Original Article

Low-Intensity Voluntary Running Lowers Blood Pressure with Simultaneous Improvement in Endothelium-Dependent Vasodilatation and Insulin Sensitivity in Aged Spontaneously Hypertensive Rats

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Our objective is to examine the effects of voluntary running at different intensity levels on blood pressure, endothelium-dependent vessel dysfunction and insulin resistance in aged spontaneously hypertensive rats (SHR) with severe hypertension. Ten-month-old male and female SHR with severe hypertension were assigned to voluntary running at either low intensity (30% of maximal aerobic velocity) or moderate intensity (60% of maximal aerobic velocity) on a motor-driven treadmill for 6 weeks, 20 min per day and 7 days per week. Age-matched Wistar-Kyoto rats and SHR were kept under sedentary conditions as controls. Blood pressure and heart rate were measured by the tail-cuff method. At the end of the exercise training, blood samples were collected for glucose, insulin and lipids assay, and aortae were isolated to examine their function *in vitro*. Low-intensity but not moderate-intensity running significantly lowered blood pressure in both male and female SHR ($p<0.01$). There was significant impairment in acetylcholine-induced vasorelaxation in SHR ($p<0.01$), which was improved by low-intensity training ($p<0.05$). Nitric oxide synthase blockade abrogated the improvement in endothelium-dependent relaxation. Hypertensive rats had elevated blood glucose and insulin levels with lowered insulin sensitivity that was ameliorated by low-intensity running. A significant increase in blood high-density lipoprotein (HDL)-cholesterol and a significant decrease in triglycerides were found in exercised SHR. In conclusion, low-intensity voluntary exercise lowers hypertension in aged SHR with severe hypertension. Exercise-induced simultaneous improvement in endothelium-dependent vessel relaxation and insulin sensitivity may act concomitantly in attenuating cardiovascular risk factors in aged hypertensive rats with significantly high blood pressure. (*Hypertens Res* 2008; 31: 543–552)

***Key Words*: spontaneously hypertensive rat, low intensity exercise, vasodilatation, nitric oxide, insulin sensitivity**

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Introduction

Hypertensive humans and animals exhibit impaired endothelium-dependent vasorelaxation, and endothelial nitric oxide synthase (eNOS) dysfunction has been suggested to play a role in this impairment (*1–4*). In addition, hypertension is frequently accompanied by insulin resistance and dyslipidemia that occur together as in metabolic syndrome and that have been implicated in the pathogenesis of hypertension and its cardiovascular complications (*5–8*).

Endurance exercise training has been proved to enhance up-regulation of eNOS, thereby contributing to increase of peripheral systemic endothelium-dependent vasorelaxation and higher blood flow (*9–11*). While some studies found that aerobic exercise promoted blood pressure–lowering in hypertensive rats (*12–14*), others found that it did not (*15–17*). Graham and Rush found that exercise training in spontaneously hypertensive rats (SHR) beginning at 11 week of age improved endothelium-dependent vasorelaxation but did not ameliorate hypertension (*15*). The mechanisms responsible for the differential effects of exercise at different intensities on blood pressure and vessel function remain controversial. However, the relatively high intensity of exercise employed in previous studies showing no reduction of blood pressure in SHR with established hypertension may increase sympathetic nervous activity that offsets the beneficial effect of exercise on vessels (*15–18*).

To date, it has not been clarified whether low- and moderate-intensity exercise would have differential effects on endothelium-dependent vessel function and associated metabolic state in aged hypertensive rats with severe hypertension, in addition to a blood pressure–lowering effect. Therefore, we designed this study to test the hypothesis that voluntary running at low and moderate intensity would have different impacts on blood pressure in 10-month-old SHR with severe hypertension. We also examined whether different intensity levels of exercise could improve insulin sensitivity and simultaneously attenuate endothelium-dependent vasorelaxation impairment in aged hypertensive rats.

Methods

Animals and Exercise Training

Male and female spontaneously hypertensive rats and Wistar-Kyoto rats (WKY) at 10 months of age were obtained from Shanghai Laboratory Animal Center, Chinese Academy of Science and housed at three animals per cage in a temperature-controlled room with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum. The methods employed in this study were approved by the Shanghai Jiao Tong University Institutional Animal Care and Use Committee and all experimental procedures were performed under protocols approved by the Animal Care Committee of the Animal Center at the Chinese Academy of Sciences in Shanghai.

