Original Article

Effects of Antihypertensive Drugs and Exercise Training on Insulin Sensitivity in Spontaneously Hypertensive Rats

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We examined the effects of antihypertensive drugs, exercise training, and combinations thereof on insulin sensitivity (IS), and the association between this relation and sympathetic activity, muscle fiber composition, and capillary density in spontaneously hypertensive rats (SHR). Six-week-old male SHR were allocated to 7 groups: a control group (C), and groups treated with azelnidipine (Aze) (a calcium channel blocker), olmesartan (Olm) (an angiotensin II type 1 receptor blocker), exercise training (Exe), and combinations of drugs and exercise training (Aze+Exe, Olm+Exe, and Olm+Aze+Exe). At age 18 weeks, IS and sympathetic activity were evaluated by an euglycemic hyperinsulinemic glucose clamp technique and power spectral analysis of systolic blood pressure, respectively. After the experiments, capillary density and muscle fiber composition in soleus muscle were examined. Aze or Exe alone significantly increased IS associated with a significant reduction in sympathetic activity. Olm alone tended to increase IS with little change in sympathetic activity. Aze, Olm, or Exe significantly increased the capillary density and percentage of insulin-sensitive type I fiber. A combination of Aze and Exe or a combination of Olm and Exe tended to increase IS compared with each drug therapy alone. There were significant correlations between IS and sympathetic activity, capillary density, and the percentage of type I fiber in all the rats. We found that Aze improved IS more substantially compared with Olm in SHR. We also found that Aze, Olm, Exe, and combinations thereof improved IS, probably through the modulation of sympathetic activity or capillarity and muscle fiber type in skeletal muscles. (*Hypertens Res* 2008; 31: 525–533)

***Key Words*: insulin sensitivity, calcium channel blocker, angiotensin II receptor blocker, exercise, sympathetic nerve activity**

Introduction

Insulin sensitivity (IS) is decreased in hypertensive subjects (*1*) and spontaneously hypertensive rats (SHR) (*2*). Although the precise mechanisms responsible for reduced IS in hypertensive subjects or animals are not fully understood, abnormalities in skeletal muscle, a major regulator of systemic IS (*1*), may be involved. It is known that the capillary density in skeletal muscle, which is related to the glucose supply, or the percentage of insulin-sensitive type I muscle fiber is reduced in hypertensive subjects (*3*, *4*) and SHR (*5*, *6*). In addition to skeletal muscle abnormalities, increased sympathetic activity may also be related to decreased IS in some forms of hypertension (*7*).

Impairment in glucose metabolism is associated with a high risk of cardiovascular diseases (*8*), and antihypertensive drugs are expected to have a beneficial effect on IS. It has been consistently shown that α_1-receptor antagonists and angiotensin-converting enzyme inhibitors have beneficial

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effects on glucose metabolism, whereas β-blockers and diuretics have adverse effects (*9*). On the other hand, the results of previous clinical studies examining the effects of calcium channel blockers (CCBs) or angiotensin II receptor blockers (ARBs) on IS are equivocal, with IS variously reported as being ameliorated (*10*, *11*) or unchanged (*9*, *11*–*14*). Therefore, in the present study, we examined the effects of a CCB (azelnidipine) or an ARB (olmesartan) on IS, and the relation between these effects and changes in sympathetic activity, capillary density and percentage of type I fiber in skeletal muscle in SHR. We also examined the effects of exercise training, a well known intervention to increase IS (*15*), and its combination with drug therapies.

Methods

Animal Care, Drug Treatment, and Exercise Training

Six-week-old male SHR (Charles River Japan, Atsugi, Japan) were divided into 7 groups: a sedentary control group (C, $n=10$), an azelnidipine group (Aze, $n=8$), an olmesartan group (Olm, $n=9$), an exercise-trained group (Exe, $n=9$), an Olm with Exe group (Olm+Exe, $n=9$), an Aze with Exe group (Aze+Exe, $n=8$) and an Olm plus Aze with Exe group (Olm+Aze+Exe, $n=8$). The rats were fed standard laboratory chow and water ad libitum while housed at a controlled temperature (24°C) with a 12-h light-dark cycle. Azelnidipine dissolved in 0.5% carboxymethylcellulose was administered by gavage daily at a dose of 3 mg/kg/day in the Aze, Aze+Exe, and Olm+Aze+Exe groups. Olmesartan dissolved in water was given by gavage daily at a dose of 10 mg/kg/day in the Olm, Olm+Exe, and Olm+Aze+Exe groups. Exercise training was performed on a rodent treadmill (KN-73 Tread-Mill; Natsume Industries Co., Tokyo, Japan) 5 days/week for 12 weeks. Rats ran at 20 m/min, 0 grade incline, for 60 min/day. Systolic blood pressure (SBP) and heart rate (HR) were measured each week from 6 weeks to 17 weeks of age by the tail-cuff method. All procedures in the present study were performed in accordance with the institutional guidelines.

Surgical Procedures

At the age of 18 weeks, under ether anesthesia, an arterial catheter (PE 100) and venous catheter (PE 20), filled with heparinized saline (100 IU/mL), were implanted into the left carotid artery and right jugular vein, respectively. The free ends of these catheters were brought subcutaneously to the back of the neck. The rats were returned to individual cages and allowed to recover for 2 days after the surgery.

