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Effect of Exercise Training on Aortic Tone in Chronic Renal Insufficiency

Stan Shelkovnikov[1], Scott M. Summers[1], Reza Elahimehr[2], Gregory Adams[3], Ralph E. Purdy[1] and Nosratola D. Vaziri[2,3]

BACKGROUND

Chronic renal insufficiency (CRI) is associated with a high incidence of hypertension (HTN), endothelial dysfunction, atherosclerosis and cardiovascular disease. Sedentary life style increases, whereas regular exercise reduces the risk of cardiovascular disease. This study was designed to test the effect of regular exercise on vasodilatory and vasoconstrictive responses of the thoracic aorta in rats with renal mass reduction.

METHODS

One week after 5/6 nephrectomy (CRI) or sham operation (control), rats were housed in either regular cages or cages equipped with running wheels for 4 weeks. Thereafter, thoracic aorta was harvested and contractile response to potassium and phenylephrine (PhE), and relaxation response to acetylcholine (ACh) and sodium nitroprusside (SNP) were determined.

RESULTS

Compared with the control animals, sedentary CRI animals exhibited significant azotemia, proteinuria, HTN, oxidative stress, and increased sensitivity to potassium and PhE, and reduced sensitivity to ACh and SNP. Exercise training for 4 weeks reduced oxidative stress, reversed CRI-induced heightened sensitivity of the aorta to PhE and potassium, and restored its sensitivity to ACh (but not SNP) without affecting arterial pressure or renal function.

CONCLUSIONS

CRI results in heightened sensitivity to potassium- and alpha-1 adrenergic-mediated contractility and depressed sensitivity to endothelium-dependent relaxation in the aorta. Regular exercise improves these abnormalities without affecting arterial pressure or renal function. These observations suggest that exercise training can improve vascular function in animals, and perhaps humans, with chronic kidney disease.

Am J Hypertens 2008; **21**:564-569 © 2008 American Journal of Hypertension, Ltd.

Chronic renal insufficiency (CRI) is associated with a high incidence of endothelial dysfunction, atherosclerosis, and cardiovascular disease.[1,2] These events are associated with and primarily caused by oxidative stress,[3] inflammation,[4,5] reduced nitric oxide (NO) availability,[6,7] upregulation of tissue renin–angiotensin system,[8,9] hypertension (HTN), and dyslipidemia.[10]

Sedentary life style and poor physical fitness increase, whereas, physical fitness reduces the risk of atherosclerotic cardiovascular disease[11,12] and lowers the cardiovascular risk factors in the general population.[13–15] The beneficial effects of physical activity are especially pronounced in high-risk individuals such as those with insulin resistance and obesity.[11]

Regular exercise can reduce the risk of cardiovascular disease by several mechanisms. First, regular exercise can lower arterial pressure in hypertensive individuals.[16] Second, exercise has been shown to improve endothelial function in patients with type 2 diabetes and congestive heart failure.[17,18] Third, in high-risk populations, exercise with or without dietary modifications can improve insulin resistance, inflammation, oxidative stress, and lipid disorders.[11,14,15,19] Finally, long-term exercise training is reported to attenuate HTN, improve endothelial function, and increase NO availability in patients with cardiovascular disease.[20,21] The beneficial effects of long-term exercise on endothelial function appears to be, in part, due to improvement of antioxidant capacity and reduction of reactive oxygen species and thereby increased NO availability in the vascular tissue.[22] In fact, exercise training increases superoxide dismutase, which is a major part of the antioxidant system and as such, exercise can be viewed as an effective antioxidant therapy.[22]

Physical activity is invariably curtailed in patients with advanced chronic kidney disease.[23] Several factors contribute to the reduction of physical activity in this population. These include impaired cardiac function, skeletal muscle weakness, anemia, malnutrition, peripheral neuropathy, deconditioning, among others.[23,24]

In view of the deleterious effects of sedentary life style and favorable impact of exercise on cardiovascular system in the general population, we sought to explore the effect of exercise training on contractile and vasodilatory responses in the thoracic aorta of rats with CRI. We chose to study thoracic

[1]Department of Pharmacology, University of California Irvine, Irvine, California, USA; [2]Division of Nephrology and Hypertension, University of California Irvine, Irvine, California, USA; [3]Department of Physiology and Biophysics, University of California Irvine, Irvine, California, USA. Correspondence: Nosratola D. Vaziri (ndvaziri@uci.edu)

Received 18 October 2007; first decision 18 November 2007; accepted 6 February 2008; advance online publication 20 March 2008. doi:10.1038/ajh.2008.24

aorta because it is commonly affected by atherosclerosis, soft tissue calcification, and loss of elasticity, events that have significant hemodynamic and pathological impacts. The thoracic aorta is the most compliant vessel in the arterial circulation and serves to convert intermittent flow from the heart to a continuous pulsatile flow downstream. The compliance of the aorta is decreased by sympathetic stimulation and increased by endothelium-derived NO. Measurements of phenylephrine (PhE)-induced contraction and acetylcholine (ACh)-induced relaxation were used as surrogates for these aortic functions in this study.