Sixteen male and sixteen female SHR were assigned to voluntary running of either low intensity or moderate intensity on a motor-driven treadmill (5° incline) continuously for 6 weeks, 7 days per week and 20 min per day. Training intensity was set at about 30% of maximal aerobic velocity (MAV) for the rats running at low intensity (9–10 m/min) (running about 150–200 m/day); for rats running at moderate intensity, the same initial setting was used, but the intensity was gradually increased to 60% of MAV within 1 week, then maintained at this level for the remainder of the exercise period (18–20 m/min) (running about 450–500 m/day). The MAV was evaluated for each rat according to the method reported by Reboul *et al*. (*19*) with slight modification. Briefly, after 1 week of acclimatization to treadmill running, rats were allowed to run on a treadmill at a beginning speed of 10 m/min for 30 s, after which the speed was increased by 4 m/min every 30 s until 85–90% of the expected MAV was reached. The speed was then increased by 2 m/min every 30 s. The highest velocity obtained before the animal was unable or unwilling to continue was used as the MAV. The whole evaluation process took about 4 to 5 min. The rats were kept to the fixed percentage of this MAV at the beginning of the exercise protocol to maintain a constant level of exercise intensity throughout the experiment in each group. Eight age-matched WKY and eight SHR of each sex were kept under sedentary conditions as controls and handled 7 days per week in the same manner as the rats in the exercise groups, except that they were not permitted regular running. Blood pressure and heart rate were measured in a conscious state before exercise and every 10 days after the beginning of exercise by the tail-cuff method. Training and pressure measurement were managed carefully to avoid extra stimulation of the rats. At the end of the exercise period, a blood sample was collected for insulin and glucose assay at the time of sacrifice.

Assessment of Vasomotor Function *In Vitro*

Vascular responses were determined 12 to 24 h after the last exercise bout. The method was modified slightly from our previous description (*20*). Briefly, after anesthesia, the thoracic aorta was removed carefully, cleaned of fat and adherent connective tissues, cut into segments of 2 to 3 mm in length, and mounted on two stainless-steel stirrups in a 10-mL organ chamber with Krebs'-Henseleit buffer (KHB) containing (in mmol/L): NaCl 118.0, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25.0, glucose 11.0, and Na_2-EDTA 0.5, at 37°C. One stirrup was connected to an isometric force transducer for tension measurement. The rings were stretched to a resting tension of 2.0 g and allowed to equilibrate for 50 min, during which the bath solution was changed every 10 min. The contraction response was evaluated twice with 60 mmol/L of KCl obtained by substituting an equimolar amount of KCl for NaCl in KHB and then 1 μmol/L of phenylephrine.

Table 1. Basic Data of Wistar-Kyoto Rats (WKY) and Spontaneously Hypertensive Rats (SHR) and Average Exercise Intensity of Exercise (Ex) Training with Low- or Moderate-Intensity

	WKY	SHR		
	Sedentary	Sedentary	Ex-low	Ex-moderate
Male				
Systolic blood pressure, mmHg	145±22	249±27**	254±28**	252±26**
Heart rate, beat/min	400±48	410±59	409±78	412±79
Body weight, g	526±68	377±64**	381±79**	386±72**
Running distance, m/day	—	—	212±32	473±82††
Running duration, min/day	—	—	20±16	20±17
Female				
Systolic blood pressure, mmHg	127±12	240±19**	239±22**	238±17**
Heart rate, beat/min	403±51	420±56	419±53	415±49
Body weight, g	489±58	341±70**	339±76**	343±81**
Running distance, m/day	—	—	205±32	465±62††
Running duration, min/day	—	—	21±14	20±16

**$p<0.01$ *vs*. WKY sedentary, ††$p<0.01$ *vs*. SHR sedentary.

Endothelium-dependent and non–endothelium-dependent vessel relaxation were assessed by acetylcholine (ACh) and sodium nitroprusside (SNP) when the contractile plateau was reached after phenylephrine. To investigate possible mechanisms responsible for exercise-induced relaxation, the rings were incubated with the nitric oxide synthase (NOS) inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME; 10 μmol/L, 20 min) before phenylephrine and acetylcholine administration.

Biochemical Determinations

Blood samples were collected after 6-week exercise training before the *in vitro* study and plasma glucose and lipids were measured automatically using a Hitachi biochemistry analyzer (Tokyo, Japan). Serum insulin was determined with a rat insulin RIA kit (Linco Research, St. Charles, USA). Insulin sensitivity was assessed using the quantitative insulin-sensitivity check index (1/log(insulin)+log(glucose)) as reported previously (*6*).

Statistical Analysis

Data are presented as the means±SD. One way ANOVA was used to determine differences among groups followed by Student-Newman-Keuls post hoc analysis, and repeated measures ANOVA was used for concentration-response relations of vessel activity. Values of $p<0.05$ were considered to indicate statistical significance.

Results

General Activity after Treadmill Exercise

All exercise trainings were carried out during the afternoon. While SHR with moderate-intensity exercise rested calmly after 20 min of treadmill running, rats with low-intensity exercise generally chased and played with each other for about 5 to 10 min after running. There were no changes in water or food intake after exercise training. One male SHR in the moderate-intensity group died at night after 1-week of treadmill running. There were no deaths in female SHR subjected to exercise.

Basic Data of SHR

SHR at 10 months of age showed severe hypertension (Table 1, $p<0.01$). Table 1 also shows the mean daily exercise intensity and duration in the low- or moderate-intensity groups of SHR. SHR had significantly lower body weight than age-matched control WKY, and this relation was not changed by exercise training. Hypertensive rats of both sexes had significantly higher blood glucose and insulin level with lower insulin sensitivity (Fig. 1), indicating insulin resistance with well established severe hypertension.