Evaluation of Sympathetic Activity

The method used to estimate sympathetic activity by power spectral analysis of SBP has been described elsewhere (*16*). Briefly, after the arterial catheter was connected for measurements in their home cage, the rats were allowed to habituate to the experimental condition for at least 1 h. Arterial pressure was monitored from the arterial catheter with a strain-gauge transducer and amplifier. Arterial pressure data were also stored on a magneto-optical disk, and the wavelet transformation was computed using software running on a personal computer (Fluclet; Dai-nippon Pharmaceutical, Osaka, Japan). In the present study, the spectral component, the low frequency (LF; 0.26–0.74 Hz) of SBP, was produced for every 1-s interval with continuous wavelet transform. The LF amplitude of SBP (SBP-LFamp) was determined as the mean of the LFamp values obtained over each successive 5 s. Of these data, the mean of six successive SBP-LFamp measurements when the rat was quiet was used as an index of sympathetic activity (*17*).

Euglycemic Hyperinsulinemic Glucose Clamp Technique

After overnight fasting, IS was evaluated by a euglycemic hyperinsulinemic glucose clamp technique in conscious rats. Before the start of the glucose clamp, the fasting blood glucose was measured by a blood glucose test meter (GT-1641; Arkray Inc., Kyoto, Japan). The initial load of insulin (25 mU/kg of novolin R; Novo Nordisk, Tokyo, Japan) was infused through a venous catheter as a bolus, and this was followed by a constant infusion of insulin at a rate of 4 mU/kg/min for 150 min. During the glucose clamp, 12.5% glucose solution was infused as needed to maintain the blood glucose at the fasting level. Ten microliters of arterial blood were sampled through an arterial catheter at 7-min intervals for determination of the blood glucose. The average of the rate of glucose infusion (mg/kg/min) for the last 35 min was taken as an index of IS (*M* value) (*18*).

Tissue Preparation and Histological Examination

After the completion of all experiments, the rats were sacrificed by intraperitoneal injection of pentobarbital (100 mg/kg). The heart and soleus muscle were rapidly dissected and weighed. Each soleus muscle was sliced transversally in the midbelly region into 6-mm sections. These samples were rapidly frozen in isopentane cooled by dry ice and stored at −80°C until use. Serial transverse cross sections (10 μm thick) near the midbelly portion of the soleus muscle were cut in a microtome cryostat at −22°C, mounted on glass slides and air dried. The muscle fiber type was determined by a myofibrillar adenosine triphosphatase staining method (*19*) after preincubation in alkaline (pH 10.7 to 10.9) and acid (pH 4.3 to 4.6) solutions. For examination of the capillary density, another section from each sample was analyzed by a combined alkaline phosphatase and dipeptidylpeptidase IV staining reaction (*20*). Images were captured by an optical microscope connected to a CCD video camera (BX51,

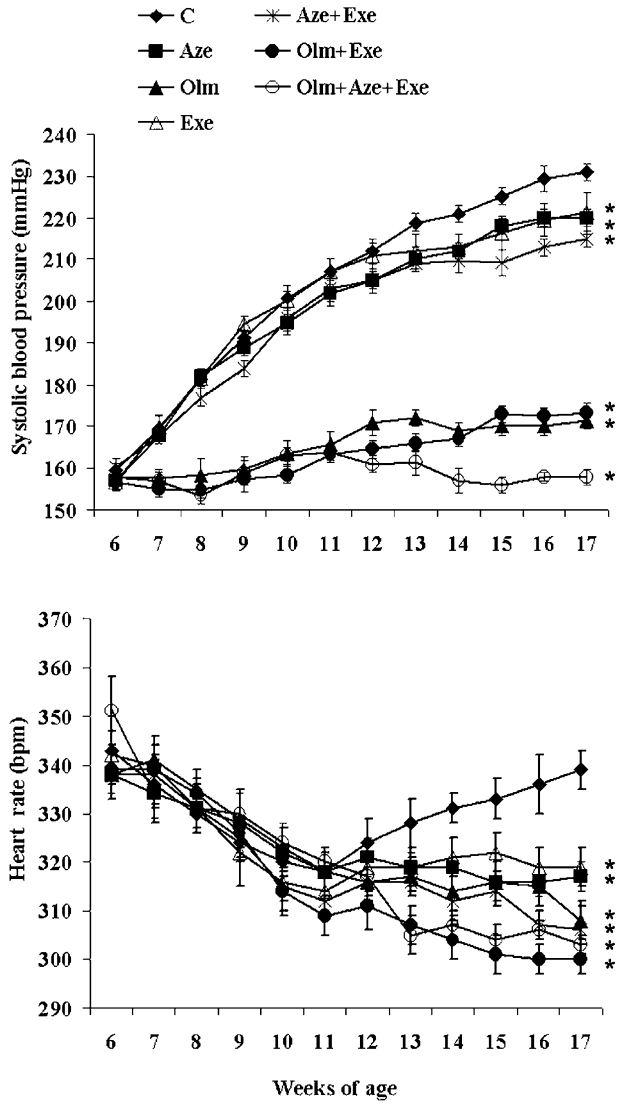

Fig. 1. *Average systolic blood pressure (SBP) and heart rate (HR) from 6 to 17 weeks of age in SHR. C, sedentary control; Olm, treatment with olmesartan; Aze, treatment with azelnidipine; Exe, exercise training; Olm+Exe, exercise training in combination with olmesartan; Aze+Exe, exercise training in combination with azelnidipine; Olm+Aze+Exe, exercise training in combination with azelnidipine and olmesartan. n=8 to 10 per group. Data are the means±SEM. *p<0.05 vs. C.*

CS900; Olympus Optical Co., Tokyo, Japan) at a magnification of ×100 (for fiber) or ×200 (for capillaries). The proportions of each fiber type, capillary-to-fiber ratio (C/F) and capillary density (CD) were estimated using image processing software (WinROOF ver.5.0; Mitani Corporation, Tokyo, Japan). For each muscle, at least 200 fibers and their associated capillaries were measured using the maximal number of non-overlapping fields.