METHODS

Study groups. Male Sprague-Dawley rats with an average body weight of 224 ± 15 g (Harlan Sprague Dawley, Indianapolis, IN) were used in this study. Animals were housed in a climate-controlled vivarium with 12-h day and night cycles and were fed a standard laboratory diet (Purina Rat Chow; Purina Mills, Brentwood, MO) and water *ad libitum*. All rats were initially placed in wheel-equipped cages for 7 days in order to become familiar with the running exercise. During this familiarization period, animals whose running distances were >1 s.d. from the mean value of the entire cohort were removed from the study. The remaining rats were then returned to standard cages and randomly assigned to the CRI and sham-operated control groups. The CRI groups underwent 5/6 nephrectomy by surgical resection of the upper and lower thirds of left kidney, followed by right nephrectomy 4 days later. The control group underwent sham operations. The procedures were carried out under general anesthesia (pentobarbital sodium 50 mg/kg IP) using strict hemostasis and aseptic techniques. The nephrectomy procedures were accomplished via dorsal incisions as described previously.[25]

After a 7-day recuperation period, the CRI rats were randomized to exercise (CRIE) and sedentary (CRI) subgroups. Five animals were included in each subgroup. The animals assigned to the exercise group were placed in the wheel-equipped cages, whereas those assigned to the sedentary subgroups were housed in the standard laboratory cages. Only one animal was placed in each standard or wheel-equipped cage throughout the study period. The animals were observed for 4 weeks. At the conclusion of the study period, animals were placed in individual metabolic cages for a timed urine collection. Arterial blood pressure was measured by tail plethysmography as described previously.[26] The animals were then anesthetized (pentobarbital 50 mg/kg, IP) and killed by exsanguination using cardiac puncture. Blood samples were removed at this time and used for the assays in **Table 1**. The thoracic aorta was also removed immediately for utilization in concentration–response studies. The experimental protocol used in the study was approved by the Institutional Animal Care and Use Committee of the University of California, Irvine.

Exercise metrics. Running wheels were instrumented to record the number of revolutions completed as described previously.[27] These data were collected daily and, in conjunction with the wheel diameter, used to calculate the daily running distance for each rat. The animals were observed for 4 weeks. At the conclusion of the 4-week observation period, the animals were killed as described earlier.

Thoracic aorta preparation. The aorta was placed in a modified Krebs bicarbonate solution bubbled with 95% O_2 and 5% CO_2 and consisting (in mmol/l): NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.6; KH_2PO_4, 1.2; $NaHCO_3$, 24; and D-glucose, 10. Tissues were cleaned to remove fat adhering to the adventitia. The aortas were then sectioned into two transverse rings of 3 mm in length per rat. In some experiments, endothelial cells were removed from the rings by gently rubbing the intimal surface with a steel rod. Removal of the endothelium was later determined by the inability of ACh (1 μmol/l) to elicit relaxation after precontraction by PhE (1 μmol/l).

Tension registration. The aortic rings were carefully suspended between two triangles of 28-gauge stainless steel. The rings were then immediately placed in an organ bath containing 10 ml of Krebs solution, heated to 37 °C, and bubbled with O_2 and CO_2. One of the two triangles was attached to a post in the tissue bath and the other to a Fort 10 isometric force transducer (World Precision Instruments, Sarasota, FL). The transducer was connected to a TMB-4 amplifier transbridge (World Precision Instruments, Sarota, FL) which was itself attached to a MacLab digital to analog converter (AD Instruments, Colorado Springs, CO) which converted tension to a tracing on a computer screen. The aortic rings were then slowly stretched using a micrometer to a tension of 2 g over the course of 10 min. The tissue was allowed to equilibrate in the bath for 2 h while a constant tension of 2 g was maintained.