Blood Pressure and Metabolic Parameters after Exercise Training

Hypertensive rats of both sexes showed increased blood glucose and insulin with lower insulin sensitivity, which was attenuated by exercise training with a more prominent effect in the low-intensity group (Fig. 1). Both low- and moderate-intensity training improved the lipid profile by increasing high-density lipoprotein (HDL)-cholesterol and lowering triglycerides (Table 2). While moderate-intensity training did not have a marked influence on blood pressure, low-intensity training significantly attenuated hypertension in both male and female hypertensive rats (Fig. 2A, $p<0.05$), with no significant changes in resting heart rate (Fig. 2B).

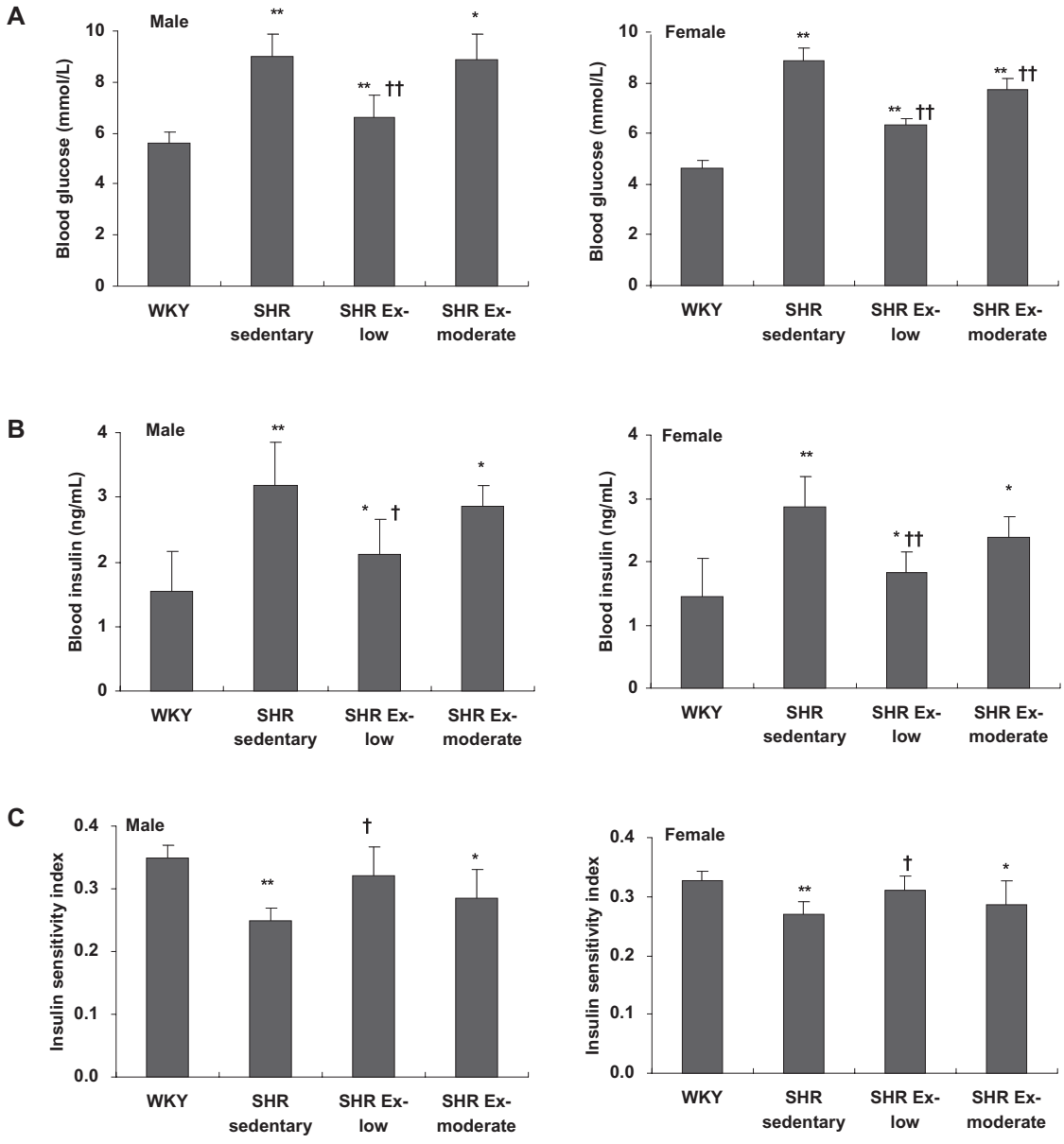

Fig. 1. *Changes in blood glucose (A), insulin (B) and insulin sensitivity index (C) after low- or moderate-intensity exercise training in spontaneously hypertensive rats (SHR) and age-matched sedentary Wistar-Kyoto rats (WKY). *$p<0.05$, **$p<0.01$ vs. WKY sedentary, †$p<0.05$, ††$p<0.01$ vs. SHR sedentary.*

Aortic Vessel Relaxation in Aged SHR with and without Exercise Training

As shown in Fig. 3, endothelium-dependent vessel relaxation was significantly blunted in aged SHR as compared with age-matched WKY ($p<0.01$). While low-intensity exercise ameliorated endothelium-dependent relaxation impairment (Figs. 3A and 4A), moderate-intensity exercise showed no significant benefit on vasorelaxation (Figs. 3A and 4A). The amelioration was abrogated by the NOS blockade with L-NAME (Fig. 4B). Non–endothelium-dependent relaxation was also decreased slightly in aged SHR of either sex (decreased SNP-induced relaxation, $p<0.05$ at 3 pmol/L to 0.1 μmol/L of SNP) and was not affected by exercise training (Fig. 3B).

