Effects of combined therapy of ACE inhibitor and exercise on cardiovascular functions and morphology of the heart and kidneys in SHR

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Abstract An exercise regimen rarely causes organ damage in humans or rats with established essential hypertension. However, daily exercise is essential for elderly people to prolong a healthy lifespan. This study was designed to examine the effects of exercise training alone and the combined treatment of exercise and anti-hypertensive medication on morphological characteristics of the heart and kidneys, as well as to examine the response to pressor agents in the spontaneously hypertensive rat (SHR). SHRs were divided into the following four groups and control group: the voluntary wheel running exercise (SHR-Ex), angiotensin converting enzyme inhibitor (ACE-I), captopril (Capt) administration (SHR-Capt), Capt administration and exercise (SHR-Capt & Ex), and sedentary control (SHR-Sed) groups. Wister-Kyoto (WKY) rats were also used and divided into either exercise (WKY-Ex) or sedentary control (WKY-Sed) groups. These treatments were conducted from 8 to 23 weeks of age. Approximately 20 mg/kg/day of Capt dissolved in tap water was given daily in Capt-treated animals. After the treatment period, both the SHR-Sed and SHR-Ex groups exhibited hypertension, hyperpressor responsivity, cardiac hypertrophy, cardiomyocyte fibrosis, and renal hypertrophy, accompanied by an enlargement of glomerular and mesangial areas. On the other hand, the Capt-treated SHR groups showed an inhibition of blood pressure elevation, no organ damage, and suppression of pressor responsivity to levels comparable to those of the normotensive WKY rats. The present results suggest that a combined treatment with exercise and an ACE-I such as Capt is recommended for middle-aged and elderly essential hypertensive patients to avoid secondary risks of cardiovascular related diseases induced by exercise alone.

Keywords: SHR, exercise, organ damage, ACE inhibitor, combined treatment of captopril and exercise

Introduction Hypertension is associated with an increased incidence of critical cardiovascular (CV) diseases such as heart attacks, stroke, peripheral arterial disease, and renal insufficiency1). It is well known that gene mutations are involved in an initiation of essential hypertension (EHT) and that environmental factors exacerbate it1,2). These factors induced by daily life are causes of high prevalence of not only EHT, but also other lifestyle-related diseases such as obesity, diabetes, and dyslipidemia.

The most recent meta-analysis3) reported that systolic (SBP) and diastolic blood pressure (DBP) were reduced by 8.3/5.2 (SBP/DBP) mmHg in hypertensive subjects after exercise regimens. However, Seals and Hagberg4) suggested that the extent of hypotensive effect (-9/-7 mmHg) by exercise is inadequate for the recommendation of exercise as a replacement for pharmacological intervention. The Trial of Preventing Hypertension (TROPHY) study5) for participants with pre-hypertension reported that life-
style modifications alone, including diet and exercise regimens, coincided with a 40.4% incidence of hypertension after 2 years, and a 63.0% incidence after 4 years. Our previous studies\(^6,7\), using the obese diabetic model, OLETF rats, reported that an exercise regimen failed to lower the blood pressure (BP) and led to progression of diabetic nephropathy (DN).

The spontaneously hypertensive rat (SHR) is well known as an EHT model of rats and is considered to have increased basal sympathetic nerve activity (SNA)\(^8\). The meta-analysis study\(^9\) concluded that exercise does not reduce BP in SHRs with established hypertension and may increase the incidence of myocardial hypertrophy. Indeed, da Cost Rebelo et al.\(^10\) reported that long-term voluntary exercise even accelerated heart disease in old female SHRs. The underlying mechanisms of these negative effects by daily exercise may involve sympathoexcitation and high blood pressure during physical exercise. All told, an exercise regimen alone in established hypertension may not be the ideal therapy to reduce basal BP levels and incidence of hypertension-related CV.

However, physical activity is essential for the elderly to prevent disuse atrophy of muscle and bone and to prolong a healthy lifespan. Therefore, it is necessary to develop an exercise regimen without causing organ damage due to BP elevation during exercise. This study was commenced to confirm the adverse effects of an exercise regimen on morphological findings of the heart and kidneys in SHRs with established hypertension. The results observed in the exercise alone group were also compared to those in the exercised SHR group with inhibited BP elevation by taking captopril (Capt). The pressor responsivity (PR) test to pressor agents was also performed because it is known that the cardiovascular response to physical exercise is abnormally exaggerated in hypertension\(^11\), whereas there are reports that aerobic exercise improves vascular endothelial function\(^12,13\). Moreover, circulating plasma volume (CPV), which is known as a factor in hypertensive effects caused by exercise\(^14\), was also examined. Our findings suggest that a combined treatment of exercise and antihypertensive agents such as Capt is recommended for established hypertensive patients to avoid secondary risks of cardiovascular-related diseases induced by exercise alone.