Table 1 | Body weight, systolic blood pressure (BP), plasma concentrations of creatinine, urea, triglyceride, cholesterol, and malondialdehyde (MDA), creatinine clearance (C_{cr}), and urine protein/creatinine ratio in sham-operated controls (SC), chronic renal failure controls (CRI), and chronic renal insufficiency exercisers (CRIE)

Groups	BW (g)	BP (mm Hg)	Creatinine (mg/dl)	C_{cr} (ml/min)	Urea (mg/dl)	Chol mg/dl	Triglyceride mg/dl	MDA (μmol/l)	U-Prot/Cr (mg/mg)
SC	374 ± 11*	121 ± 5*	0.45 ± 0.03*	2.08 ± 0.5*	43 ± 3*	69 ± 3*	20 ± 30*	92 ± 6	0.07 ± 0.008*
CRI	331 ± 8	154 ± 2	1.35 ± 0.09	0.5 ± 0.14	98 ± 8	186 ± 37	143 ± 36	126 ± 9*	6.3 ± 2.2
CRIE	321 ± 7	162 ± 7	1.26 ± 0.06	0.44 ± 0.07	118 ± 9	148 ± 14	141 ± 20	84 ± 8	12.7 ± 3.5**

*$P < 0.05$ vs. other groups, **$P < 0.05$ vs. CRI group, $n = 5$ in each group.

In preliminary experiments, we found that a 2-g pre-stretch for 2 h provides optimal condition for subsequent induction of maximal contraction. Additional preliminary work indicated that these tissues hold contraction upon stimulation with either PhE or KCl for a minimum of 45 min, which is well under the time required for the experiments.

Concentration–response curves. All concentration–response curves (CRCs) were obtained by the cumulative addition of compounds, allowing force to reach steady-state before the addition of the next concentration. Using endothelium denuded rings, CRCs were first obtained for potassium chloride (KCl). Then tissues were washed once with fresh Krebs solution, allowed to equilibrate back to baseline for 1 h, and then PhE CRCs were obtained. After this, the tissues were washed six times with fresh Krebs and again allowed to re-equilibrate for 1 h. Finally, the rings were contracted to a stable steady state with 1 μmol/l PhE, and sodium nitroprusside (SNP) relaxation curves were obtained. In separate experiments, endothelium-intact thoracic aorta rings were contracted with 1 μmol/l PhE and ACh relaxation CRCs were obtained. Between 70 and 90% of maximal contraction was evoked by 1 μmol/l PhE in the thoracic aorta. Contractions remained stable for at least 40 min in all three treatment groups, a duration longer than that required for completion of the relaxation CRCs. Indomethacin (10 μmol/l) and L-arginine (50 μmol/l) were used with the study of the relaxing effect of ACh to block the formation of prostaglandins and avoid substrate limitation for the formation NO, respectively. The concentration causing 50% of maximal response (EC_{50}) was used as the measure of sensitivity.

Creatinine and urea assays. Plasma creatinine concentration was determined using the DICT-500 kit purchased from BioAssay Systems (Hayward, CA). This colorimetric assay is based on the Jaffe reaction. Plasma urea concentration was measured by the DIUR-500 kit (BioAssay Systems) utilizing the *o*-phthaldialdehyde reagent.

Plasma malondialdehyde and lipid assays. Plasma malondialdehyde (MDA) concentration was measured using high-performance liquid chromatography as described previously.[26] Plasma cholesterol and triglyceride concentrations were measured by colorimetric assays in 96-well plates using reagents purchased from Thermo Electron (Waltham, MA) and the Spectra Max, Gemini XS plate reader (Molecular Devices, Sunnyvale, CA).

Materials. Acetylcholine iodide, PhE, SNP, D-glucose, sodium chloride, KCl, magnesium sulfate, calcium chloride, potassium phosphate monobasic, and sodium bicarbonate were purchased from Sigma Chemical (St Louis, MO).

Statistical analysis. All values are reported as means and s.e.m. Differences among the CRCs of the three groups were determined using two-way ANOVA employing the Prism software package (Graphpad, San Diego, CA). Prism was also used for calculating EC_{50}s and performing unpaired, two-tailed *t*-tests on single dose response. For all statistical tests, $P < 0.05$ level of confidence was accepted for significance.

RESULTS

General data

Data are shown in **Table 1**. Body weight obtained at the conclusion of the study was significantly lower in all CRI rats than in the control animals. As expected, serum creatinine and urea concentrations were significantly elevated in the CRI groups as compared with the corresponding values found in the control rats. Voluntary exercise for 4 weeks did not significantly affect serum urea or creatinine concentrations. Plasma triglyceride and cholesterol concentrations were significantly elevated in the sedentary CRI (CRI) group and were not significantly altered by exercise training. Arterial blood pressure was significantly higher in CRI rats than in the sham-operated animals and was not significantly altered by exercise regimen. As expected, urinary protein excretion was significantly greater in the CRI rats than in the control animals. Urinary protein excretion increased further with exercise training in the CRI group. Physical exercise is well known to raise protein excretion transiently in subjects with proteinuria. This phenomenon is attributed to the alteration of renal hemodynamics and does not appear to reflect deterioration of kidney structure.[28] Plasma lipid peroxidation product, MDA, was significantly elevated, denoting presence of oxidative stress in the sedentary CRI rats. Exercise training reversed CRI-associated elevation of plasma MDA. The latter observation points to amelioration of oxidative stress with exercise training in the CRI animals.