Aortic Vessel Contraction to PE in Aged SHR

Aortic contraction to the α-receptor agonist PE was significantly higher in aged SHR than WKY (Fig. 5), and the difference was diminished by blockade of NOS with L-NAME, indicating that an impairment of nitric oxide formation degraded the vessel function of 10-month-old SHR. Although

Table 2. Lipids of Wistar-Kyoto Rats (WKY) and Spontaneously Hypertensive Rats (SHR) Underwent Low- and Moderate-Intensity Exercise (Ex) Training

	WKY	SHR		
	Sedentary	Sedentary	Ex-low	Ex-moderate
Male				
Total cholesterol, mmol/L	2.07±0.22	2.08±0.19	2.25±0.09*,†	2.24±0.13*,†
LDL-cholesterol, mmol/L	0.53±0.09	0.49±0.17	0.52±0.03	0.47±0.11
HDL-cholesterol, mmol/L	1.12±0.20	1.05±0.10	1.54±0.07**,††	1.41±0.09**,††
Triglyceride, mmol/L	1.17±0.23	1.16±0.21	0.78±0.09**,††	0.80±0.08**,†
Female				
Total cholesterol, mmol/L	2.01±0.30	1.98±0.17	2.37±0.11*,†	2.34±0.18*,†
LDL-cholesterol, mmol/L	0.46±0.08	0.43±0.10	0.47±0.03	0.46±0.17
HDL-cholesterol, mmol/L	1.25±0.22	1.14±0.09	1.70±0.08**,††	1.71±0.16**,††
Triglyceride, mmol/L	1.09±0.21	1.06±0.20	0.71±0.12**,††	0.79±0.13**,†

*$p<0.05$, **$p<0.01$ *vs*. WKY sedentary, †$p<0.05$, ††$p<0.01$ *vs*. SHR sedentary. LDL, low-density lipoprotein; HDL, high-density lipoprotein.