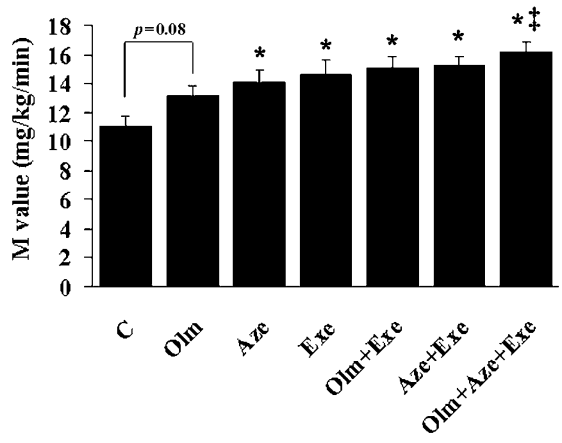

Fig. 2. *Comparisons of the index of insulin sensitivity (M value) in the seven groups. C, sedentary control; Olm, treatment with olmesartan; Aze, treatment with azelnidipine; Exe, exercise training; Olm+Exe, exercise training in combination with olmesartan; Aze+Exe, exercise training in combination with azelnidipine; Olm+Aze+Exe, exercise training in combination with azelnidipine and olmesartan. n=8 to 10 per group. Data are the means±SEM. *p<0.01 vs. C; ‡p<0.05 vs. Olm.*

Data Analysis

All results were expressed as the means±SEM. SBP and HR were analyzed by two-way analysis of variance with Fisher's probability least significant difference (PLSD) test. Correlations were tested by single and multiple regression analyses. Another data analysis among the groups was performed by Fisher's PLSD test for multiple comparisons after one-way analysis of variance. Values of $p<0.05$ were considered to indicate statistical significance.

Results

SBP and HR

Figure 1 shows the age-related changes in SBP and HR. Azelnidipine alone, olmesartan alone, exercise training alone, and their combinations significantly attenuated the development of hypertension and decreased HR. The antihypertensive effects of Olm were significantly greater than those of Aze ($p<0.0001$).

M Value

The *M* values in the seven groups are shown in Fig. 2. The *M* values in Exe and Aze were significantly higher than that in C ($p<0.01$). Although the *M* value in Olm tended to increase compared with that in C, the difference was not significant ($p=0.08$). The *M* values in Aze+Exe and Olm+Exe tended to

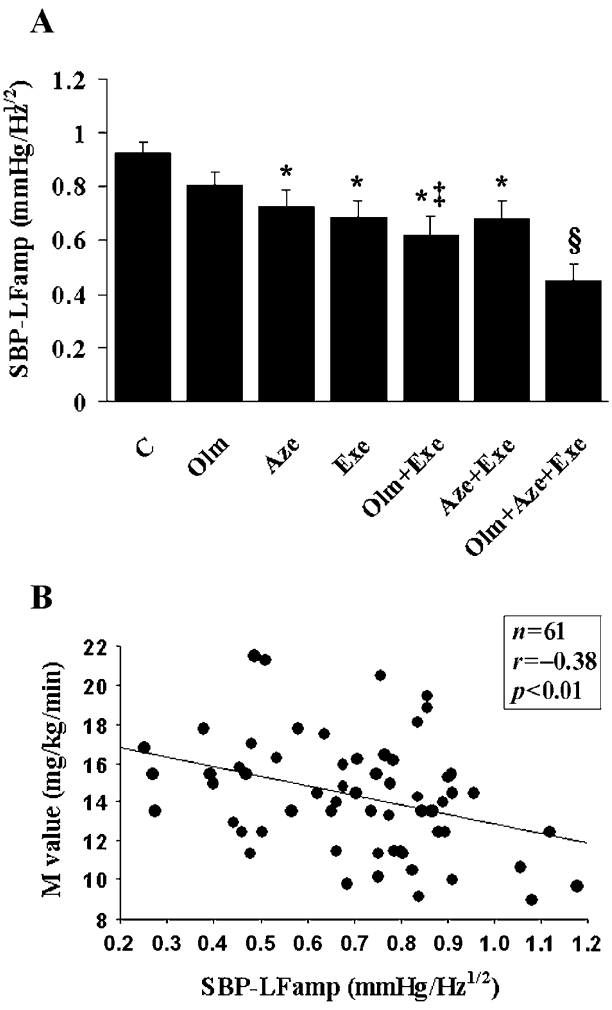

Fig. 3. *Comparisons of SBP-LFamp, a marker of sympathetic activity, in the seven groups under quiet conditions (A), and correlation between the M value and SBP-LFamp (B). C, sedentary control; Exe, exercise training; Aze, treatment with azelnidipine; Olm, treatment with olmesartan; Aze+Exe, exercise training in combination with azelnidipine; Olm+Exe, exercise training in combination with olmesartan; Olm+Aze+Exe, exercise training in combination with azelnidipine and olmesartan. n=8 to 10 per group. Data are the means±SEM. *p<0.05 vs. C; ‡p<0.05 vs. Olm; §p<0.05 vs. the other six groups.*

be higher than those in Aze and Olm, respectively, but the differences were not significant. The *M* value in the Olm+Aze+Exe group tended to be higher than that in Olm+Exe or Aze+Exe group.

SBP-LFamp

SBP-LFamp in Aze, Exe, Aze+Exe, Olm+Exe, and Olm+Aze+Exe was significantly lower than that in C (Fig. 3A). On the other hand, there was no significant difference in SBP-LFamp between Olm and C (0.81±0.05 *vs.* 0.92±0.04 mmHg/Hz$^{1/2}$, $p=0.14$). SBP-LFamp in Olm+Aze+Exe was lowest among the six treated groups.

