Effects of exercise training on the vascular reactivity of the whole kidney circulation in rabbits

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De Moraes, Roger, Giovanni Gioseffi, Antonio C. L. Nóbrega, and Eduardo Tibirica. Effects of exercise training on the vascular reactivity of the whole kidney circulation in rabbits. J Appl Physiol 97: 683–688, 2004. First published April 16, 2004; 10.1152/japplphysiol.00923.2003.—Exercise training is known to improve vasodilating mechanisms mediated by endothelium-dependent relaxing factors in the cardiac and skeletal muscle vascular beds. However, the effects of exercise training on visceral vascular reactivity, including the renal circulation, are still unclear. We used the experimental model of the isolated perfused rabbit kidney, which involves both the renal macro- and microcirculation, to test the hypothesis that exercise training improves vasodilator mechanisms in the entire renal circulation. New Zealand White rabbits were pen confined (Sed; n = 24) or treadmill trained (0% grade) for 5 days/wk at a speed of 18 m/min during 60 min over a 12-wk period (ExT; n = 24). Kidneys isolated from Sed and ExT rabbits were continuously perfused in a nonrecirculating system under conditions of constant flow and precontracted with norepinephrine (NE). We assessed the effects of exercise training on renal vascular reactivity using endothelial-dependent [acetylcholine (ACh)] and -independent [nitric oxide (NO)] vasodilators. ACh induced marked and dose-related vasodilatation induced by acetylcholine (ACh), which is known to produce NO-dependent vascular relaxation, has been used to characterize endothelium function in different physiological and pathophysiological conditions both in animal and human studies (7). Vascular reactivity can also be modulated by an endothelium-derived hyperpolarizing factor (EDHF), which probably acts through the activation of Ca2+·sensitive K+ channels (3). EDHF seems to be essentially involved in the local regulation of blood flow in small resistance vessels (10, 37), with the exception of the coronary and renal vascular beds where it also contributes to the modulation of vascular reactivity in conduit arteries (3). Endothelium dysfunction, which can be evidenced by an impairment in endothelium-dependent relaxation, plays a pivotal role in the pathogenesis of cardiovascular diseases, such as arterial hypertension and coronary heart disease (54), as well as in diabetic angiopathy (13). Moreover, NO interferes with key events involved in the development of atherosclerosis, such as smooth muscle cell proliferation, platelet adhesion and vessel wall interaction, and monocyte and leucocyte adhesion (54).

Several studies demonstrated that chronic aerobic exercise alters endothelial function, improving vasodilating mechanisms mediated by NO (15, 38, 59), EDHF (33, 36), and prostanoid metabolites (21), mainly in resistance vessels of the cardiac and skeletal muscle vascular beds (36, 47). It is suggested that increased vascular wall shear stress associated with acute bouts of aerobic exercise may represent the main stimulus for vascular adaptations induced by chronic aerobic exercise (3, 39, 41). Accordingly, chronic aerobic exercise of moderate intensity is considered to have beneficial effects in cardiovascular diseases involving endothelial dysfunction (11, 14, 16, 17).

During dynamic exercise, cardiac output is redistributed, increasing blood flow to active muscle, including the myocardium, and decreasing the perfusion of splanchic and renal circulations (12), which represents a physiological challenge to the control mechanisms of the cardiovascular system. When...
repeated over time, these modifications in blood flow induce structural and functional vascular adaptations, which have been well characterized in coronary (22), pulmonary (19), and skeletal muscle vascular beds (43). On the other hand, few studies have focused on the chronic effects of exercise on the visceral circulation, such as the kidney, where blood flow is known to be reduced during acute exercise (1, 9). However, it is noteworthy that the kidneys, which play a pivotal role in whole body homeostasis, undergo dramatic changes in their blood flow patterns during exercise (12) and are involved in the course of disease states such as primary arterial hypertension (20) and diabetes (13).

Armstrong and Laughlin (1) reported that the reduction in renal blood flow observed during treadmill running in trained animals is lesser than that measured in sedentary ones. Moreover, exercise training reduces norepinephrine (NE)-induced contractile responses in renal arteries of miniature swine, an effect that appears to be abolished by endothelium removal (31). In addition, endothelium-independent relaxation of the renal artery induced by sodium nitroprusside (SNP) was not affected by the training program, thus suggesting that the reduced renal artery vasoconstriction observed in trained animals results from increased release of endothelium-derived relaxing substances. The authors hypothesized that this phenomenon could be relevant in the preservation of renal blood flow during acute exercise in trained animals (31). Nevertheless, the control of regional blood flow depends essentially on the functional characteristics of the microcirculation resistance vessels, whose adaptations to exercise training have not been investigated so far.