Materials and Methods

**Experimental animals.** Fifty-four male SHRs and eighteen male Wistar Kyoto (WKY) rats were purchased from Hoshino Experimental Animals (Saitama, Japan) when they were 6-7 weeks old. The animals were housed under conditions at 20-25°C, humidity of 40-60%, and lighting from 6:00 AM to 6:00 PM. The animals were supplied with standard rat chow (CE-II, CLEA, Tokyo, Japan) and tap water ad libitum. The weight of food consumption (FC) and volume of water drunk (WD) were measured weekly throughout the experimental period. The experimental protocols were approved by the Committee for the Care and Use of Animals of Jikei University School of Medicine (Issue Number: H18-1). This study was conducted under the Guidelines on Animal Care of Jikei University School of Medicine.

**Experimental design.** For pre-treatment examinations, first, a PR test (see below for methodology) was performed for SHRs (n = 6) and WKY rats (n = 6) after 1 week of the habitation period in individual standard cages (i.e. when the animals were 8 weeks of age), and then sampling for morphological analysis of the heart and kidney (see below for methodology) was done 4 or 5 days after the PR test. Another group of SHRs (n = 6) was used for only CPV measurement (see below for methodology) after 1 week of habitation period (i.e. when animals were 8 weeks of age). All animals used for pre-treatment measurements were humanely euthanized. The remaining 42 SHRs were randomly divided into the four groups. The exercise treatment group (SHR-Ex, n = 12) performed daily wheel running exercise. The Capt treatment group (SHR-Capt, n = 12) was given approximately 20 mg/kg/day of Capt (Wako Pure Chemical Industries, Osaka, Japan) dissolved in tap water. The combined treatment with Capt and exercise group (SHR-Capt & Ex, n = 6) exercised while taking Capt daily. The sedentary control group (SHR-Sed, n = 12) was also assigned. Twelve WKY rats were assigned to an exercise group (WKY-Ex, n = 6) and a sedentary group (WKY-Sed, n = 6). The SHR-Ex, SHR-Capt & Ex and WKY-Ex groups were allowed to run on a rotatory wheel (Shinano, Tokyo, Japan), and their running distances were recorded weekly from 8-23 weeks of age. All animals were housed individually, and the following parameters were measured.

**BW, BP and heart rate (HR) measurements.** BW, resting BP, and HR were measured weekly throughout the experimental period (from 7-23 weeks of age). BP and HR were measured 5 times using a tail-cuff plethysmography (BP-98; Softron, Tokyo, Japan) between 10:00 AM and 12:00 PM, and the average was recorded for each animal.

**PR test.** The PR test was performed in 8-week-old SHRs and WKY rats, and 24-week-old rats after the treatment period. The animals were anesthetized by pentobarbital sodium, which was intraperitoneally infused at a dose of 50 mg/kg (BW). The left carotid was exposed and a cannula (SP-45; NATSUME, Tokyo, Japan) filled with saline was inserted. The end of the arterial cannula was connect- ed to the transducer to continuously monitor the arterial pulsatile pressure (AP) on a polygraph (366; San-ei Electronics, Tokyo, Japan). Another cannula was inserted into the left jugular vein for infusion of the pressor agents. SBP and DBP were measured from the pulse wave, and mean blood pressure (MBP) was calculated mathemati-
cally. After the AP stabilized, the norepinephrine (NE)-like substance, phenylephrine (Phen; Wako, Japan), was infused in doses of 1, 2, 4, 6, and 8 μg/kg each in a single bolus, based on our preliminary study. After each trial, the subsequent one was started once the AP had returned to the pre-trial level. After this experiment, Ang II (Sigma, USA) was infused in doses of 12.5, 25, 50, 75, and 100 ng/kg in a single bolus once the AP stabilized. After the infusions of pressor agents, maximal changes of MBP were measured and changes from the baseline levels (⊿MBP) were calculated and used as the PR indicator. The trial was repeated at each infusion rate at least twice; the two closest values were selected, and the average was determined for the identical value for each animal.

**Analysis of hematological and biochemical components in blood.** Three to 5 days after the post-treatment PR test, whole blood was drawn completely under pentobarbital anesthesia via a catheter placed in the external jugular vein for hematological components (Sysmex NE-8000; TOA Medical Electronics Co., Kobe, Japan) and serum lipids and electrolyte concentrations (Automatic analyzer JCA-BM 2250; JEOL Ltd., Tokyo, Japan).

**Morphological analysis of the heart and kidney.** After treatments, the rats were killed by exsanguination under deep anesthesia with diethyl ether. Subsequently, their hearts and kidneys were removed and weighed. The hearts were cut in a cross-section along the border of the atrium and ventricle of the heart, and the left kidneys were halved into longitudinal sections. These portions were fixed in 10% formalin solution for a week and embedded in paraffin. These paraffin sections were stained with periodic acid silver methenamine (PASM) and Masson’s trichrome stain by the standard method. Long and short axis dimensions of the heart, interventricular septal (IVST), left ventricular posterior wall (LVPWT), and right ventricular posterior wall thickness (RVPWT) of each rat were measured on photographs of heart preparation that were magnified five to eight times. These structural measurements of the heart were performed post-treatment only. Morphological analysis of myocardial sections stained with Masson trichrome was performed using an automatic image analyzer system fitted to the optical microscope IX71 (SP-500, Olympus, AVIO, Tokyo, Japan). These myocardial section images were scanned using an automatic image analyzer system (SP-500, Olympus, AVIO, Tokyo, Japan) fitted to the microscope6,7). In each animal, 50 randomly selected glomerular profiles were analyzed and averaged as the individual variables of mean glomerular area [A(G)], mesangium area [A(M)], mean glomerular volume [V(G)], and the ratio of A(M) to A(G) [A(M) / A(G)], calculated using previously described equations6,7). For pretreatment control data, morphological analysis of the heart and kidney was also performed in 8-week-old SHRs and WKY rats as described above.