Capillary Density and Muscle Fiber Composition

The C/F and CD in the six treated groups were significantly higher than those in C (Fig. 4A and B). Both C/F and CD in Aze+Exe were significantly higher than those in Aze. The percentage of type I fiber in soleus muscle was significantly higher in the six treated groups than in C (Fig. 4C). The percentages of type I fibers in Aze+Exe and Olm+Exe were significantly higher compared with those in Aze and Olm, respectively. The percentage of type I fibers in Olm+Aze+Exe was highest among the six treated groups.

Regression Analyses

There was a significantly inverse correlation between the *M* values and SBP-LFamp values (Fig. 3B), while there were significantly positive correlations between the *M* values and C/F, CD, or the percentage of type I fiber from all the rats (Fig. 5). On the other hand, multiple regression analysis indicated that the *M* values were predicted by the percentage of type I fiber ($F=4.08$) but not by C/F ($F=0.06$), CD ($F<0.01$), or SBP-LFamp ($F=0.9$) ($F>4$ was significant).

Left Ventricular Weight and Body Weight

Table 1 shows the data of body compositions. At the age of 6 weeks, there were no significant differences in body weight (BW) among the groups. However, at the age of 18 weeks, the BW values in Olm+Exe and Olm+Aze+Exe were significantly lower than that in C. The left ventricular weight (LW) and LW/BW ratio were significantly lower in Aze, Olm, Aze+Exe, Olm+Exe and Olm+Aze+Exe compared with those in C. The LW and LW/BW ratio in Olm+Aze+Exe were lowest among the six treated groups. There was a significantly positive correlation between SBP at the age of 17 weeks and LW/BW ratio from all the rats ($r=0.9$, $p<0.0001$).

Discussion

In the present study, we demonstrated that azelnidipine alone and exercise training alone significantly increased IS to a similar level, while olmesartan induced only a slight but nonsignificant increase in IS in SHR. The effect of exercise training on IS tended to be additive to those of azelnidipine or olmesartan. We also found that improvements of IS were associated with decreases in SBP-LFamp, a marker of sympathetic activity, and increases in the percentage of type I fiber and capillary density in skeletal muscle. These findings suggest that azelnidipine, olmesartan, exercise training, and their combinations improved IS at least in part *via* a modulation of

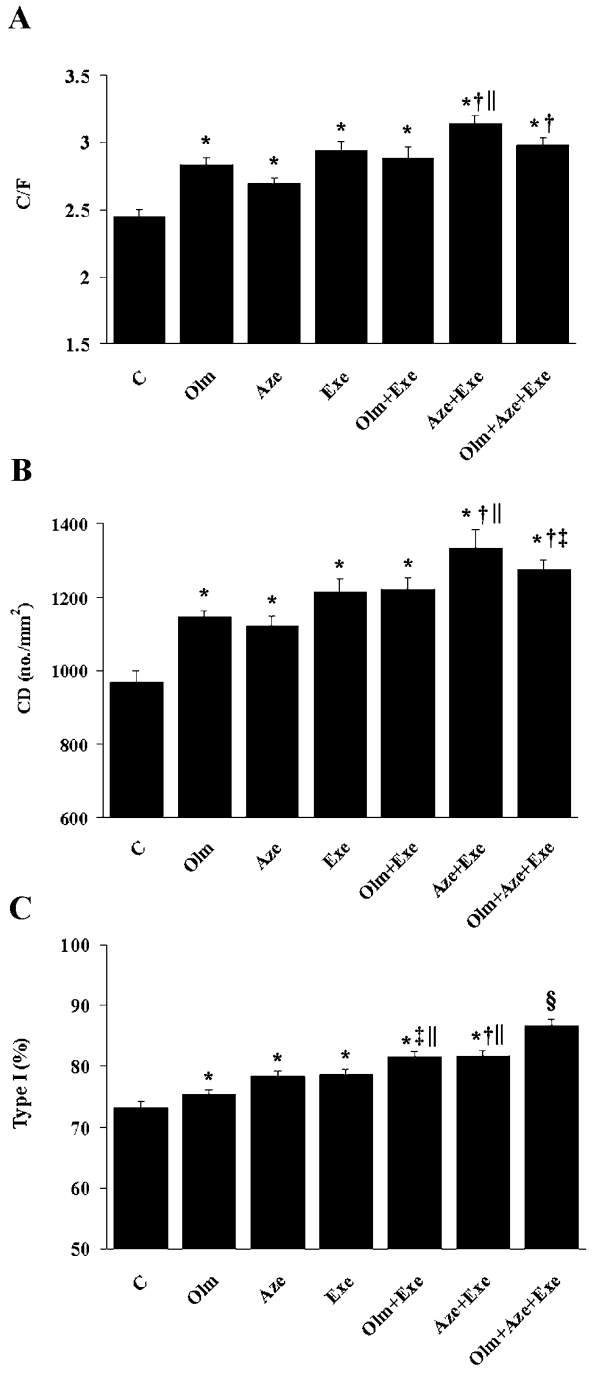

Fig. 4. *Comparisons of the capillary-to-fiber ratio (C/F) (A), capillary density (CD) (B) and percentage of type I fibers (C) in the seven groups. C, sedentary control; Olm, treatment with olmesartan; Aze, treatment with azelnidipine; Exe, exercise training; Olm+Exe, exercise training in combination with olmesartan; Aze+Exe, exercise training in combination with azelnidipine; Olm+Aze+Exe, exercise training in combination with azelnidipine and olmesartan. n=8 to 10 per group. Data are the means±SEM. *p<0.05 vs. C; †p<0.01 vs. Aze; ‡p<0.01 vs. Olm; ‖p<0.05 vs. Exe; §p<0.001 vs. the other six groups.*

