrees of lipid peroxidation and body cells attacked by free radicals, and it can be used as an indicator of cell membrane injury (29). Compared with the CD group, the MDA concentration was increased significantly in the HD group, and this result was consistent with Jia *et al* (13). It also indicated the high level of oxidative stress in these rats. However, in the CIE group the MDA concentration was reduced further and the peroxidation of lipids was reduced. In the HIE group, the MDA level decreased significantly to the control level, indicating there was less oxidative stress in these rats and indicating that the damage to membrane system was reduced.

SOD is an important antioxidant enzyme and it can remove superoxide anion radicals specifically to protect cells. Its activity may indirectly reflect the ability of the body to clear oxygen free radicals. The effects of exercise on SOD activity were not consistent, as different types, intensity and duration of exercise may have different roles. Aerobic exercise can improve SOD activity, and enhance the ability of the body against superoxide anion (30). Aerobic exercise increases the oxygen consumption and generation of free radicals in the body, resulting in elevated intracellular antioxidant enzymes levels produce acute adaptive changes (31). Long-term aerobic exercise will cause the antioxidant levels in the body to produce chronic adaptive changes. The experiment found that in the HD group the SOD activity decreased indicating that HD enhanced the lipid peroxidation; in the HIE group the SOD activity was significantly increased, suggesting that intermittent exercise could improve antioxidant enzyme activity and reduce lipid peroxidation in HD rats. When compared with the CD, intermittent exercise significantly improved the SOD level.

Endothelium-dependent dilation is enhanced following exercise training (15,32). NO produced by endothelial NO synthase promoted endothelium-dependent dilation. Exercise-induced improvement in vessel relaxation is mainly mediated by NO. During oxidative stress, superoxide (O_2^-) reduced the function of the endothelial NO synthase and decreased the half-life of NO by improving the production of peroxynitrite from O_2^- and NO. While in the normal state, O_2^- is quenched by SOD. In the HIE groups in the present study, the level of NO was increased, similar to the CD group, suggesting that intermittent exercise improved endothelial function and its mechanism may increase the bioavailability of NO. In the CIE group, an increased content of SOD could reduce O_2^- levels, thereby, the NO level was increased.

In the present study, Sprague-Dawley rats were fed with HD feed formulation in order to establish the HD model. The HD lead to changes in lipid metabolism, including increased levels of serum TC, TG and LDL, and a decreased HDL level, which is a major risk factor for cardiovascular disease such as arterial injury (21). Effects of aerobic exercise on high cholesterol metabolism have been investigated for numerous years, although they have certain differences in the results due to the different sport modes, intensity, research subjects and methods (33), a large number of clinical studies have found that long-term regular aerobic exercise can effectively improve the poor structure so that the risk of cardiovascular disease is significantly reduced (34). Simultaneously, aerobic exercise can improve lipid metabolism in the CD, particularly intermittent exercises. The experimental results showed that in the HD group, improved blood lipids lead to the decreased levels of LDL, TC and TG, while the HDL level was increased, which may be associated with the increased lipoprotein lipase activity, so that the lipoprotein releases more fatty acids (35). In the CIE group, blood lipids were also improved, which reduced the LDL, TC and TG levels, suggesting that intermittent exercise could improve lipid metabolism in the two diets. Contraction tension of thoracic aortic rings to NA was weakened, indicating that intermittent exercise improved arterial function and blood vessel elasticity in the two diets through increased liquid metabolism. Artery H&E staining suggested that cells were arranged more regularly in the medial smooth muscle in the CIE group, and vascular function was further improved. Compared with the HD group, the medial smooth muscle was arranged more regularly in the HIE group, reversing the atherosclerosis effects.

In conclusion, the present study showed that intermittent exercise could improve vasoreactivity in rats fed with a HD or CD, and the mechanism may be associated with enhanced antioxidant enzyme activity, reduced the quantity of free radicals

and the damage of lipid peroxidation, and furthermore, the medial smooth muscle was arranged more regularly. However, the detailed mechanism of its regulation requires further study. In addition, intermittent exercise could decrease the food utilization rate, improve antioxidant enzyme activity and decrease the damage of lipid peroxidation. Therefore, intermittent exercise should be encouraged in subjects consuming a HD or CD.

Acknowledgements

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