

# Exercise training protects the renal circulation against high glucose challenge

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## Keywords

diabetes mellitus,  
hyperglycemia,  
isolated rabbit kidney,  
vascular reactivity

Received 25 January 2005;  
revised 24 March 2005;  
accepted 9 May 2005

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## ABSTRACT

It has been shown previously that high glucose causes direct and acute endothelial dysfunction in non-diabetic isolated rabbit kidney. This study assessed whether exercise training is able to maintain normal renal vascular endothelial function despite high glucose exposure. Animals were pen confined (SED) or treadmill trained over a 12-week period (ExT). Kidneys isolated from SED and ExT rabbits were continuously perfused *ex vivo* during 3 h with Krebs–Henseleit solutions containing normal (5.5 mM) or high (15 mM) concentrations of D-glucose. In the SED 5.5 group, acetylcholine (ACh) induced dose-related vasodilator responses, reaching the maximum of  $41 \pm 2\%$  ( $n = 10$ ;  $P < 0.05$ ). In the kidneys perfused with high concentrations of glucose (SED 15), endothelium-dependent vasodilation was significantly blunted. Maximal relaxation in the presence of 15 mM glucose was of  $19 \pm 2\%$ , which was significantly different from the SED 5.5 group ( $41 \pm 2\%$ ,  $n = 10$ ,  $P < 0.01$ ). In the ExT 5.5 group, ACh-induced vasodilation was significantly enhanced when compared with the SED 5.5 group, reaching the maximum of ( $52 \pm 2\%$ ,  $n = 10$ ,  $P < 0.05$ ). Moreover, the exposure of the renal circulation of ExT animals to high glucose did not change endothelium-dependent vasodilation induced by ACh ( $46 \pm 3\%$ ,  $n = 6$ ), when compared with the ExT 5.5 group. Finally, exercise training prevented the deleterious effects of high glucose on endothelium-dependent renal vasodilation (SED 15:  $19 \pm 2\%$  vs. ExT 15:  $46 \pm 3\%$ ;  $P < 0.05$ ). It is concluded that exercise training protects the rabbit renal circulation against endothelial dysfunction elicited by acute exposure to moderately elevated glucose levels, corresponding to the postprandial glycemia of diabetes type 2 patients under treatment. The enhanced renal vasodilator reserve elicited by exercise training turns out to be a response that protects the kidney from the deleterious effects of glycemic peaks.

## INTRODUCTION

Diabetes mellitus (DM) is a highly prevalent disease that have a great impact on morbidity and mortality of the general population [1,2]. Cardiovascular diseases, including accelerated atherosclerosis and microangiopathy, are typical diabetic complications related to an early endothelial dysfunction process [3], which is generally diagnosed by reduced endothelium-dependent vasodila-

tion. In this context, extensive clinical and experimental evidence has already demonstrated the existence of endothelial dysfunction not only in patients with established DM but also in individuals with insulin resistance and impaired glucose tolerance (IGT) [for review, see 4].

In recent years, post-challenge or postprandial hyperglycemia has been found to be an independent risk factor for cardiovascular comorbidities and all-cause mortality in IGT and type 2 diabetes (DM2) [5]. Most likely,

hyperglycemia induces endothelial dysfunction by reducing nitric oxide (NO) bioavailability either by decreasing its production and/or by increasing its inactivation by oxygen-derived free radicals [6]. The endothelial function can also be altered by a decreased production of an endothelium-derived hyperpolarizing factor (EDHF) and vasodilating prostanoids or eventually by increased production of endothelium-derived contracting factors [3,4]. Moreover, hyperglycemia-induced endothelial dysfunction in the renal circulation is known to be involved in the pathophysiology of diabetic nephropathy [7], one of the major clinical complications of the disease. The adverse effects of elevated plasma glucose levels suggest that a tight glycemic control in patients with diabetes could possibly reduce the risk of renal and cardiovascular complications [8]. In fact, many studies have investigated various strategies, including therapeutic agents and regular physical aerobic exercise, aimed at reducing the risk of cardiovascular disease both in patients with diabetes and in subjects with IGT [9].

Using the experimental model of the isolated perfused rabbit kidney, which includes both the renal macro- and microcirculation, Fonteles *et al.* [10] showed that endothelium-dependent vasodilation of the renal circulation is impaired in diabetic animals. Moreover, we recently demonstrated that, even in rabbits without diabetes, acute exposure of renal circulation to high glucose concentrations (15 mM) induces significant impairment of renal endothelium-dependent vasodilation [11]. These results suggest that moderately elevated glucose levels can induce direct and acute endothelial damage in the renal circulation, an effect that turns out to be independent of other metabolic complications associated with diabetes.