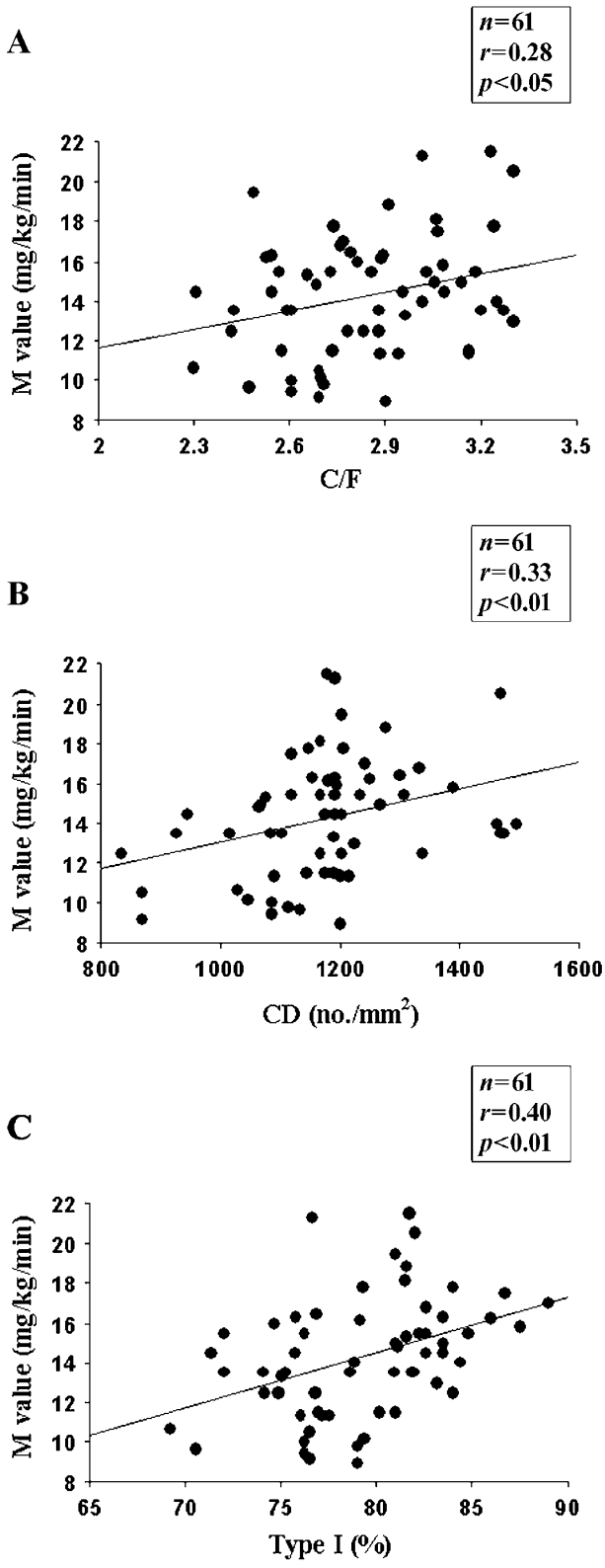

Fig. 5. *Correlation between the M value and capillary-to-fiber ratio (C/F) (A), capillary density (CD) (B) and percentage of type I fibers (C).*

Table 1. Body Compositions

	C	Olm	Aze	Exe	Olm+Exe	Aze+Exe	Olm+Aze+Exe
BW (g)	325±5	321±3	326±3	326±3	305±4*,†	324±3	311±3*,‡
LW (mg)	1,005±24	777±12*	895±15*	998±13	739±11*	929±13*	601±15§
LW/BW (mg/g)	3.10±0.07	2.42±0.04*	2.75±0.04*	3.05±0.03	2.43±0.04*	2.87±0.03*	1.93±0.04§

BW, body weight; LW, left ventricular weight; C, sedentary control; Olm, treatment with olmesartan; Aze, treatment with azelnidipine; Exe, exercise training; Olm+Exe, exercise training in combination with olmesartan; Aze+Exe, exercise training in combination with azelnidipine; Olm+Aze+Exe, exercise training in combination with azelnidipine and olmesartan. $n=8$ to 10 per group. Data are the means±SEM. *$p<0.01$ *vs*. C; †$p<0.01$ *vs*. Olm; ‡$p<0.01$ *vs*. Aze+Exe; §$p<0.001$ *vs*. the other six groups.

the sympathetic activity, muscle fiber composition and capillary density in SHR.

Sympathetic Activity

It is well known that sympathetic activity is increased in SHR (*21*). In the present study, there was a significant inverse correlation between IS and SBP-LFamp, suggesting that drug treatments, exercise training, and their combinations improve IS in part by decreasing the sympathetic activity. There are several mechanisms by which sympathetic activity affects IS. First, sympathetic activation induces vasoconstriction, which in turn reduces the microcirculatory supply and glucose delivery in skeletal muscle (*22*). Second, sympathetic activation impairs tissue IS, acting primarily through β-adrenergic receptors (*23*) or α_1-adrenergic receptors on adipocytes (*24*). Third, sympathetic activation seems to deteriorate IS by decreasing adiponectin (*25*) and leptin (*26*, *27*) in adipose tissue.

Concerning the effects of CCBs on IS, it has been demonstrated that short-acting CCBs such as nicardipine or nifedipine do not affect IS (*12*, *13*). It has been thought that short-acting CCBs activate the sympathetic nervous system through the baroreflex mechanism, which offsets their favorable effect on IS, such as vasodilation. However, despite its long-acting property, amlodipine does not improve IS (*11*), probably because it activates the sympathetic nervous system (*28*). On the other hand, the long-acting dihydropyridine CCB, cilnidipine, which suppresses norepinephrine release from sympathetic nerve endings by acting on N-type calcium channel receptors, has been shown to improve IS in subjects with essential hypertension (*10*) and fructose-fed rats (FFR) (*18*). In the present study, the azelnidipine-improved IS was associated with a significant reduction of SBP-LFamp. Although azelnidipine does not have an N-type calcium channel blocking action, it has a unique long-acting dihydropyridine-based calcium antagonistic action with sympathoinhibitory effects (*29*, *30*). Taken together, these results suggest that the sympathoinhibitory action is necessary for CCBs to exert a beneficial effect on IS.