**CPV and CBV.** In separate groups of SHRs that were used for the PR test and morphological analysis, CPV and CBV were measured after the treatment period of sedentary control (n = 6), exercise (n = 6), and Capt administration (n = 6), but not exercise with Capt administration. BW, FC, WD, running distance, BP, and HR of these animals were not measured weekly during the treatment period; therefore, these data were not used in the analytical process. The CPV was determined by dilution of 131I radioiodinated human serum albumin (131I-RISA)16). The CPV measurement was performed on SHRs at 8 weeks of age, and at 24 weeks for the SHR-Sed, SHR-Ex and SHR-Capt groups. The animals were anesthetized by pentobarbital sodium, intraperitoneally infused at a dose of 50 mg/kg. Approximately 1.0 MBq of 131I-RISA was injected through the right external jugular venous catheter. Exactly 10 minutes after injection, 0.2 mℓ of blood was taken from the left external jugular venous catheter. After centrifugation of the blood sample, hematocrit (Hct, %) was measured, and radioactivity in an aliquot (50 μℓ) of plasma was counted in a Packard gamma scintillation spectrometer (COBRA II, Packard Instrument Co, USA). The CPV and circulating blood volume (CBV) were calculated using the following equations: CPV (mℓ) = (Total count of radioactivity of 131I-RISA injected) / (average count of radioactivity per mℓ of plasma), CBV = CPV × 100 / (100-Hct)16). For pretreatment control data, CVP and CBV were also measured in 8-week-old SHRs as described above.

**Statistical procedures.** Results are expressed as mean ± standard error (SE). Data were analyzed by either one- or two-way repeated measures ANOVA using Ekusuru-Toukei 2015 (Social Survey Research Information Co. Ltd., Tokyo, Japan). In the latter case, significant interaction and simple main effects were also evaluated. The Bonferroni post-hoc test was used as appropriate. In some parameters, statistical determination of correlation coefficients was also performed using Pearson’s test. In all cases, the level of significance was set at p < 0.05.

**Results**

**Changes in BW.** Changes in BW throughout the experi-
ment are shown in Fig. 1. BW in all groups gradually increased during the treatment period. There was a significant interaction effect between groups and treatment period \[ F(40, 270) = 3.254, p < 0.001 \], and significant simple main effects of groups in each week were also found from 11 weeks of age to the end of the treatment period \[ e.g. 23^{rd} \text{ week}, F(5, 50) = 6.400, p < 0.001 \]. The WKY-Sed group showed a higher BW than that of the other groups throughout the treatment period, whereas SHR-Capt & Ex exhibited lower levels throughout the treatment period.

**Changes in FC and WD.** FC increased with aging in each group. FC change from the start of the treatment (at 8 weeks of age) to 12 weeks of age was the lowest in the SHR-Capt & Ex group (18.6 ± 2.6 g/day), and it was the highest in the WKY-Ex group (22.2 ± 0.8 g/day). Additionally, there was a significant difference between these two groups. Thereafter, there were no significant differences in FC change up to the completion of the treatment among the 6 groups. WD also increased with aging in each group, and the amount of WD at the beginning of the treatment was lowest in the SHR-Capt & Ex group (24.9 ± 2.4 ml/day), and the highest in the WKY-Ex group (30.3 ± 2.5 ml/day). Again, there was a significant difference between the two groups. Moreover, there was also a significant difference of WD at 16 weeks of age between the SHR-Ex group (42.0 ± 4.9 ml/day) and the SHR-Capt group (34.4 ± 2.7 ml/day), but no differences in WD change were shown among the other groups.

**Changes in BP and HR throughout the treatment.** Changes in resting SBP, DBP, and HR every 2 weeks are shown in Fig. 1. There was a significant interaction effect between groups and treatment periods in all parameters (see text). *p < 0.05, **p < 0.01, ***p < 0.001 shows significant simple main effects of groups in each week.