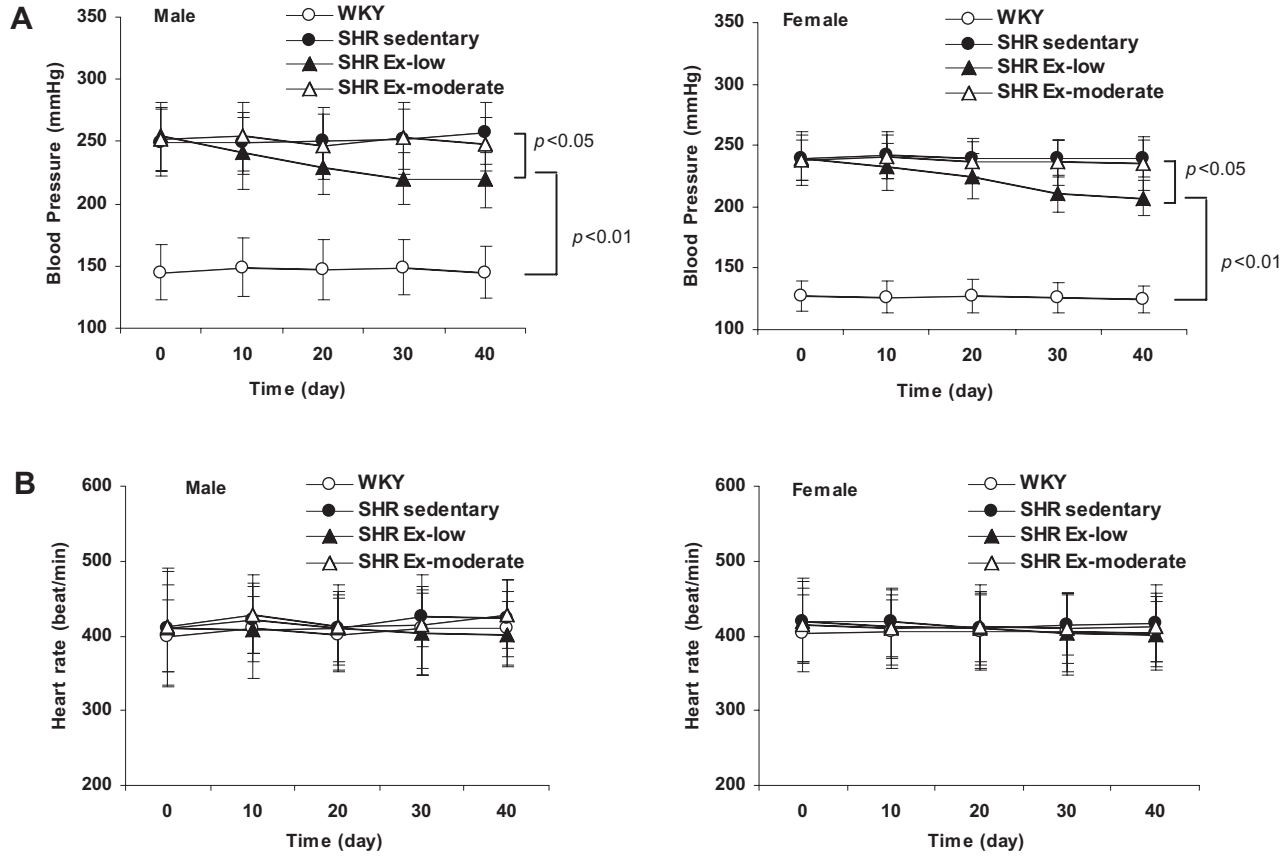

Fig. 2. *Changes in blood pressure (A) and heart rate (B) after low- or moderate-intensity exercise training in spontaneously hypertensive rats (SHR) and age-matched sedentary Wistar-Kyoto rats (WKY). Differences between the blood pressure of the SHR and WKY groups were statistically significant at both 30 and 40 days ($p<0.05$).*

both the vessels from SHR with low-intensity and those from SHR with moderate-intensity exercise showed a significant increase in responses to phenylephrine as compared to the control WKY aorta, L-NANE–induced enhancement of the contraction was only observed in the vessels from SHR with low-intensity exercise (Fig. 5).