Thus the present study was designed to test the hypothesis that exercise training modifies vascular reactivity in the entire renal circulation, using the ex vivo experimental model of the isolated perfused rabbit kidney, which involves both macro- and microcirculation.

MATERIALS AND METHODS

Experimental animals. All procedures were approved by the Oswaldo Cruz Foundation’s Animal Welfare Committee and were consistent with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1996). New Zealand White rabbits of both sexes (from the Oswaldo Cruz Foundation’s breeding farm) weighing from 2.0 to 2.5 kg were housed under controlled conditions of light (12:12-h light-dark cycle) and temperature (22 ± 1°C) with free access to water and standard rabbit food.

Training program. The rabbits were randomly allocated to two groups: 1) exercise rabbits (ExT) assigned to a chronic aerobic training program and 2) sedentary rabbits (Sed) confined to their cages during the same time period. Exercise training was performed on a low-speed motorized treadmill (Universidade de São Carlos, São Paulo, Brazil) and consisted of 12-wk period of running at a speed of 18 m/min during 60 min at no incline (0%). The training program was preceded by a 2-wk period of adaptation to the aerobic exercise, during which the running time and speed of the treadmill were gradually increased from 10 min at 12 m/min to the above-described training schedule. Effectiveness of training was assessed by using treadmill exercise performance tests. After 12 wk of the training program and/or sedentary pen confinement respectively, ExT and Sed animals were submitted to a maximal treadmill running test. The standard exercise test consisted of starting the treadmill at 10 m/min (0% grade) for 1 min, followed by 3 m/min increases each minute up to exhaustion.

Preparation of the isolated perfused rabbit kidney. The rabbits were anesthetized with pentobarbital sodium (40 mg/kg) administered via a marginal ear vein and received an intravenous injection of heparin (500 IU/kg). After a midline laparotomy, the kidneys were isolated, and both the renal arteries and veins were cannulated with polyethylene catheters (Pharmacia Biotech, external diameter 1.8 mm, internal diameter 1.1 mm) and flushed immediately with Krebs-Henseleit solution (50 ml) to remove blood elements. The kidneys were transferred to a humidified petri dish and perfused continuously in a nonrecirculating system under conditions of constant flow at 3.0 ml/min by means of a peristaltic pump (Masterflex model 7518, Cole-Porter Instrument, Vemon Hills, IL) with warm (37°C) Krebs-Henseleit solution gassed with 95% O2-5% CO2. The perfusion line was connected to a pressure transducer (model 7016, Ugo Basile, Comerio, Italy) via a three-way stopcock, and the changes in perfusion pressure were continuously monitored with a preamplifier and recorder system (Gemini 7070, Ugo Basile, Comerio, Italy). The composition of the Krebs-Henseleit solution was (in mM) 118 NaCl, 4.7 KCl, 1.17 MgSO4, 2.5 CaCl2·6H2O, 1.2 NaH2PO4, 25 NaHCO3, and 5.5 glucose (pH 7.4).

Experimental protocols. The preparation was allowed to equilibrate for 30 min before addition of test substances (basal period). Because the isolated kidney is a denervated preparation with a low vascular tone, the renal circulation was submaximally precontracted with a continuous infusion of NE (~10 μM). The concentration of NE was adjusted subsequently to ensure stable tracings during at least 10–15 min. Only those kidneys that maintained a steady perfusion pressure above 100 mmHg in the presence of NE were selected for study. The vasodilating agents were always injected into the perfusion circuit immediately adjacent to the kidney in a constant volume of 100 μl. Kidneys from the same rabbit were always used in different experimental protocols. Cumulative dose-response curves to the vasodilator effects of ACh and SNP were performed in distinct experimental groups, and the peak effects of each dose were calculated. Because the repetitive administration of bradykinin (BK) to the renal circulation induces tachyphylaxis phenomenon, it is not possible to perform dose-response curves. Thus the vasodilator effect of BK was tested using a continuous infusion of BK (10–10 M) during 20 min. In a separate experimental group, we evaluated whether NO is involved in the enhanced renal vascular response resulting from exercise training using a continuous infusion of the NO-synthase inhibitor Nω-nitro-L-arginine methyl ester (L-NAME; 300 μM). The same protocol was used in kidneys obtained from sedentary animals.