In the present study, olmesartan had little effect on the sympathetic activity in SHR. Although some ARBs can suppress sympathetic activity through the modulation of norepinephrine release from sympathetic nerve endings, not all ARBs exert such an effect (*31*). These different effects of ARBs on sympathetic activity may be in part explained by differences in the affinity for the pre-synaptic angiotensin II type 1 (AT1) receptor. In addition, differences in the animal models used in the studies may also have affected the results. Kamide *et al*. (*32*) have demonstrated that olmesartan suppresses the sympathetic activity in FFR but not in normal chow–fed rats. It has been shown that a fructose-rich diet increases the sympathetic activity and induces hyperinsulinemia, insulin resistance and hypertension (*18*, *32*). The less potent effect of olmesartan on the sympathetic activity in SHR may be due to a difference in the mechanism for sympathetic activation in FFR.

It is well known that exercise training reduces the sympathetic nerve activity in hypertensive patients (*15*, *33*). In accordance with the results of previous studies, exercise training in the present study significantly reduced SBP-LFamp, a marker of sympathetic activity. Thus, the improvement of IS by exercise training may be at least partly mediated by a decrease in sympathetic activity.

Capillary Density

It is well known that the capillary density in skeletal muscle is decreased in hypertensive subjects (*3*) and hypertensive animals (*5*). A decrease in the capillary density is associated with a longer diffusion distance between the nutritional blood vessels and the skeletal muscle cells. This would impede the delivery of glucose to the muscle cells and thereby decrease the IS (*22*). In the present study, azelnidipine, olmesartan, exercise training, and their combinations improved IS, which was associated with significant increases in the capillary density. Moreover, there was a significant positive correlation between the IS and capillary density values from all the rats. Thus, azelnidipine, olmesartan, exercise training, and their combinations increased IS, at least in part by increasing the capillary density.

Capillary rarefaction associated with hypertension has been considered to be a form of "structural autoregulation," reflecting the long-term adaptation of the microcirculation to the elevated blood pressure or the initial increase of blood flow in hypertension. In this respect, capillary rarefaction would

enable the tissue blood flow to be regulated without consumption of the energy necessary for active vasoconstriction (*5*). On the other hand, it has been shown that vasodilatation and increased blood flow promote angiogenesis in skeletal muscle (*34*). Thus, it is possible that azelnidipine (*30*) or olmesartan (*35*) increased the capillary density through their vasodilator action or hypotensive effect. In addition to the vasodilator or hypotensive effect, the proangiogenic effect of olmesartan may also be mediated by the following mechanism: ARB blocks AT1 receptors while unbound angiotensin II stimulates angiotensin II type 2 (AT2) receptors, which in turn increases angiogenic factors such as vascular endothelial growth factor (VEGF) receptors, Tie-2 expression and the angiopoietin-1/angiopoietin-2 ratio (*36*). In this regard, however, Scheidegger *et al*. (*37*) reported that treatment with valsartan for 4 weeks did not increase the capillary density in SHR. The reason for the different results between this previous study and the present study is not clear, but the difference in the treatment period may be involved.

Consistent with previous studies (*38*), in the present study exercise training significantly increased the capillary density. Thus, in the present study the exercise-induced increase in the capillary density may have partially contributed to the improvement of IS.

Muscle Fiber Composition

Skeletal muscle consists of slow twitch oxidative fibers (type I) and fast twitch non-oxidative fibers (type II). Type I fibers have an abundance of mitochondria and work oxidatively. On the other hand, type II fibers have fewer mitochondria and use more glycolytic pathways. Type I fiber is more insulin-sensitive than type II fiber (*39*). Although the results of previous studies examining the composition of muscle fiber types in SHR are equivocal, some studies have reported that SHR show a lower percentage of type I fibers compared with normotensive rats (*6*). In the present study, azelnidipine, olmesartan or exercise training significantly increased the percentage of type I fiber in SHR. These findings are in accordance with the results of previous studies which examined the effects of CCBs (*18*), ARBs (*40*), or exercise training (*19*) on the muscle fiber composition in FFR. Moreover, there was a significantly positive correlation between IS and the percentage of type I fiber from all the rats. Thus azelnidipine, olmesartan, exercise training, and their combinations improved IS at least in part by increasing the percentage of type I fibers.

The mechanisms responsible for the decreased percentage of type I fiber in SHR remain unclear, but an increased level of plasma catecholamines may be involved (*6*). Therefore, the reduction in sympathetic activity by azelnidipine or exercise training may partially contribute to the increase in the percentage of type I fibers. Recently, it has been found that the conversion of muscle fibers from type II to type I in response to exercise training is mediated by a calcium-signaling pathway that involves calcineurin, calmodulin-dependent kinase and the transcriptional cofactor peroxisome proliferator–activated receptor-γ coactivator-1 (PGC-1α) (*41*). On the other hand, the mechanisms by which ARBs increase the percentage of type I fibers are not yet clear.

It is of interest that the values of BW in the Olm+Exe and Olm+Aze+Exe groups were significantly lower than those in the Olm and Aze+Exe groups, respectively. Although it has been shown that telmisartan, an ARB, has the ability to markedly reduce BW *via* peroxisome proliferators–activated receptor (PPAR)-γ action, other ARBs do not exhibit such activity (*42*). Taken together, the results of the present study suggest that there is some interaction between olmesartan and exercise training in terms of their effects on BW, which may have contributed, at least in part, to the increased IS in the Olm+Exe and Olm+Aze+Exe groups.