Exercise activity data

The CRI rats assigned to the exercise group ran an average of 3.3 ± 0.2 km/day during the 4-week observation period. This included an initial low volume of running upon re-admittance to the running wheels (1.8 ± 0.16 km/day) and subsequent recovery to 5.5 ± 1.4 km/day by the end of the study.

Contractile response to potassium and PhE

KCl and PhE induced a concentration-dependent contraction in aortic rings from all groups (**Figures 1** and **2**). The sensitivity

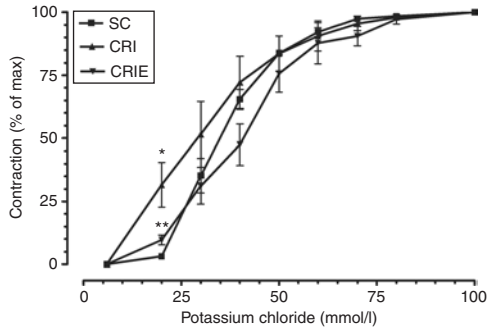

Figure 1 | Cumulative concentration–response curve for potassium chloride (KCl) in denuded rat thoracic aorta rings from sham-operated control (SC), sedentary chronic renal insufficiency (CRI) and CRI-exercise (CRIE) groups. *$P < 0.05$ vs. SC, #$P < 0.05$ vs. CRI, $n = 5$ in each group.

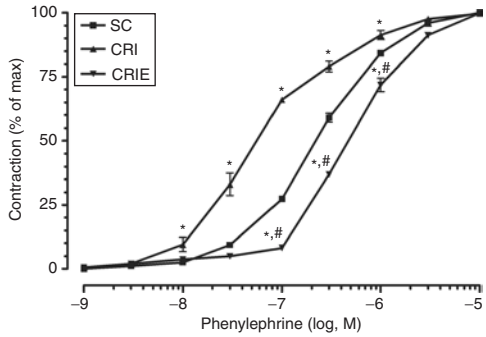

Figure 2 | Cumulative concentration–response curve for phenylephrine (PhE) in denuded rat thoracic aorta rings from sham-operated control (SC), sedentary chronic renal insufficiency (CRI) and CRI-exercise (CRIE) groups. *$P < 0.05$ vs. SC, #$P < 0.05$ vs. CRI, $n = 5$ in each group.

Table 2 | Aortic sensitivity

Groups	KCl	PhE	ACh	SNP
SC	1.52 ± 0.02 (30)	6.62 ± 0.02 (240)	7.74 ± 0.06 (18)	8.82 ± 0.08 (1.5)
CRI	1.45 ± 0.05 (35)	7.26 ± 0.04* (55)	7.15 ± 0.07* (70)	8.14 ± 0.05* (7.2)
CRIE	1.41 ± 0.04 (39)	6.24 ± 0.03* (580)	7.77 ± 0.04 (17)	7.96 ± 0.06* (11)

Contractile sensitivity (pD2 ± s.e.) for potassium chloride (KCl) and phenylephrine (PhE), and relaxing effect of acetylcholine (ACh), and sodium nitroprusside (SNP) in thoracic aorta from different groups: sham-operated control (SC), chronic renal insufficiency control (CRI), and chronic renal insufficiency exercisers (CRIE). Except for ACh, aortic rings were endothelium denuded. Numbers in parenthesis are EC_{50}s expressed in mmol/l for KCl and nmol/l for all others.
*$P < 0.05$ vs. SC, $n = 5$ in each group.

Table 3 | Maximum contraction and relaxation

Groups	KCl (mN/mm)	PhE (mN/mm)	ACh (%)	SNP (%)
SC	12 + 0.5	11.3 + 0.5	97 + 2	86 + 6
CRI	9.5 + 0.5	8.6 + 0.7	82 + 10	77 + 9
CRIE	10.3 + 0.8	11 + 2	71 + 10	62 + 6

No significant differences amongst sham-operated control (SC), chronic renal insufficiency (CRI) control or chronic renal insufficiency exercisers (CRIE), $n = 5$ in each group. Maximum contraction (mN/mm) for potassium chloride (KCl) and phenylephrine (PhE), and maximum relaxation (% of max PhE response) for acetylcholine (ACh), and sodium nitroprusside (SNP), in thoracic aorta from different groups: SC, CRI, and CRIE. Except for ACh, aortic rings were denuded. No significant differences amongst SC, CRI or CRIE ($P > 0.05$), $n = 5$ in each group.

to high concentrations of KCl was not significantly different among the study groups. However, the CRI groups showed increased sensitivity to KCl at 20 mmol/l concentration. The EC_{50} of responses to KCl and PhE are presented in Table 2. There was a significant difference in the EC_{50} between control and CRI with PhE. Maximum responses to KCl and PhE were not significantly different among the study groups (Table 3).