![Fig. 1](https://via.placeholder.com/150)

**Fig. 1** Changes in body weight (BW), heart rate (HR), systolic blood pressure (SBP), and diastolic blood pressure (DBP) throughout the experimental period. Values are presented as mean ± SE. n of each group = 6. Comparisons were made among the six groups. BW of all groups significantly increased with aging. The sedentary WKY rat (WKY-Sed) group showed the highest BW change throughout the treatment. HR change in all groups tended to decrease with aging, and the daily exercise WKY rat (WKY-Ex) group showed a significantly lower HR change compared to the WKY-Sed and the four SHR groups from 17-23 weeks of age. SBP and DBP in the sedentary SHR (SHR-Sed) and exercise SHR (SHR-Ex) groups changed with significantly higher levels compared to those of the captopril-treated SHRs (SHR-Capt), captopril- and exercise-treated SHRs (SHR-Capt & Ex), and two WKY rat groups from 9-23 weeks of age. There were significant interaction effects between groups and treatment periods in all parameters (see text). *p < 0.05, **p < 0.01, ***p < 0.001 shows significant simple main effects of groups in each week.
effect between groups and treatment period [SBP: F (40, 270) = 5.715, P < 0.001; DBP: F (40, 270) = 4.184, P < 0.001], and significant simple main effects of groups in each week were also found throughout the treatment period in both SBP and DBP. BP in the SHR-Sed and SHR-Ex groups increased from the first week, and higher levels lasted till the end of the treatment period, whereas BP elevation in the Capt-treated SHR groups was inhibited throughout the treatment. BP levels of 2 WKY rat groups exhibited lower levels compared to SHR groups during the latter period of treatment.

The HR of all groups tended to gradually decrease with aging. There was a significant interaction effect between groups and treatment period [F (40, 270) = 2.725, P < 0.001] and significant simple main effects of groups in each week were also found from 17 weeks of age to the end of the treatment period. The WKY-Ex group showed a significant decrease from 17 weeks of age to the end of the treatment period, compared to the WKY-Sed group and four SHR groups.

**Effects on PR test.** Comparisons of ∆MBP responses to Phen and/or Ang II infusions measured at 8 and 24 weeks of age are shown in Fig. 2. There were significant interaction effects in ∆MBP between groups (i.e. 8 weeks SHR, 24 weeks SHR, 8 weeks WKY, and 24 weeks WKY) and dosages of Phen [F (15, 120) = 13.035, p < 0.001] and Ang II [F (15, 120) = 3.613, p < 0.001].

![Fig. 2](https://via.placeholder.com/150)

**Fig. 2** Effects of aging on BP responses to phenylephrine (Phen) and angiotensin II (Ang II) infusions. Values are presented as mean ± SE. n of each group = 6. Changes in mean blood pressure (MBP) from the basal level (∆MBP) by intra-venous infusions of Phen and Ang II were compared among four groups (i.e. 8-week old SHRs, 24-week old SHRs, 8-week old WKY rats, 24-week old WKY rats). A-(a) shows ∆MBP in response to Phen infusion in SHR groups and A-(b) shows WKY rat groups. B-(a) and B-(b) show ∆MBP in response to Ang infusion in SHR and WKY rat groups, respectively. There were significant interaction effects in ∆MBP between groups and dosages of Phen and Ang II (see text for details). Pressor responses to both agents were significantly higher in older groups of rats within the same strain, and the responses were also found to be higher in SHR groups compared to the age-matched WKY groups. wks: weeks, BW: body weight. ***p < 0.001 vs 8 wks SHR; *p < 0.05, **p < 0.01, ***p < 0.001 vs 8 wks SHR; +p < 0.05, ++p < 0.01, +++p < 0.001 vs 24 wks SHR; $p < 0.05, $$$p < 0.001 vs 8 wks WKY.
simple main effects of groups at relevant dosage of Phen and Ang II were also found. \( \Delta \)MBP responses to Phen at 24 weeks of age in both SHRs and WKY rats were significantly higher than those at 8 weeks of age. \( \Delta \)MBP responses to Ang II at 24 weeks of age in SHRs also were significantly higher than those at 8 weeks of age [Fig. 2, B-(b)]. However, the difference of \( \Delta \)MBP responses to Ang II between 8-week- and 24-week-old WKY rats were less obvious than in SHRs. It should be noted that cardiovascular responses in younger and older SHRs were larger than in age-matched WKY rats.

\( \Delta \)MBP responses to Phen and Ang II after treatment conditions are shown in Fig. 3. There were significant interaction effects between groups of animals and dosages of both Phen [F (25, 180) = 6.084, p < 0.001] and Ang II [F (25, 180) = 3.031, p < 0.001]. Significant simple main effects of groups in relevant dosages of Phen and Ang II were also found. \( \Delta \)MBP to Phen in the exercised SHRs was significantly lower than sedentary control SHRs (at 6 and 8 μg/kg), although the response in SHRs remained significantly higher than WKY rats. The Capt-treated SHR groups showed significantly lower responses of MBP to Phen in the exercised SHRs was significantly lower than sedentary control SHRs (at 6 and 8 μg/kg), although the response in SHRs remained significantly higher than WKY rats. The Capt-treated SHR groups showed significantly lower responses of