Study Limitations

There were several notable limitations in the present study. First, azelnidipine (3 mg/kg/day) significantly decreased blood pressure in SHR, but its depressor effect was smaller than that of olmesartan. With regard to the antihypertensive effects of azelnidipine, it has been reported that treatment of SHR with azelnidipine (3 mg/kg/day) beginning at the age of 23 weeks decreased SBP by about 30 mmHg (*43*). On the other hand, treatment with azelnidipine (3 mg/kg/day) did not significantly decrease blood pressure in 12-week-old stroke-prone SHR (*44*). Thus, to obtain the same antihypertensive effect as olmesartan, a much larger dose of azelnidipine than that used in the present study may be needed. Further studies will be needed to clarify whether treatment with a larger dose of azelnidipine, an agent which has been shown to decrease blood pressure to a similar level as olmesartan, could bring about different effects on sympathetic activity, skeletal muscle, and IS. Second, although there were significant correlations between the *M* values and SBP-LFamp, C/F, CD, and the percentage of type I fiber from all the rats, multiple regression analysis indicated that the *M* values were predicted only by the percentage of type I fiber. This, however, does not necessarily mean that SBP-LFamp, C/F, and CD are not related with IS, because there were significant correlations between the percentage of type I fiber and SBP-LFamp ($r=-0.56$, $p<0.0001$), C/F ($r=0.54$, $p<0.0001$), and CD ($r=0.56$, $p<0.0001$).

In conclusion, the results of this study suggest that azelnidipine, olmesartan, and exercise training, either alone or in various combinations, can improve IS in SHR, and that this improvement is at least partially attributable to a modulation of sympathetic activity, capillary density or muscle fiber composition. The combination of azelnidipine, olmesartan, and exercise training may be especially useful for the treatment of hypertensive subjects with insulin resistance.

References

1. Natali A, Santoro D, Palombo C, *et al*: Impaired insulin action on skeletal muscle metabolism in essential hypertension. *Hypertension* 1991; **17**: 170–178.
2. Melancon S, Bachelard H, Badeau M, *et al*: Effects of high-sucrose feeding on insulin resistance and hemodynamic responses to insulin in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* 2006; **290**: H2571–H2581.
3. Hedman A, Reneland R, Lithell HO: Alterations in skeletal muscle morphology in glucose-tolerant elderly hypertensive men: relationship to development of hypertension and heart rate. *J Hypertens* 2000; **18**: 559–565.
4. Frisk-Holmberg M, Essen B, Fredrikson M, Strom G, Wibell L: Muscle fibre composition in relation to blood pressure response to isometric exercise in normotensive and hypertensive subjects. *Acta Med Scand* 1983; **213**: 21–26.
5. Prewitt RL, Chen II, Dowell R: Development of microvascular rarefaction in the spontaneously hypertensive rat. *Am J Physiol* 1982; **243**: H243–H251.
6. Ben Bachir-Lamrini L, Sempore B, Mayet MH, Favier RJ: Evidence of a slow-to-fast fiber type transition in skeletal muscle from spontaneously hypertensive rats. *Am J Physiol* 1990; **258**: R352–R357.
7. Arauz-Pacheco C, Lender D, Snell PG, *et al*: Relationship between insulin sensitivity, hyperinsulinemia, and insulin-mediated sympathetic activation in normotensive and hypertensive subjects. *Am J Hypertens* 1996; **9**: 1172–1178.
8. Scherrer U, Sartori C: Insulin as a vascular and sympathoexcitatory hormone: implications for blood pressure regulation, insulin sensitivity, and cardiovascular morbidity. *Circulation* 1997; **96**: 4104–4113.
9. Lithell HO: Effect of antihypertensive drugs on insulin, glucose, and lipid metabolism. *Diabetes Care* 1991; **14**: 203–209.
10. Yagi S, Goto S, Yamamoto T, Kurihara S, Katayama S: Effect of cilnidipine on insulin sensitivity in patients with essential hypertension. *Hypertens Res* 2003; **26**: 383–387.
11. Tomiyama H, Yambe M, Yamada J, *et al*: Discrepancy between improvement of insulin sensitivity and that of arterial endothelial function in patients receiving antihypertensive medication. *J Hypertens* 2007; **25**: 883–889.
12. Kihara A: Effect of the calcium antagonist nicardipine hydrochloride on glucose tolerance and insulin secretion. *Am Heart J* 1991; **122**: 363–369.
13. Lind L, Berne C, Pollare T, Lithell H: Metabolic effects of anti-hypertensive treatment with nifedipine or furosemide: a double-blind, cross-over study. *J Hum Hypertens* 1995; **9**: 137–141.
14. Moan A, Hoieggen A, Seljeflot I, *et al*: The effect of angiotensin II receptor antagonism with losartan on glucose metabolism and insulin sensitivity. *J Hypertens* 1996; **14**: 1093–1097.
15. Kohno K, Matsuoka H, Takenaka K, *et al*: Renal depressor mechanisms of physical training in patients with essential hypertension. *Am J Hypertens* 1997; **10**: 859–868.
16. Minami N, Mori N, Nagasaka M, *et al*: Mechanism behind augmentation in baroreflex sensitivity after acute exercise in spontaneously hypertensive rats. *Hypertens Res* 2006; **29**: 117–122.
17. Brown DR, Brown LV, Patwardhan A, Randall DC: Sympathetic activity and blood pressure are tightly coupled at 0.4 Hz in conscious rats. *Am J Physiol* 1994; **267**: R1378–R1384.
18. Takada M, Ura N, Higashiura K, *et al*: Effects of cilnidipine on muscle fiber composition, capillary density and muscle blood flow in fructose-fed rats. *Hypertens Res* 2001; **24**: 565–572.
19. Kawamura T, Yoshida K, Sugawara A, *et al*: Regulation of skeletal muscle peroxisome proliferator–activated receptor gamma expression by exercise and angiotensin-converting enzyme inhibition in fructose-fed hypertensive rats. *Hypertens Res* 2004; **27**: 61–70.
20. Grim M, Carlson BM: Alkaline phosphatase and dipeptidylpeptidase IV staining of tissue components of skeletal muscle: a comparative study. *J Histochem Cytochem* 1990; **38**: 1907–1912.
21. Cabassi A, Vinci S, Cantoni AM, *et al*: Sympathetic activation in adipose tissue and skeletal muscle of hypertensive rats. *Hypertension* 2002; **39**: 656–661.
22. Julius S, Gudbrandsson T, Jamerson K, Tariq Shahab S, Andersson O: The hemodynamic link between insulin resistance and hypertension. *J Hypertens* 1991; **9**: 983–986.
23. Deibert DC, DeFronzo RA: Epinephrine-induced insulin resistance in man. *J Clin Invest* 1980; **65**: 717–721.
24. McCarty MF: Elevated sympathetic activity may promote insulin resistance syndrome by activating alpha-1 adrenergic receptors on adipocytes. *Med Hypotheses* 2004; **62**: 830–838.
25. Nowak L, Adamczak M, Wiecek A: Blockade of sympathetic nervous system activity by rilmenidine increases plasma adiponectin concentration in patients with essential hypertension. *Am J Hypertens* 2005; **18**: 1470–1475.
26. Trayhurn P, Duncan JS, Hoggard N, Rayner DV: Regulation of leptin production: a dominant role for the sympathetic nervous system? *Proc Nutr Soc* 1998; **57**: 413–419.
27. Shimomura I, Hammer RE, Ikemoto S, Brown MS, Goldstein JL: Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 1999; **401**: 73–76.
28. Lindqvist M, Kahan T, Melcher A, Ekholm M, Hjemdahl P: Long-term calcium antagonist treatment of human hypertension with mibefradil or amlodipine increases sympathetic nerve activity. *J Hypertens* 2007; **25**: 169–175.
29. Shokoji T, Fujisawa Y, Kiyomoto H, *et al*: Effects of a new calcium channel blocker, azelnidipine, on systemic hemodynamics and renal sympathetic nerve activity in spontaneously hypertensive rats. *Hypertens Res* 2005; **28**: 1017–1023.
30. Kimura Y, Hirooka Y, Sagara Y, Sunagawa K: Long-acting calcium channel blocker, azelnidipine, increases endothelial nitric oxide synthase in the brain and inhibits sympathetic nerve activity. *Clin Exp Hypertens* 2007; **29**: 13–21.
31. Nap A, Balt JC, Pfaffendorf M, Van Zwieten PA: Sympatholytic properties of several AT1-receptor antagonists in the isolated rabbit thoracic aorta. *J Hypertens* 2002; **20**: 1821–1828.
32. Kamide K, Rakugi H, Higaki J, *et al*: The renin-angiotensin and adrenergic nervous system in cardiac hypertrophy in