Relaxation response to ACh and SNP

Acetylcholine and SNP induced a 62–97% reduction in contraction evoked by 1 µmol/l PhE in the aortic rings from all animals. Maximum relaxations evoked by ACh and SNP did not differ significantly among the study groups (Table 3). In order to determine aorta sensitivities to the relaxing effects of ACh and SNP, *y* axes for the respective CRCs were normalized as follows. The maximal precontractions to 1 µmol/l PhE were assigned the value of 0% and all maximal relaxations to either ACh or SNP were assigned the value of 100%. The respective resulting CRCs are shown in Figures 3 and 4 and the pD2 (–log [EC_{50}]) values derived from these curves are given in Table 2. Sensitivity to ACh was significantly lower in the sedentary CRI rats compared with the control group but was completely restored by exercise in the exercised CRI (CRIE) animals.

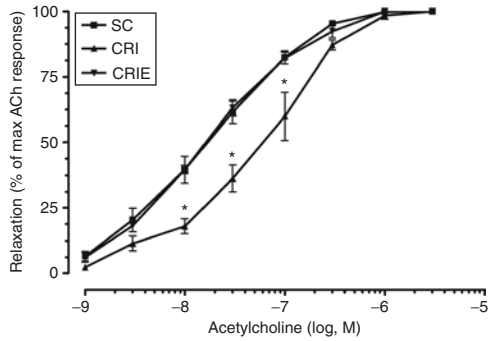

Figure 3 | Cumulative concentration–response curve for acetylcholine after precontraction with phenylephrine (1 µmol/l) in endothelium intact rat thoracic aorta rings from sham-operated control (SC), sedentary chronic renal insufficiency (CRI) and CRI-exercise (CRIE) groups. *$P < 0.05$ vs. SC, $n = 5$ in each group.

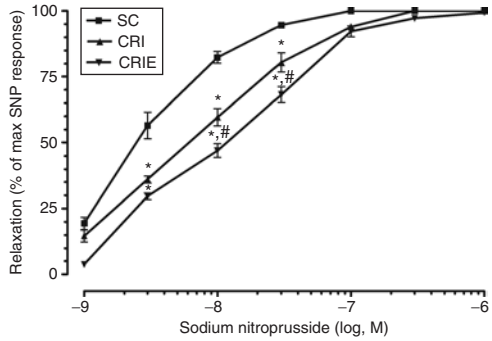

Figure 4 | Cumulative concentration–response curve for sodium nitroprusside (SNP) after precontraction with phenylephrine (1 µmol/l) in denuded rat thoracic aorta rings from sham-operated control (SC), sedentary chronic renal insufficiency (CRI) and CRI-exercise (CRIE) groups. *$P < 0.05$ vs. SC, #$P = 0.05$ vs. CRI, $n = 5$ in each group.

The aorta rings from both sedentary CRI and CRIE rats showed lower sensitivity to SNP as compared with those from the control animals (Table 2, Figure 4). Thus, exercise training failed to reverse the effect of CRI on aorta sensitivity to SNP. In fact, exercise training resulted in further reduction of sensitivity of the aorta to the NO donor. The pD2 values for SNP are presented in Table 2. No significant differences were found between maximal responses to SNP among the study groups (Table 3).

DISCUSSION

The CRI rats placed in the wheel-equipped cages demonstrated progressive increase in the running behavior during the study

period. This observation points to positive effects of chronic exercise training in preserving functional capacity of animals with moderate renal insufficiency.

The sedentary CRI animals used in this study exhibited systemic oxidative stress as evidenced by significant elevation of plasma lipid peroxidation product, MDA. Exercise training attenuated CRI-associated elevation of plasma MDA. This observation points to amelioration of oxidative stress with exercise training in the CRI animals. There is increasing evidence that oxidative stress and reduced NO availability in the kidney and cardiovascular tissues contribute to endothelial dysfunction, HTN, inflammation, cardiovascular remodeling, and atherosclerosis.[29,30] For instance, reactive oxygen species avidly react with and inactivate NO. In addition, uncontained reactive oxygen species inhibit NO production by uncoupling endothelial NO synthase, by depleting NO synthase cofactor, tetrahydrobiopterin, and by raising the level of asymmetrical dimethyl-arginine, which is a potent endogenous NO synthase inhibitor.[31,32] The reduction in endothelium-derived NO availability, in turn, lowers vasodilatory tone and depresses the physiological response to shear stress *in vivo* and ACh *in vitro*. In addition to oxidative stress, vascular calcification and severe dyslipidemia contribute to altered vascular function and structure in CRI.[10] Together, these conditions can affect vascular function and structure. In this study we examined the effects of CRI and exercise training on the contractile and relaxation responses in the thoracic aorta of rats with CRI. We chose thoracic aorta because it is commonly affected by atherosclerosis, soft tissue calcification, and loss of elasticity—events that have significant hemodynamic and pathological implications. Moreover, thoracic aorta accounts for a significant portion of the total arterial bed compliance.[33]