fig 3

Effects of each treatment in SHRs and WKY rats, including sedentary, daily exercise, captopril, and combined exercise and captopril treatments on \( \Delta \)MBP responses to Phen and Ang II infusions. Values are presented as mean ± SE. n of each group = 6. There were significant interaction effects between groups of animals and dosages of both Phen [A-(a) and A-(b)] and Ang II [B-(a) and B-(b), see text for details]. A-(a) shows the \( \Delta \)MBP response to Phen infusion in the SHR groups after treatment. Although \( \Delta \)MBP response to Phen gradually increased, accompanied by an increasing dosage of Phen in all SHR groups, the \( \Delta \)MBP responses in both the SHR-Sed and SHR-Ex groups were significantly higher than those in both the SHR-Capt and SHR-Capt & Ex groups. It also should be noted that the \( \Delta \)MBP responses in the SHR-Ex were lower than those in the SHR-Sed at 6 and 8 μg/kg. A-(b) shows that \( \Delta \)MBP response to Phen infusion in the WKY-Sed and WKY-Ex groups after treatment. At only the highest concentration of Phen, \( \Delta \)MBP in the exercise group was significantly higher than in the sedentary groups. B-(a) and B-(b) show \( \Delta \)MBP corresponds to Ang II infusion after treatment. The \( \Delta \)MBP response gradually increased with an increasing dosage of Ang II in all groups. The SHR-Capt group showed a significantly lower response than the other 3 SHR groups [B-(a)]. In WKY groups [B-(b)], \( \Delta \)MBP in the exercise group was significantly higher than in the sedentary groups. *p < 0.05, **p < 0.01, ***p < 0.001 vs. SHR-Sed; +++p < 0.001 vs. SHR-Ex; Sp < 0.05, SSp < 0.01, SSSp < 0.001 vs. SHR-Capt; #p < 0.05, ##p < 0.01, ###p < 0.001 vs. SHR-Capt & Ex; && p < 0.01, &&&p < 0.001 vs. WKY-Sed.
Combined therapy of medication and exercise in SHR

A significant higher concentration of serum K (6.4-6.7 mEq/L) and significantly lower ratio of Na/K (20.8-21.6) were shown in the four SHR groups compared to those in the WKY rat groups (K, 5.1-5.6 mEq/L, Na/K ratio, 25.9-28.5), although there was no significant difference in either variable in each group.

**Effects on morphological findings of the heart and kidney.**

Heart weight (HW/BW, g/kg) and structural variables of the heart after treatment are shown in Table 1. The HW/BW and LVPWT in the non Capt-treated SHR groups were significantly larger than those of the Capt-treated SHR groups. The HW/BW in the WKY-Ex group was significantly heavier than in the WKY-Sed group. The relationship between SBP, which averaged readings measured from 20-23 weeks of age, and the HW/BW was 0.800 (p < 0.001) in combined SHR and WKY rat groups.

A typical morphological analysis of myocardial sections stained with Masson trichrome is shown in Fig. 4 (A). The areas more stained with blue are shown in older SHR and WKY rat groups. Myocardial fibrotic areas (MFA) and the ratio of MFA ($\mu$m$^2$) to measured cardiomyocyte area (1,228 $\mu$m$^2$) (MFR) are shown in Fig. 4 (B). The younger SHR and WKY rat groups show a significantly smaller MFA and MFR than the older SHR-Sed and WKY rat groups in each strain.

Both MFA and MFR were significantly smaller in the SHR-Ex and Capt-treated SHR groups than the SHR-Sed group. These variables in the Capt-treated SHR groups tended to be smaller than the SHR-Ex group, but were not significant. Morphological findings of the kidney are shown in Fig. 5. The structural variables were significantly lower in the younger than in the older SHR and WKY rat groups.

**Effects on hematological and biochemical components in blood.** There was no significant difference in the hematological findings among all groups after treatment. Serum HDL-C concentration in the 2 WKY rat groups (49.2-54.5 mg/dl) was significantly higher than in the four SHR groups (39.0-46.5 mg/dl). The SHR-Ex (46.5 ± 5.4 mg/dl) and SHR-Capt & Ex (44.7 ± 7.2 mg/dl) groups showed a significantly higher HDL-C than the non-exercised SHR-Sed (42.3 ± 5.2 mg/dl) and SHR-Capt (39.0 ± 4.2 mg/dl) groups. Serum Tcho and TG concentrations were not significantly different among all groups. A significantly higher concentration of serum K (6.4-6.7 mEq/L) and significantly lower ratio of Na/K (20.8-21.6) were shown in the four SHR groups compared to those in the WKY rat groups (K, 5.1-5.6 mEq/L, Na/K ratio, 25.9-28.5), although there was no significant difference in either variable in each group.

**Effects on CPV and CBV.** Since BW contributed to CPV and CBV, these measurements were expressed as per kg of BW. Post-treatment CPV and CBV in the three SHR groups (i.e. SHR-Sed, SHR-Ex, and SHR-Capt) were not significantly different from the pre-treatment SHR group. After treatment, the SHR-Ex group showed a significantly lower CBV (59.4 ± 0.6 ml/kg) by 4.8% compared to the other SHR groups (62.4 ± 1.6 ml/kg in the SHR-Sed; 62.6 ± 1.4 ml/kg in the SHR-Capt). The CPV (28.9 ± 0.7 ml/kg) in the SHR-Ex group tended to be 4.5% lower compared to other SHR groups, but it was not significant.