fructose-fed rats. *Am J Hypertens* 2002; **15**: 66–71.
33. Laterza MC, de Matos LD, Trombetta IC, *et al*: Exercise training restores baroreflex sensitivity in never-treated hypertensive patients. *Hypertension* 2007; **49**: 1298–1306.
34. Hudlicka O: Is physiological angiogenesis in skeletal muscle regulated by changes in microcirculation? *Microcirculation* 1998; **5**: 5–23.
35. Igase M, Strawn WB, Gallagher PE, Geary RL, Ferrario CM: Angiotensin II AT1 receptors regulate ACE2 and angiotensin-(1–7) expression in the aorta of spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol* 2005; **289**: H1013–H1019.
36. Kitayama H, Maeshima Y, Takazawa Y, *et al*: Regulation of angiogenic factors in angiotensin II infusion model in association with tubulointerstitial injuries. *Am J Hypertens* 2006; **19**: 718–727.
37. Scheidegger KJ, Nelissen-Vrancken MH, Leenders PJ, *et al*: Structural adaptation to ischemia in skeletal muscle: effects of blockers of the renin-angiotensin system. *J Hypertens* 1997; **15**: 1455–1462.
38. Melo RM, Martinho E Jr, Michelini LC: Training-induced, pressure-lowering effect in SHR: wide effects on circulatory profile of exercised and nonexercised muscles. *Hypertension* 2003; **42**: 851–857.
39. James DE, Jenkins AB, Kraegen EW: Heterogeneity of insulin action in individual muscles *in vivo*: euglycemic clamp studies in rats. *Am J Physiol* 1985; **248**: E567–E574.
40. Higashiura K, Ura N, Takada T, *et al*: The effects of an angiotensin-converting enzyme inhibitor and an angiotensin II receptor antagonist on insulin resistance in fructose-fed rats. *Am J Hypertens* 2000; **13**: 290–297.
41. Lin J, Wu H, Tarr PT, *et al*: Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature* 2002; **418**: 797–801.
42. Benson SC, Pershadsingh HA, Ho CI, *et al*: Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. *Hypertension* 2004; **43**: 993–1002.
43. Oizumi K, Nishino H, Miyake S, *et al*: Hemodynamic changes following long-term administration of CS-905, a novel dihydropyridine calcium blocker, in conscious SHR. *Jpn J Pharmacol* 1990; **54**: 1–6.
44. Nakamoto M, Ohya Y, Sakima A, Yamazato M, Takishita S: Azelnidipine attenuates cardiovascular and sympathetic responses to air-jet stress in genetically hypertensive rats. *Hypertens Res* 2007; **30**: 359–366.