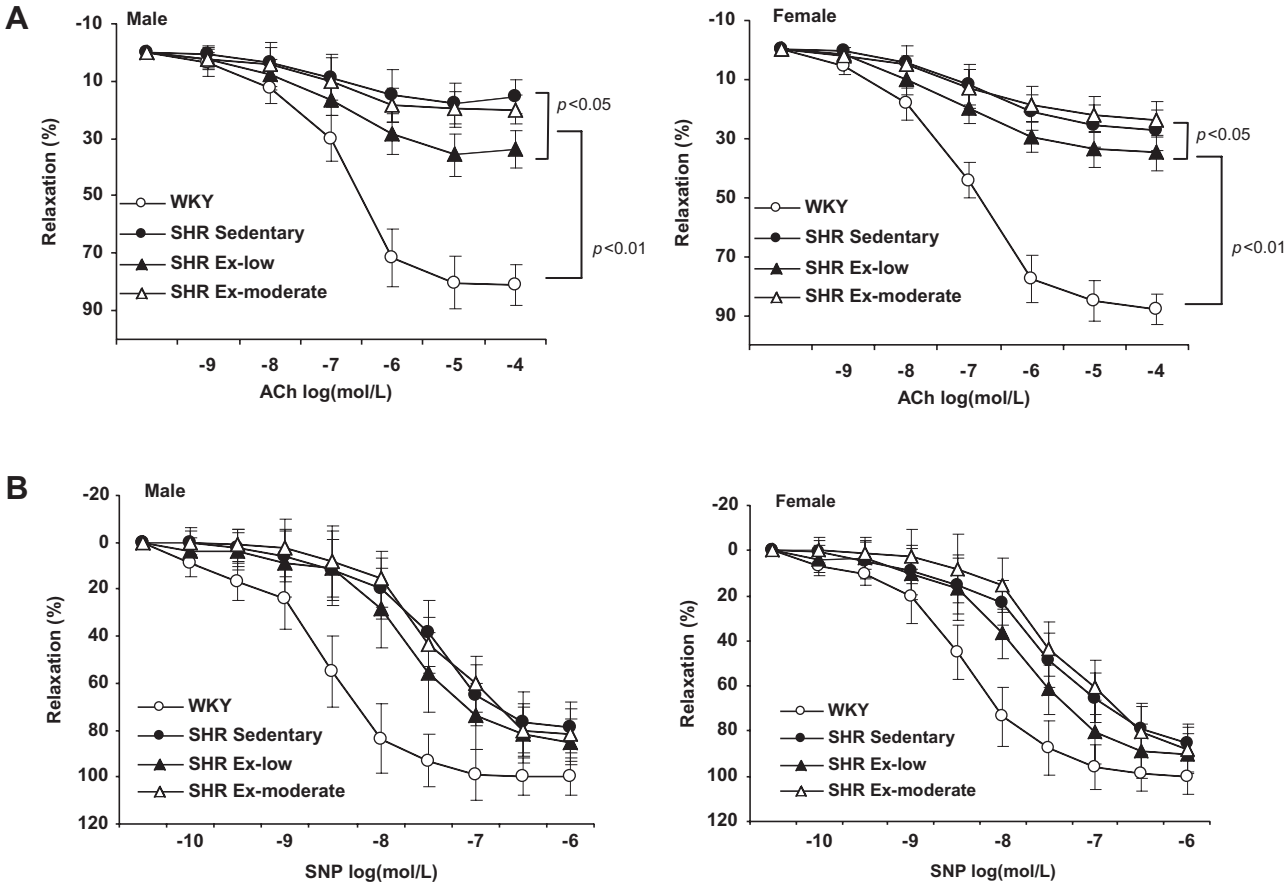

Fig. 3. *Endothelium-dependent (A) and non–endothelium-dependent (B) relaxation after exercise with different training intensity levels in spontaneously hypertensive rats (SHR) and age-matched sedentary Wistar-Kyoto rats (WKY). A: Dose-dependent relaxation to acetylcholine (ACh). B: Dose-dependent relaxation in response to sodium nitroprusside (SNP). Relaxation induced by SNP at concentration of 3 pmol/L to 0.1 μmol/L in SHR group was reduced statistically significant at $p < 0.05$ as compared with that in WKY group.*

Discussion

The main findings of the present investigation are that exercise training of an intensity as low as 30% of maximal aerobic velocity for 20 min/day for 6 weeks attenuated severe hypertension and the impairment of endothelium-dependent vessel relaxation in aged male and female SHR. The improvement of vessel relaxation was abrogated by NOS blockade. It is also interesting that moderate-intensity exercise (60% of maximal aerobic velocity) showed no significant effect on blood pressure and endothelium-dependent vessel relaxation. Hypertensive rats with exercise training also showed reduced hyperglycemia and hyperinsulinemia with improved insulin sensitivity. A significant exercise-induced increase in HDL-cholesterol was evident as well.

Although there is little doubt about the beneficial effects of exercise training on essential hypertension, it remains uncertain what level of exercise intensity is required to achieve an antihypertensive effect. In clinical settings, there is evidence that a higher level of physical activity or fitness is associated with a lower incidence of hypertension (*21*). However, studies reporting the effectiveness of aerobic exercise on blood pressure lowering in hypertension have mainly employed low to moderate exercise intensity (*13*, *14*, *22*), and some studies that did not show an antihypertensive effect used a relatively long duration of daily moderate exercise, or high intensity exercise (*15–17*). In agreement with previous studies, the present data confirmed that continuous, low-intensity exercise is effective in attenuating hypertension in SHR with well established severe hypertension. The novelty of the present study is the finding that low- rather than moderate-intensity exercise training conferred beneficial effects on blood pressure and vessel function in aged animals with severe hypertension, with concomitant improvement in vessel function and metabolic state.

The exercise intensity and duration employed in the present study were chosen based on the willingness of individual