### Table 1. Morphological findings of the heart measured after treatment

<table>
<thead>
<tr>
<th></th>
<th>HW/BW (g/kg)</th>
<th>IVST (mm)</th>
<th>LVPWT (mm)</th>
<th>RVPWT (mm)</th>
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<tbody>
<tr>
<td>SHR-Sed (n=6)</td>
<td>4.00±0.06</td>
<td>1.00±0.06</td>
<td>1.10±0.04</td>
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<td>3.97±0.09</td>
<td>1.06±0.08</td>
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<td>0.81±0.06</td>
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<td>3.39±0.05</td>
<td>1.05±0.01</td>
<td>1.02±0.05</td>
<td>0.84±0.09</td>
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<tr>
<td>SHR-Capt &amp; Ex (n=6)</td>
<td>3.47±0.06</td>
<td>1.02±0.07</td>
<td>1.07±0.07</td>
<td>0.81±0.07</td>
</tr>
<tr>
<td>WKY-Sed (n=6)</td>
<td>2.89±0.05***</td>
<td>1.11±0.05</td>
<td>0.98±0.05**</td>
<td>0.94±0.04**</td>
</tr>
<tr>
<td>WKY-Ex (n=6)</td>
<td>3.37±0.07***</td>
<td>1.23±0.08#</td>
<td>1.22±0.09</td>
<td>0.97±0.09#</td>
</tr>
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</table>

Values are presented as mean ± SE. n of each group = 6. HW/BW, heart weight per body weight (g/kg); IVST, interventricular septal thickness; LVPWT, left ventricular posterior wall thickness; RVPWT, right ventricular posterior wall thickness. The HW/BW in the non Capt-treated SHR groups were significantly larger than those of the Capt-treated SHR groups. The HW/BW in the WKY-Ex group was significantly heavier than that in the WKY-Sed group. *p < 0.05, **p < 0.01, ***p < 0.001. #p < 0.05, ##p < 0.01, ###p < 0.001 compared to SHR rat group with the same condition.
groups in each. After treatment, the Capt-treated SHR groups showed significantly lower kidney weight (KW), A(M), A(G), and A(M)/A(G) than those of the non Capt-treated SHR groups, whereas there was no significant difference in these variables in the WKY rat groups.

**Discussion**

In this study, effects of daily voluntary wheel exercise on PR, CPV, and morphological properties of the heart and kidneys in SHR were measured and compared to findings in the exercised SHR with lowered BP by long-term administration of Capt. Our findings suggest that combined treatment with exercise and anti-hypertensive agents is recommended for established hypertensive patients to avoid secondary risks of cardiovascular related diseases and support their physical fitness.

**BW, BP and HR changes.** BW reduction by daily exercise was obvious in WKY rats, but not in SHRs. This might be due to a lesser volume of exercise in the SHR-Ex groups compared to the WKY-Ex group. As expected,
the Capt-treated SHR groups showed a BP reduction level comparable to that of WKY rat groups throughout the treatment period, demonstrating the validity of our experimental protocol to reduce BP levels in SHRs with anti-hypertensive agents. The WKY-Ex group showed a significant decrease in HR throughout the treatment. This finding may reflect exercise-induced bradycardia, the mechanisms of which have recently been reported by Boyett et al.\textsuperscript{17}. However, such bradycardia did not occur in the EHT model, SHR, which performed regular exercise. Possibly, the SHR-Ex group did not perform enough exercise to induce a cardiac response.

**PR test.** Like a physiological ligand, NE, Phen selectively activates the $\alpha_1$-receptor and causes vasoconstriction, but it does not affect cardiac or peripheral vascular $\beta$-receptors. $\Delta$MBP response to Phen was significantly enhanced in the older SHR and WKY rat groups compared with younger groups in each, although it was more enhanced in the SHR than the WKY rat groups. Supiano et al.\textsuperscript{18} demonstrated that normotensive humans show an age-associated increase in SNA combined with appropriate downregulation of $\alpha$-adrenergic responsiveness, whereas older hypertensive humans have an impaired downregulation of $\alpha$-adrenergic responsiveness, and this could contribute to higher BP. It is thought that SNA increased with aging in the WKY rat and SHR groups, but older SHRs produced an enhanced $\Delta$MBP response to Phen and resting BP elevation compared to younger SHRs and age-matched WKY rats due to an impaired downregulation of $\alpha$-adrenergic responsiveness\textsuperscript{18}. Responses of $\Delta$MBP to Ang II as well as Phen were higher in the older SHR group than the younger SHR group and age-matched WKY rats. The angiotensin type 1 receptor (AT\textsubscript{1}R) is the main target receptor of Ang II, and it plays a key role in vasoconstriction, whereas the AT\textsubscript{2}R typically plays an opposing and protective role in such responses\textsuperscript{19}. Consistent with our findings, Dinh et al.\textsuperscript{19} reported that aged mice have a markedly enhanced pressor response to Ang II. Schiffrin et al.\textsuperscript{20} observed that the density of the mesenteric vascular AT\textsubscript{1}R in pre-hypertensive SHR was significantly higher than in age-matched WKY rats, although they failed to see the difference in 12-week-old rats. However, more recent studies by Romero-Nava et al.\textsuperscript{21} demonstrated that gene and protein expression of AT\textsubscript{1}R in the aorta of matured SHRs was higher than in age-matched WKY rats, suggesting that age-related changes in AT\textsubscript{1}R expression in SHRs depend on localiza-