Statistical analysis. The results are expressed as means ± SE. Repeated-measures ANOVA was used to test within-group variations, and the Student-Newman-Keuls test was used for post hoc analysis. To evaluate differences between the control and trained groups, Student’s t-test for unpaired samples was used. Differences with P values of <0.05 were considered significant. All calculations were made by computer-assisted analyses using a commercially available statistical package (Graphpad Instat, Graphpad Software, University of London, London, UK).

RESULTS

Exercise training efficacy. ExT rabbits were able to run longer than Sed ones, achieving exhaustion at 770 ± 64 s, compared with 499 ± 24 s for Sed animals (n = 12; P < 0.05), thus confirming that the training program employed was effective to increase exercise capacity.

Basal values of renal perfusion pressure. The renal perfusion pressure measured before NE infusion ranged from 40 to 60 mmHg. Precontraction of the renal circulation with a continuous infusion of NE increased renal perfusion pressure to similar values (P > 0.05) in the different experimental
groups during the baseline period (before injection of vasodilating agents; Table 1).

Effects of exercise training on vascular reactivity of the renal circulation. Figure 1 illustrates the effects of exercise training on the vascular reactivity of the isolated rabbit renal circulation precontracted with a continuous infusion of NE. In the Sed group, ACh induced significant and dose-related vasodilator responses, the reduction in perfusion pressure reaching 41% \(\pm\) 8% \((n = 6; P < 0.05)\). On the other hand, in the kidneys from ExT rabbits, endothelium-dependent vasodilation was enhanced, reaching 54% \(\pm\) 6\% \((n = 6; P < 0.05)\). Continuous perfusion of the isolated kidneys with L-NAME (300 \(\mu\)M) completely blunted the additional vasodilation induced by ACh in the group of ExT animals, compared with Sed ones. Renal perfusion pressure variation in the ExT animals was reduced from 54\% to 38\% in the kidneys perfused with L-NAME \((n = 6; P < 0.05)\). In contrast, L-NAME (300 \(\mu\)M) did not affect ACh-induced vasodilation in kidneys from sedentary animals (Table 1). The maximum variation in renal perfusion pressure induced by BK given as a continuous infusion \((10^{-6} \text{M})\) did not affect ACh-induced vasodilation in kidneys from sedentary animals (Table 1)

<table>
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<tr>
<th>Group</th>
<th>0</th>
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<th>30</th>
<th>100</th>
<th>300</th>
<th>1,000</th>
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<tbody>
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<td>Sed</td>
<td>149 (\pm) 9</td>
<td>136 (\pm) 7</td>
<td>125 (\pm) 8</td>
<td>118 (\pm) 9</td>
<td>102 (\pm) 7*</td>
<td>87 (\pm) 8*</td>
</tr>
<tr>
<td>Sed and L-NAME</td>
<td>156 (\pm) 8</td>
<td>148 (\pm) 8</td>
<td>134 (\pm) 9</td>
<td>113 (\pm) 7*</td>
<td>100 (\pm) 7*</td>
<td>92 (\pm) 8*</td>
</tr>
<tr>
<td>ExT</td>
<td>139 (\pm) 9</td>
<td>122 (\pm) 10</td>
<td>104 (\pm) 10</td>
<td>91 (\pm) 7*</td>
<td>76 (\pm) 6*†</td>
<td>65 (\pm) 7†</td>
</tr>
<tr>
<td>ExT and L-NAME</td>
<td>146 (\pm) 7</td>
<td>136 (\pm) 4</td>
<td>120 (\pm) 4*</td>
<td>110 (\pm) 4*</td>
<td>101 (\pm) 3‡</td>
<td>90 (\pm) 2‡</td>
</tr>
</tbody>
</table>

Sodium nitroprusside, \(\mu\)mol

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<thead>
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<th>0.3</th>
<th>1</th>
<th>3</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sed</td>
<td>127 (\pm) 7</td>
<td>114 (\pm) 7</td>
<td>108 (\pm) 6</td>
<td>103 (\pm) 5</td>
<td>96 (\pm) 6*</td>
</tr>
<tr>
<td>ExT</td>
<td>135 (\pm) 5</td>
<td>111 (\pm) 6</td>
<td>99 (\pm) 7†</td>
<td>89 (\pm) 7†</td>
<td>80 (\pm) 6†</td>
</tr>
</tbody>
</table>

Values are means \(\pm\) SE of 6 experiments. Sed, sedentary animals; ExT, exercise-trained animals; L-NAME, N\(^\omega\)-nitro-L-arginine methyl ester. The renal circulation was submaximally precontracted with norepinephrine (see MATERIALS AND METHODS). *P < 0.05 vs. basal values. †P < 0.05 vs. Sed group. ‡P < 0.05 vs. ExT group.