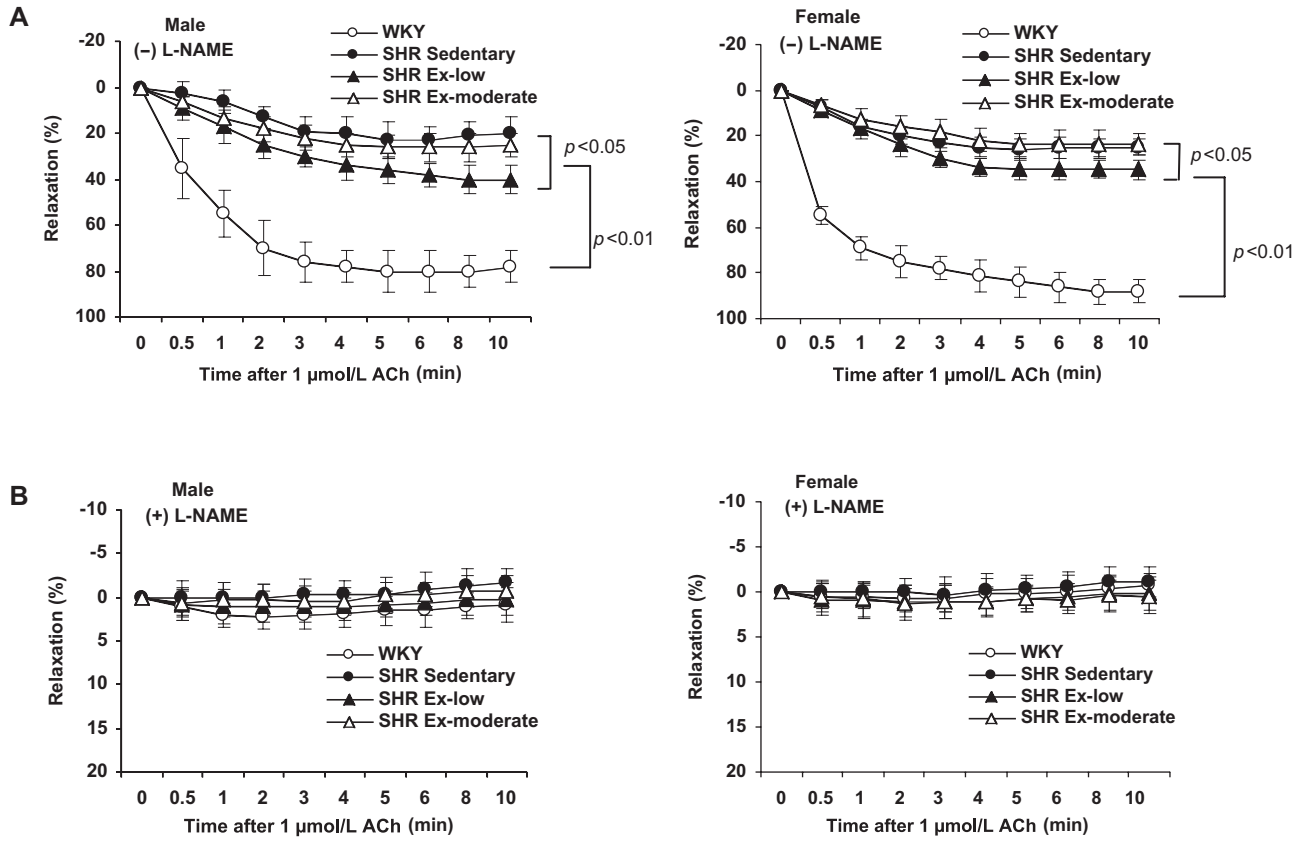

Fig. 4. *Endothelium-dependent vessel relaxation with (A) and without (B) nitric oxide synthase blockade by N^{ω}-nitro-L-arginine methyl ester (L-NAME, 10 μmol/L, 20 min) after exercise with different training intensity levels in spontaneously hypertensive rats (SHR) and age-matched sedentary Wistar-Kyoto rats (WKY).*

hypertensive rats to run during the exercise training, in order to ensure that the animals would exercise on their own will. The general observation of rat activity also showed that SHR with low-intensity exercise generally chased and played with each other for about 5 to 10 min after running, while those with moderate-intensity exercise rested calmly after 20 min of treadmill running. This phenomenon indicated that healthy emotional changes after mild exercise could help to reduce blood pressure in SHR after exercise with low-intensity running. And moderate exercise, although shown to be helpful for blood pressure–lowering in relatively young subjects, may increase blood pressure to an even more severe level in severely hypertensive rats during exercise, which could be detrimental to the already activated sympathetic nervous system, resulting in accelerated systemic overload and initiating a vicious cycle. However, additional experiments designed to investigate the relevant emotional effects will be needed before reaching a conclusion. Based on the results from hypertensive animals, it could be inferred that it is crucial to set an appropriate exercise intensity to obtain beneficial effects in severe hypertension.

There is evidence showing that exercise could induce reduction in cardiac output, decrease in peripheral resistance and reduced blood volume, which in turn, attenuate high blood pressure (*12–15*, *21*, *23*, *24*). Here we demonstrated that low intensity exercise resulted in reduced blood pressure in 11- to 12-month-old SHR, along with improved endothelium-dependent and NO-related vessel relaxation, indicating decreased peripheral resistance that may contribute to the exercise-induced antihypertension effect in aged hypertensive rats. This result is in agreement with the finding by Spier *et al*. (*25*) that training ameliorated ageing-induced reduction in endothelium-dependent vasodilatation in the soleus muscle arteries. The contraction response to submaximal phenylephrine was increased after NO blockade in SHR with low-intensity exercise. This also suggested that an improvement of the endothelial-dependent vessel relaxation through NO pathway was involved in enhanced vasodilatation response in low-intensity trained SHR. Therefore, when the NO pathway was blocked with L-NAME, the vessel contraction force in SHR receiving low-intensity exercise was greater than without L-NAME. The decrease of SNP-induced relaxation in SHR may be the result of smooth muscle impairment due to long-term severe hypertension. This decrease was not ameliorated by