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**Fig. 5** Effects of each treatment in SHRs and WKY rats on kidney weight and morphological findings of the kidneys. Values are presented as mean $\pm$ SE. n of each group = 6. $A(M)$, mean mesangial area; $A(G)$, mean glomerular area; $A(M)/A(G)$, the ratio of $A(M)$ to $A(G)$. All variables shown were significantly less in the younger (8 weeks of age) than in the older (24 weeks of age) SHR and WKY rat groups. After treatment (24 weeks of age), the Capt-treated SHR groups showed significantly lower kidney weight (KW), $A(M)$, $A(G)$, and $A(M)/A(G)$ than those of the non Capt-treated SHR groups, whereas there was no significant difference in these variables between the WKY rat groups. $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$. $^#p < 0.05$, $^{##}p < 0.01$, $^{###}p < 0.001$ compared to the SHR rat group with the same condition.
tion of vessels. Therefore, it could not be ruled out that the enhanced responses of $\Delta$MBP to Ang II in old SHRs (24 weeks old) found in this study might be due to an increased expression of AT$_1$R. Moreover, these findings observed in the SHR$^{18,19,21}$ might not occur with aging in the WKY rat groups as age-related changes in $\Delta$MBP responses to Ang II were less obvious than SHRs. Further examinations will be required to confirm these hypotheses.

After the treatment period, the $\Delta$MBP responses to Phen at higher dosages in SHR-Ex were significantly lower than SHR-Sed, although these exercise-induced effects were not found between WKY groups, demonstrating strain-specific effects. Previous studies$^{12,13,22}$ reported that aerobic exercise increased the production of nitric oxide (NO), and that a negative correlation was shown between changes in BP and plasma NO metabolite concentration$^{22}$. It is also reported that plasma endothelin-1 (ET-1) concentration, which is one of the vasoconstrictors produced by vascular endothelial cells, increases with aging$^{29}$, and that a decrease in plasma ET-1 is accompanied by a lowered BP due to aerobic exercise training$^{22}$. In SHRs, the basal activity and protein expression of endothelial NO synthase in the aorta are known to be lower, whereas plasma ET-1 concentration is higher than WKY$^{10,24}$. Therefore, we speculate that alteration of these parameters by daily exercise is more sensitive in SHRs than in WKY, and these physiological changes may contribute to alteration of the basal contractile characteristics in blood vessels, although this needs to be elucidated. However, we failed to see an attenuation of $\Delta$MBP responses to Ang II in SHRs after exercise training. Moreover, $\Delta$MBP responses to Ang II in WKY-Ex were greater than in WKY-Sed. Both AT$_1$R and $\alpha_1$-receptors are Gq/11-coupled receptors, which increase the cytosolic Ca$^{2+}$ concentrations by activating phospholipase C. Therefore, the differences of cardiovascular responses induced by Phen and Ang II may be due to the different expression levels of these receptors, which specifically respond to either Phen or Ang II. Further experiments are required to confirm this hypothesis. It should be noted that voluntary exercise used in this study led to no effects on resting BP in SHR-Ex compared to SHR-Sed. These findings indicate that improved pressor responses mediated by $\alpha_1$-receptors after the exercise regimen used in this study are not sufficient to reduce the basal level of BP.

The $\Delta$MBP response to Phen in the Capt-treated SHR groups was significantly lower compared to the non Capt-treated groups, and it was comparable with that in the WKY rat groups. Antonaccio et al.$^{25}$ reported that pressor responses to both NE and Ang II were inhibited in the SHRs, which were administered with ACE-I for a long time. Compared to WKY rats, the renin-angiotensin system (RAS) activity in circulating blood, peripheral vascular tissues$^{26,28,27}$ and the brain$^{25}$ is enhanced in SHRs. Furthermore, SHRs are characterized by an increased SNA due to enhanced RAS activity in the brain$^{25}$, as well as an increased number of AT$_1$R and $\alpha_1$-adrenergic receptors in the vasculature as described above$^{29}$. Although these variables$^{20,25-28}$ were not measured in this study, the suppression of $\Delta$MBP response to Phen observed in the Capt-treated SHR groups might be due to the suppression of basal RAS activity in the blood, organs, and peripheral vascular tissues$^{20,25-28}$ caused by long-term Capt administration. On the other hand, the SHR-Capt & Ex groups failed to show a suppression of $\Delta$MBP response to Ang II, although the cardiovascular responses in the SHR-Capt were attenuated after the treatment period. These findings again suggest that exercise used in this study does not affect AT$_1$R expression levels in the blood vessels, or it may even induce counteractive effects to Capt administration-induced physiological changes of blood vessels. Further studies are needed for confirmation.

**Effects of voluntary exercise on CPV and CBV.** After treatment, CBV in the SHR-Ex group was significantly lower by 4.8% than in the SHR-Sed and SHR-Capt groups, although CPV in the SHR-Ex group tended to be lower than in the other 2 SHR groups. Arakawa$^{14}$ suggested that the exercise-induced BP reduction might be caused by decreases in CPV and plasma NE concentration. However, resting BP reduction was not shown, regardless of the reduced CBV in the SHR-Ex group. Conversely, there is a report$^{10}$ that plasma volume was elevated with exercise training in healthy athletes. Exercise-induced circulating fluid volume variation may differ depending on the type of exercise in humans and other species.