Table 1. Mean absolute values of renal perfusion pressure

DISCUSSION

The results of the present study demonstrate that exercise training significantly improves both endothelium-dependent and -independent vascular reactivity in the rabbit kidney circulation. These original findings are particularly relevant because the experimental model of the isolated perfused kidney encompasses both the renal macro- and microcirculation.

It has already been shown that chronic aerobic exercise enhances vascular reactivity in different levels of the circulatory system, from large conduit vessels to the microcirculation in different vascular beds (8, 22, 23). Nevertheless, most studies focused on vascular beds that show an increased blood flow during acute exercise such as the coronary and skeletal muscle circulations (8, 22, 23). On the other hand, the effects of chronic aerobic exercise on visceral vascular reactivity, including the renal circulation, where the blood flow actually diminishes during acute exercise, are not well understood.

Armstrong and Laughlin (1), using radiolabeled microspheres, showed that blood flow to most splanchnic organs and the kidneys during acute exercise is maintained at higher levels in trained rats, when compared with sedentary controls. These results were confirmed by Di Carlo and Bishop (9), who demonstrated a smaller increase in vascular resistance in the rabbit renal and mesenteric arteries during acute exercise in trained animals, resulting in a lesser reduction in blood flow to those vascular beds (9). As reviewed by McAllister (30), this phenomenon could be attributed to a lower sympathetic nervous system activity at the same absolute intensity of exercise, resulting from the downregulation of \(\alpha\)-adrenoreceptors or alternatively from changes at the postreceptor level.

The reduced renal vasoconstrictor response to exercise in the trained state has also been investigated in preparations of isolated conduit vessels. In this context, the reduction of the contractile response induced by NE in isolated renal arteries observed after chronic aerobic exercise in swine appears to be abolished by endothelial denudation (31), indicating the importance of endothelium-related mechanisms in the enhancement of vascular reactivity after exercise training. However, in the same study, endothelium-dependent renal artery vasodilation induced by BK was not affected by chronic exercise (31). These unexpected results could be explained by the relative importance of endothelium-dependent vasodilators in the modulation of vascular tone in different parts of the vascular tree. It is well known that the vascular tone is submitted to distinct regulatory mechanisms in large conduit vessels, such as the aorta or the renal artery, compared with small resistance vessels, which are directly involved with the local control of blood flow (26). For instance, the predominant involvement of NO over other endothelium-derived relaxing factors, such as EDHF, in endothelium-dependent vasodilation, seems to depend on some extent on the vessel diameter (10, 37). Thus it was necessary to design specific studies.
to elucidate the effects of chronic aerobic exercise in the intact renal circulation.

In the present study, we used the ex vivo experimental model of the isolated perfused kidney, which represents the renal vascular function as a whole and is classically used in the field of vascular research to investigate renal vascular reactivity in physiological situations as well as in different disease states. The usefulness of this preparation has been widely demonstrated in the study of different physiological and biochemical aspects of renal function (27). This model allows the accurate control of regional hemodynamic variables such as perfusion pressure and flow intensity, as well as the elimination of neurohumoral and blood cell influences on renal function. Actually, the model of the isolated kidney perfused at constant flow with salt solutions has been demonstrated to be a valuable tool for the study of the regulation of renal vascular tone (2, 42, 52, 57). Using this experimental setup, we demonstrated for the first time that exercise training of moderate intensity increases vasodilation of whole renal circulation elicited by endothelium-dependent and -independent vasodilating agents. These results could explain, at least in part, why renal blood flow during acute exercise at the same absolute intensity is higher after a training period in rats and rabbits (1, 9).

The mechanisms responsible for the renal vascular adaptations after chronic exercise were not investigated in the present study, but it might be a consequence of repetitive bouts of increased shear stress occurring during acute exercise. Augmented cardiac output and mean arterial pressure during exercise, associated with renal vasoconstriction, could result in increases of blood flow velocity and shear stress in the kidney circulation, despite the reduction of total renal blood flow. Repeatedly increased shear stress has long been known to be associated with the upregulation of endothelial NO synthase (eNOS) and downregulation of endothelin-1 (ET-1) (3, 4, 39, 41), thus favoring the enhanced vascular responsiveness observed after chronic aerobic exercise programs. Moreover, renal hyperemia occurring immediately after exercise is associated with the upregulation of free radical scavenger systems, which inactivate vascular reactive oxygen species and consequently prevent their interaction with NO, thus providing a better NO bioavailability (5, 45, 46).