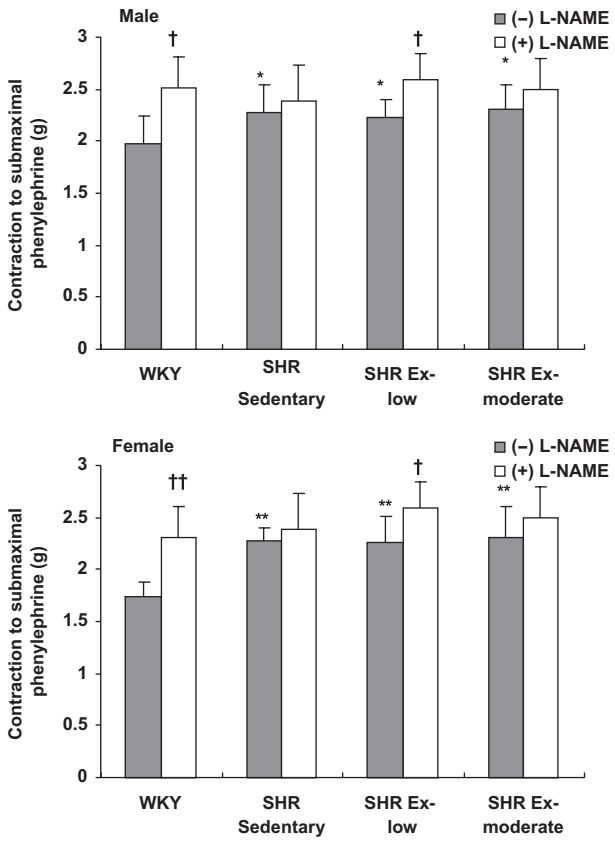

Fig. 5. *Contraction responses to submaximal phenylephrine (1 μmol/L) with and without inhibition of nitric oxide synthase after exercise with different intensity levels in male and female spontaneously hypertensive rats (SHR) and age-matched sedentary Wistar-Kyoto rats (WKY). L-NAME, N^{ω}-nitro-L-arginine methyl ester. *$p < 0.05$, **$p < 0.01$ vs. WKY sedentary, $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs. respective (–) L-NAME.*

exercise, indicating that an irreversible injury of smooth muscle already existed before exercise training.

In the study by Veras-Silva *et al*. (*13*), low- or moderate-intensity exercise for 18 weeks in SHR decreased blood pressure and cardiac output but not total peripheral vascular resistance. Further, Graham and Rush (*15*) found that exercise training for 6 weeks improved endothelium-dependent vasorelaxation with no changes in blood pressure in SHR around 4 to 5 months of age.

It is of note that the exercise intensity employed by the above-mentioned researchers, who found no consistency in blood pressure–lowering and vasorelaxation effects, was higher (55% to 70% of the animals' maximal oxygen uptake) (*13*, *15–17*) than in the present study (30% of the maximal aerobic velocity), and the duration of the daily exercise was also longer than in the present study (45 to 60 min *vs*. 20 min per day). In an experiment on female SHR, Renna *et al*. (*16*) reported that exercise resulted in no change in blood pressure despite a decrease in heart rate. They trained female SHR with a protocol of 20–25 m/min, 60 min per day. The reason for the lack of a blood pressure–reducing effect was not clear, but the relatively higher intensity employed in their experiment may have played a role. Tipton *et al*. (*26*) also found that a relatively low intensity of about 40–60% of maximal oxygen consumption lowered blood pressure in aged SHR. Therefore, it seems that pleasant and voluntary running may be of potential importance in attenuating high blood pressure in hypertensive rats. It is considered that a decrease in sympathetic tone to the heart and a decrease in resting bradycardia play an important role in the beneficial effects of low intensity exercise on hypertension (*27*, *28*), whereas high-intensity training can make animals exposed to a high sympathetic drive during exercise that could last longer after exercise bout. And sympathetic over-activation can offset beneficial effects of exercise training on vessels seen in hypertensive subjects and animals (*29*).

Although moderate-intensity aerobic exercise training (about 50% maximum oxygen uptake, 30 min per day and daily as much as possible) is recommended for patients with mild-to-moderate hypertension (*30*), the compliance with a regimen of such intensity would be low for old patients with severe hypertension and concomitant cardiovascular problems. In addition, increase in the blood pressure is more notable during exercise of enhanced intensity, and the same exercise may become anaerobic when its intensity increases. It is also reported that in previously sedentary hypertensive subjects, the exercise volume required to reduce blood pressure may be relatively small (*31*). Therefore, previous studies and the present results in relatively aged SHR with severe hypertension suggest that mild exercise may be optional for elderly patients with severe hypertension.

In addition to confirming the blood pressure–lowering effect of exercise training, the present study showed that hyperglycemia and hyperinsulinemia were ameliorated in SHR by exercise at an intensity of as low as 30% of MAV (10 m/min) for 20 min per day. It is well known that, in addition to realizing a hypotension effect with reduced renal and skeletal sympathetic activity, exercise helps to improve insulin sensitivity and other risk factors (*21*, *22*, *29–34*). However, some studies have shown that exercise did not reduce circulating insulin in genetic hypertensive rats (*35*, *36*). The exercise intensity employed in studies demonstrating no improvement in insulin sensitivity was relatively high (mostly 20 m/min for 60 min per day). It has been reported that insulin sensitivity increased after training consisting of exercise three times per week, but declined to sedentary levels with training consisting of exercise seven times per week with little further reduction in cardiovascular risk factors (*22*). Just like the mechanisms for blood pressure modulation, higher-intensity exercise may also lead to increased activity of the sympathetic nervous system, which could result in insulin resistance. Since elevated insulin is reported to accelerate vasoconstriction (*6*, *37*), the reduction in circulating insulin

level and insulin resistance observed in the present study may also contribute to the lowering of blood pressure, in addition to the direct vasodilatation of exercise. Enhanced endothelium-dependent vessel relaxation by exercise could also act concomitantly with the reduced insulin to increase blood flow to striated muscle and thus glucose utilization and insulin sensitivity.

In conclusion, low-intensity voluntary running lowers blood pressure in aged male and female spontaneously hypertensive rats with simultaneous improvement in endothelium-dependent vasodilatation and insulin sensitivity. Improvements in both endothelium-dependent vessel relaxation and insulin sensitivity may act concomitantly in attenuating high blood pressure in genetic hypertension.

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