**Effects on hematological and biochemical components in blood.** The exercised SHR groups showed significantly higher serum HDL-C than the non-exercised SHR groups. This finding might be attributed to exercise, but it is not known why no significant difference in the serum HDL-C and HDL-C/Tcho ratio between the WKY rat groups was shown. The previous study$^{31}$ reported that there was no difference in serum electrolytes, including Na and K concentrations between the SHR and WKY rat groups. However, a significantly higher concentration of serum K and a lower ratio of Na/K in the SHR groups. Serum electrolytes are thought to be involved in suppressed secretion of aldosterone in the SHR groups. But plasma aldosterone concentration was not measured in this study.

**Morphological analysis of the heart and kidney.** Heart weight (HW) was heavier in the older than younger SHR and WKY rat groups. However, HW per BW (HW/BW, g/kg) was significantly lower in the older than the younger WKY rat groups, whereas it was heavier in the older SHR. The MFA and/or MFR in older SHR (SHR-Sed group) was 6.8 times larger than in younger SHR, but it was only 3.4 times larger in older WKY rats (WKY-Sed...
group) than in younger WKY rats. In this study, cardiac hypertrophy was introduced in the SHR-Sed and SHE-Ex groups; however, it was suppressed by Capt administration with or without exercise. Moreover, the Capt-treated SHR groups showed a significantly smaller MFA and/or MFR than the non-Capt-treated SHR groups, although cardiac fibrosis in the SHR-Ex group was improved compared to that in the SHR-Sed group. It is known that the RAS in the myocardial tissues is involved in cardiac hypertrophy. It is also known that Ang II plays an important role in cardiac hypertrophy and fibrosis of myocardium, in addition to contractions of systemic blood vessels. Inhibition of cardiac hypertrophy and myocardial fibrosis observed in the Capt-treated SHR groups might be caused by a suppression of RAS activity in cardiac tissue due to Capt administration. Similar to findings reported by da Costa Rebelo et al., the finding observed in the SHR-Ex group suggested that exercise regimen per se has an effect of inhibiting myocardial fibrosis. The SHR-Ex group showed a significantly smaller MFA by 35% compared to the SHR-Sed group. Although the precise mechanism is unknown, according to a previous finding, it might be that daily repetition of an exercise-induced oxygen deficiency in the cardiac muscle cells contributes to the amelioration of myocardial fibrosis in the SHR-Ex group. On the other hand, the WKY-Ex group exhibited cardiac hypertrophy, which included an increase in HW/BW, IVST, and LVPWT. Exercise-induced cardiac hypertrophy for normal humans and animals is known as athlete’s heart, which is a physiological adaptation to aerobic exercise and physical conditioning.

After treatment, the SHR-Sed and SHR-Ex groups showed a significantly larger KW, A(M), A(G), and A(M)/A(G) ratio compared to the Capt-treated SHR groups (Fig. 5), although there were no significant differences between the WKY-Sed and WKY-Ex groups. The predominant effect of Ang II on the kidney is constriction of efferent arterioles, which induces an increase in glomerular capillary hydraulic pressure. There are several biological mechanisms for glomerular hypertension-induced renal injury, including capillary distension and mesangial cell and matrix proliferation. Kobori et al. suggest that SHR have enhanced intrarenal angiotensin production that contributes to increased Ang II levels, leading to the development of hypertension and renal injury. It may be that the long-term administration of Capt decreased systemic BP, improved renal glomerular hypertension, and suppressed mesangial cell and matrix proliferation in the kidneys through an inhibition of the RAS activity in the kidney.

It should be noted that treadmill exercise training at moderate-intensity in SHRs delayed the development of hypertension and resulted in structural and functional improvements in the kidney of SHRs by decreasing oxidative stress, inflammation, and RAS activation in the kidney itself. These findings suggest that different types of exercise may induce different effects on cardiovascular and kidney functions. Further study will be required to confirm these hypotheses.

Conclusion

Regular voluntary wheel exercise for an EHT model using SHRs, which are genetically predisposed to hypertension, reduced pressor responses to the α-receptor agonist. However, the exercise produced cardiac hypertrophy, cardiomyocyte fibrosis, and renal hypertrophy, accompanied by an enlargement of glomerular and mesangial areas of the kidneys. On the contrary, the Capt-treated SHR groups showed an inhibition of BP elevation, no organ damage, and suppression of pressor responsivity at levels comparable to those of the normotensive WKY rats. Furthermore, serum HDL-C concentrations in the SHR-Capt & Ex groups were significantly higher than those in the non-exercised SHR groups.

The present results suggest that combined treatment with exercise and an ACE-I such as Capt is the ideal therapy for supporting physical fitness and avoiding critical risks of cardiovascular related diseases in established EHT often seen in middle-aged and elderly populations.

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Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this article.

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