The contractile activity of thoracic aorta was tested using potassium and PhE in endothelium-denuded rings. Although sensitivity at EC_{50} and maximum response to potassium were similar among the study groups, the contractile response to low potassium concentration (20 mmol/l) was significantly higher in CRI than in the control animals. In addition, thoracic aorta from the CRI animals showed a more than fourfold increase in sensitivity to PhE. Similar trends have been reported in several other models of HTN and vascular diseases. For instance, vessels from stroke-prone spontaneously hypertensive rats and rats with renal vascular HTN exhibit heightened sensitivity to potassium-induced contraction. In addition, vessels from spontaneously hypertensive rats show increased sensitivity to adrenoreceptor agonists (PhE and norepinephrine) and calcium ionophore (Bay K 8644).[34–36] The reason for heightened vascular sensitivity to contractile actions of potassium and α-1 receptor agonist, PhE, in CRI animals is uncertain. However, it may be, in part, due to depressed cytosolic ionized magnesium ($[Mg^{2+}]i$) and elevated cytosolic ionized calcium concentration ($[Ca^{2+}]i$), which have been documented in CRI humans and animals.[37,38]

We used ACh and SNP to study relaxation responses in the study animals. The maximal relaxations caused by these agents ranged between 62 and 97%. However, there were no significant differences between the treatment groups. It must be noted that the magnitudes of maximal relaxation were all numerically less in the aorta rings from CRI and CRIE compared with control rats. Thus, it is possible that significant differences would be found using a larger number of animals.

Compared with controls, CRI rats showed markedly reduced sensitivity of de-endothelialized aorta rings to SNP. Moreover, the CRIE group exhibited a further reduction in sensitivity of de-endothelialized aorta rings to SNP. Because SNP is an NO donor, these results show that CRI lowers vascular smooth muscle sensitivity to NO and that this effect is magnified by exercise. CRI also decreased sensitivity to the endothelium-dependent relaxing effects of ACh. This could be, in part, due to CRI-induced reduction of vascular smooth muscle sensitivity to NO (see above). In addition, CRI could have reduced availability of endothelium-derived NO by promoting its inactivation via oxidative stress which was present in CRI animals (**Table 1**).

Exercise restored the sensitivity of endothelium-intact aorta to the relaxing effects of Ach in rats with CRI. Accordingly, the ACh relaxation CRCs in control- and CRIE-derived aortas were completely superimposed (**Figure 3**). It should be noted that the restoration of sensitivity in CRIE-derived aorta to ACh occurred in the face of a very marked decrease in vascular smooth muscle sensitivity to NO in the same tissues (**Figure 4**, **Table 2**). Because prostaglandin synthesis was fully inhibited in these experiments, the relaxation caused by ACh was likely due exclusively to the release of NO from the endothelium. We conclude that exercise markedly enhanced endothelial-dependent control of the aorta tone in tissues obtained from the CRIE rats.

A possible limitation in this study was the use of 1 μmol/l PhE to elicit precontractions in the relaxation measurements. PhE caused ~85 and 70% of maximal contraction in control and CRIE-derived aorta rings, respectively. Thus, this difference could have influenced the results of the relaxation experiment. However, we suggest that it did not on the following grounds. The sensitivity to SNP was markedly reduced in CRIE- compared with control-derived aorta rings. In contrast, the sensitivity to Ach was markedly increased in the CRIE- compared with control-derived rings. The small difference in pre-contraction cannot account for these diametrically opposed shifts in the CRCs to ACh vs. SNP. Thus, we conclude that in CRIE rats, exercise further decreased vascular smooth muscle sensitivity to NO, but enhanced endothelium-dependent relaxation by an effect on the endothelium itself. The enhanced endothelial function manifested as greater sensitivity to nearly all submaximal concentrations of ACh. We suggest that the enhanced endothelial function by exercise may reflect enhanced ACh-induced NO output. In addition, reduction of reactive oxygen species-mediated NO inactivation, occasioned by attenuation of oxidative stress, may have enhanced NO availability in CRI rats undergoing exercise training. These findings are consistent with the results of the studies conducted by Adams *et al.*[39] in humans and Chen *et al.*[40] in experimental animals. Using internal mammary artery specimens obtained during coronary bypass surgery, Adams *et al.*[39] reported a marked improvement

of endothelium-dependent vasorelaxation in patients with coronary artery disease enrolled in a 4-week exercise training program. Similarly, earlier studies have demonstrated enhanced ACh-stimulated endothelium-derived NO release with exercise training in spontaneously hypertensive[40] rats.