On the other hand, it has been recently demonstrated in two different studies that acute high-intensity exercise induces 1) an increase of the expression of ET-1 mRNA in the rat kidney (28), 2) a decrease in the renal levels of NO stable metabolites (nitrites/nitrates), and 3) a reduction of the renal expression of eNOS mRNA (34). These investigators assumed that the lower levels of shear stress in the renal vascular bed during acute exercise, although not directly measured, could result in the downregulation

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Fig. 1. Effects of exercise training on the dose-response curves of vasodilation induced by acetylcholine in the isolated perfused rabbit kidney. The renal circulation was submaximally precontracted with norepinephrine. Percent variation in renal perfusion pressure was calculated as percentage of basal perfusion pressure. Values are means ± SE of 6 experiments. Sed, sedentary animals; ExT, exercise-trained animals; L-NAME, Nω-nitro-L-arginine methyl ester. *P < 0.05 vs. basal values. #P < 0.05 vs. Sed group. §P < 0.05 vs. ExT group.

Fig. 2. Effects of exercise training on the dose-response curves of vasodilation induced by sodium nitroprusside in the isolated perfused rabbit kidney. The renal circulation was submaximally precontracted with norepinephrine. Percent variation in renal perfusion pressure was calculated as percentage of basal perfusion pressure. Values are means ± SE of 6 experiments. *P < 0.05 vs. basal values. #P < 0.05 vs. Sed group.
of eNOS and the consequent reduction of NO production in the rat kidney, along with increased ET-1 expression and release (34). An alternative interpretation for their findings could be considered. Given the pattern of expression of eNOS, which is regulated not only at the transcriptional level but also posttranscriptionally (25), it is possible that complex interactions between different endogenous mediators released acutely during exercise constitute the stimulus for several chronic adaptations. High-intensity exercise, as used in the above-mentioned study of Miyauchi et al. (34), elicits the release of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 (35, 40, 56). Interestingly, it has been shown that TNF-α induces a downregulation of eNOS, which results from a destabilization of eNOS mRNA with no effect on transcription (25, 60). Moreover, TNF-α release has been shown to increase plasma levels of ET-1 (6, 18, 58). Thus the observed reduction of renal eNOS expression and activity as well as ET-1 upregulation could be specific to high-intensity acute exercise. Because ET-1 can chronically upregulate eNOS (29), it is conceivable that repetitive exposure to vasoconstrictor stimuli would provoke a physiological adaptation in the renal circulation favoring vasodilation and thus preserving renal perfusion during acute exercise.

Despite the putative key role of shear stress in the vascular adaptations induced by exercise training, other unknown mechanisms may also be involved. For instance, it has been shown that 4 wk of bicycle ergometer training increased reactive hyperemic blood flow in the forearm of healthy subjects (48), a vascular bed not involved with the active muscles. In addition, exercise training in female miniature swines increased endothelium-dependent relaxation in brachial but not in femoral arteries, an unexpected result in quadruped animals (24).

The results of the present study demonstrate that exercise training induces an increase of NO bioavailability in the kidney circulation because the inhibition of NO production obtained with L-NAME completely blunted the additional ACh-induced renal vasodilation observed in trained animals. Moreover, ACh-induced renal vasodilation in Sed animals was not inhibited by L-NAME infusion. Taken together, these results suggest that the enhanced vasodilation is probably related to functional alterations in the vascular endothelium. On the other hand, renal vasodilation induced by BK infusion was similar in Sed and ExT animals. Although these results were unexpected, different responses to ACh- and BK-mediated vasodilation have been described previously and may suggest a specific adaptation of the cholinergic/NO pathway promoted by exercise training. In fact, it has already been reported that the vasodilator responses to ACh, but not to BK, are enhanced in the renal circulation (for review, see Ref. 44).

Interestingly, our results also showed that endothelial-independent renal vasodilation obtained with the NO donor SNP is potentiated in trained animals compared with the sedentary ones, indicating that exercise training induces an increase in vascular smooth muscle sensitivity to NO. Chronic vascular adaptations could contribute to the preservation of renal function during acute exercise and confer renoprotective effects in diseases where endothelial function is compromised. This effect could be clinically relevant because renal pathophysiological processes resulting from hypercholesterolemia (49) as well as diabetic (13) and hypertensive (55) nephropathies have been associated with dysfunction of the renal vascular endothelium.

In conclusion, we demonstrated that exercise training alters the rabbit kidney vascular reactivity, potentiating endothelium-dependent and -independent renal vasodilation. Thus this effect seems to be related not only to an increased bioavailability of NO but also to the enhanced responsiveness of the renal vascular smooth muscle to NO. The mechanisms involved in this phenomenon as well as its physiological significance deserve further investigation.

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