In conclusion, CRI results in diminished sensitivity to endothelium-dependent and independent vasorelaxation and increased sensitivity to potassium- and PhE-mediated vasoconstriction in thoracic aorta. Exercise training improves sensitivity to endothelium-dependent (but not endothelium-independent) relaxation and reduces sensitivity to α agonist- and potassium-induced contraction in thoracic aorta. These findings suggest that the favorable effect of exercise may be mediated by improvement in endothelium-derived NO availability in thoracic aorta of CRI animals.

Acknowledgments: The authors thank Paul Bodell, Phuc Tran, Alvin Yu, Jasleen Saini, Rudy Senstad, Tiffany Yu, Julianne Lynn, Phillip Bucur, Sandy Liu, and Nkiruka Ojukwv for their technical support. This work was funded, in part, by the National Institutes of Health (RO1-HL0792-04 to N.D.V. and NIH P01HD048721, Project 1 to G.A.).

Disclosure: The authors declared no conflict of interest.

1. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; 351:1296–1305.
2. Weiner DE, Tighiouart H, Amin MG, Stark PC, MacLeod B, Griffith JL, Salem DN, Levey AS, Sarnak MJ. Chronic kidney disease as a risk factor for cardiovascular disease and all-cause mortality: a pooled analysis of community-based studies. *J Am Soc Nephrol* 2004; 15:1307–1315.
3. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM. The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 2002; 62:1524–1538.
4. Kaysen GA. Inflammation: cause of vascular disease and malnutrition in dialysis patients. *Semin Nephrol* 2004; 24:431–436.
5. Stenvinkel P, Alvestrand A. Inflammation in end-stage renal disease: sources, consequences, and therapy. *Semin Dial* 2002; 15:329–337.
6. Baylis C. Arginine, arginine analogs and nitric oxide production in chronic kidney disease. *Nat Clin Pract Nephrol* 2006; 2:209–220.
7. Vaziri ND. Effect of chronic renal failure on nitric oxide metabolism. *Am J Kidney Dis* 2001; 38:S74–S79.
8. Goncalves AR, Fujihara CK, Mattar AL, Malheiros DM, Noronha Ide L, de Nucci G, Zatz R. Renal expression of COX-2, ANG II, and AT1 receptor in remnant kidney: strong renoprotection by therapy with losartan and a nonsteroidal anti-inflammatory. *Am J Physiol Renal Physiol* 2004; 286:F945–F954.
9. Vaziri ND, Bai Y, Ni Z, Quiroz Y, Rodriguez-Iturbe B. Intra-renal angiotensin II/AT_1 receptor, oxidative stress, inflammation and progressive injury in renal mass reduction. *J Pharmacol Exp Ther* 2007; 323:85–93.
10. Vaziri ND. Dyslipidemia of chronic renal failure: the nature, mechanisms, and potential consequences. *Am J Physiol Renal Physiol* 2006; 290:F262–F272.
11. Gill JM, Malkova D. Physical activity, fitness and cardiovascular disease risk in adults: interactions with insulin resistance and obesity. *Clin Sci* 2006; 110:409–425.
12. Warburton DE, Nicol CW, Bredin SS. Health benefits of physical activity: the evidence. *CMAJ* 2006; 174: 801–809.
13. Oscai LB, Patterson JA, Bogard DL, Beck RJ, Rothermel BL. Normalization of serum triglycerides and lipoprotein electrophoretic patterns by exercise. *Am J Cardiol* 1972; 30:775–780.
14. Roberts CK, Vaziri ND, Barnard RJ. Effect of diet and exercise intervention on blood pressure, insulin, oxidative stress and nitric oxide availability. *Circulation* 2002; 106:2530–2532.
15. Roberts CK, Won D, Pruthi S, Kurtovic S, Sindhu RK, Vaziri ND, Barnard RJ. Effect of a short-term diet and exercise intervention on oxidative stress, inflammation, MMP-9 and monocyte chemotactic activity in men with metabolic syndrome factors. *J Appl Physiol* 2006; 100:1657–1665.
16. Whelton SP, Chin A, Xin X, He J. Effect of aerobic exercise on blood pressure: a meta-analysis of randomized, controlled trials. *Ann Intern Med* 2002;136:493–503.
17. Maiorana A, O'Driscoll G, Cheetham C, Dembo L,Stanton K, Goodman C, Taylor R, Green D. The effect of combined aerobic and resistance exercise training on vascular function in type 2 diabetes. *J Am Coll Cardiol* 2001; 38:860–866.
18. Maiorana A, O'Driscoll G, Dembo L, Cheetham C, Goodman C, Taylor R, Green D. Effect of aerobic and resistance exercise training on vascular function in heart failure. *Am J Physiol Heart Circ Physiol* 2000; 279:H1999–H2005.
19. Varady KA, Jones PJ. Combination diet and exercise interventions for the treatment of dyslipidemia: an effective preliminary strategy to lower cholesterol levels? *J Nutr* 2005; 35:1829–1835.
20. Higashi Y, Yoshizumi M. Exercise and endothelial function: role of endothelium-derived nitric oxide and oxidative stress in healthy subjects and hypertensive patients. *Pharmacol Ther* 2004; 102:87–96.
21. Kingwell BA. Nitric oxide-mediated metabolic regulation during exercise: effects of training in health and cardiovascular disease. *FASEB J* 2000; 14:1685–1696.
22. Kojda G, Hambrecht R. Molecular mechanisms of vascular adaptations to exercise. Physical activity as an effective antioxidant therapy? *Cardiovasc Res* 2005; 67:187–197.
23. Kosmadakis GC, Zerefos N. Physical exercise in dialysis patients. *Int J Artif Organs* 2007; 30:429–434.
24. Adams G, Vaziri ND. Skeletal muscle dysfunction in chronic renal failure: Effect of exercise. *Am J Physiol Renal Physiol* 2006; 290:783–791.
25. Vaziri ND, Ni Z, Wang XQ, Oveisi F, Zhou XJ. Downregulation of nitric oxide synthase in chronic renal insufficiency: role of excess PTH. *Am J Physiol* 1998; 274: F642–F649.
26. Gonick HC, Ding Y, Bondy SC, Ni Z, Vaziri ND. Lead-induced hypertension: interplay of nitric oxide and reactive oxygen species. *Hypertension* 1997; 30:1487–1492.
27. Adams, GR, Zhan C, Haddad F, Vaziri ND. Voluntary exercise during chronic renal failure in rats. *Med Sci Sport Exer* 2005; 37:557–562.
28. Bellinghieri G, Savica V, Santoro D. Renal alterations during exercise. *J Ren Nutr* 2008; 18:158–164.
29. Schulman IH, Zhou MS, Raij L. Nitric oxide, angiotensin II, and reactive oxygen species in hypertension and atherogenesis. *Curr Hypertens Rep* 2005; 7:61–67.
30. Vaziri ND, Rodriguez-Itrube B. Mechanisms of disease: oxidative stress and inflammation in the pathogenesis of hypertension. *Nat Clin Pract Nephrol* 2006; 2:582–593.
31. Forstermann U, Munzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 2006; 113:1708–1714.
32. Sydow K, Münzel T. ADMA and oxidative stress. *Atheroscler Suppl* 2003; 4:41–51.
33. Tuday EC, Meck JV, Nyhan D, ShoukasAA, Berkowitz DE. Microgravity-induced changes in aortic stiffness and their role in orthostatic intolerance. *J Appl Physiol* 2007; 102:853–858
34. Alvarez Y, Briones AM, Balfagón G, Alonso MJ, Salaices M. Hypertension increases the participation of vasoconstrictor prostanoids from cyclooxygenase-2 in phenylephrine responses. *J Hypertens* 2005; 23:767–777.
35. Callera GE, Yeh E, Tostes RC, Caperuto LC, Carvalho CR, Bendhack LM. Changes in the vascular β-adrenoceptor-activated signalling pathway in 2Kidney-1Clip hypertensive rats. *Br J Pharmacol* 2004; 141:1151–1158.
36. Chen HI, Chiang IP. Chronic exercise decreases adrenergic agonist-induced vasoconstriction in spontaneously hypertensive rats. *Am J Physiol* 1996; 271:H977–H983.
37. Vaziri ND, Zhou XJ, Naqvi F, Smith J, Oveisi F, Wang ZQ, Purdy RE. Role of nitric oxide resistance in erythropoietin-induced hypertension in rats with chronic renal failure. *Am J Physiol Endocrinol Metab* 1996; 271:E113–E122.
38. Kaupke CJ, Zhou XJ, Vaziri ND. Cytosolic ionized magnesium concentration in ESRD: effect of hemodialysis. *ASAIO J* 1993; 39:M614–M617.
39. Adams V, Linke A, Krankel N, Erbs S, Gielen S, Mobius-Winkler S, Gummert JF, Mohr FW, Schuler G, Hambrecht R. Impact of regular physical activity on the NAD(P)H oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation* 2005; 111:555–562.
40. Chen Hi H, Chiang IP, Jen CJ. Exercise training increases acetylcholine stimulated endothelium-derived nitric oxide release in spontaneously hypertensive rats. *J Biomed Sci* 1996; 3:454–460.