Several experimental studies have already demonstrated that chronic aerobic exercise alters endothelial function, improving vasodilating mechanisms mediated by NO [12], EDHF [13] and prostanoid metabolites [14], most likely through an increase in vascular shear stress [15,16]. Accordingly, chronic aerobic exercise of moderate intensity is considered to have beneficial effects in cardiovascular diseases involving endothelial dysfunction [17,18], including DM [19]. In this context, we recently demonstrated that exercise training alters the rabbit kidney vascular reactivity, potentiating endothelium-dependent and -independent renal vasodilation, thus suggesting not only an increased bioavailability of NO but also an enhanced responsiveness of the renal vascular smooth muscle to NO [20]. Thus, it is reasonable to speculate that if the enhanced renal vasodilatory

capacity induced by exercise training also occurs under hyperglycemic environment, it would represent a direct protective effect of exercise on the renal circulation, in addition to the well-known effects of exercise on metabolic control.

Therefore, the main purpose of the present study was to investigate the putative protective effects of exercise training on the endothelial dysfunction induced by high glucose levels in rabbit isolated perfused kidney. To achieve this purpose, we used non-diabetic animals in order to test whether exercise training is able to preserve normal renal vascular endothelial function against the direct and acute deleterious effects of high glucose.

## MATERIALS AND METHODS

### Experimental animals

All procedures were approved by the Oswaldo Cruz Foundation's Animal Welfare Committee and were consistent with the USA 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 ( $22 \pm 1$  °C) with free access to water and standard rabbit food.

### Training program

Exercise training was performed on a low-speed motorized treadmill (Universidade de São Carlos, São Paulo, Brazil) and consisted of 12-week period of running for 5 days a week at a speed of 18 m/min during 60 min at no incline (0%). The training program was preceded by a 2-week 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-mentioned training schedule. Effectiveness of training was assessed using maximal treadmill running tests 24 h after the last exercise session. 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 sodium pentobarbital (40 mg/kg) administered via a marginal ear vein and received an i.v. 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 non-recirculating system under conditions of constant flow at 3.0 mL/min by means of a peristaltic pump (Masterflex® Model 7518, Cole-Porter Instrument Co., Vernon Hills, IL, USA) with warm (37 °C) Krebs–Henseleit solution gassed with 95% O<sub>2</sub>/5% CO<sub>2</sub>. 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/recorder system (Gemini 7070, Ugo Basile). The composition of the Krebs–Henseleit solution was (in mM) 118 NaCl, 4.7 KCl, 1.17 MgSO<sub>4</sub>, 2.5 CaCl<sub>2</sub>·6H<sub>2</sub>O, 1.2 NaH<sub>2</sub>PO<sub>4</sub>, 25 NaHCO<sub>3</sub>, 5.5 glucose (pH 7.4).

### Experimental protocols

The rabbits were either submitted to the chronic aerobic training program (exercise-trained rabbits, ExT) or confined to their cages during the same time period (sedentary rabbits, SED). In order to verify the efficacy of the training program, six SED and six ExT animals were randomly assigned to the standard exercise test. In separate groups of experiments the kidneys isolated from SED and ExT rabbits were perfused continuously during 3 h with solutions containing normal (5.5 mM) or high (15 mM) concentrations of D-glucose, constituting four experimental groups: SED 5.5 ( $n = 10$ ), SED 15 ( $n = 10$ ), ExT 5.5 ( $n = 10$ ) and ExT 15 ( $n = 6$ ). As the isolated kidney is a denervated preparation with a low vascular tone, the renal circulation was pre-contracted with a continuous infusion of norepinephrine (NE,  $\approx 10 \mu\text{M}$ ). The concentration of NE was adjusted subsequently in order to assure stable tracings during at least 10–15 min. Dose–response curves to the endothelial-dependent vasodilator effects of acetylcholine (ACh) were performed by way of injection into the perfusion circuit immediately adjacent to the kidney in a constant volume of 100  $\mu\text{L}$ .

### Glucose concentrations

Glucose levels used in the present study correspond to 2 h post-breakfast median [272.5 mg/dL (15 mM)] values obtained from a cohort of 780 Brazilian type 2 diabetic outpatients regularly attending the diabetes clinic at State University of Rio de Janeiro (M.B. Gomes, personal communication).

### Drugs

The following drugs were used: acetylcholine chloride, ( $\pm$ )-arterenol hydrochloride (noradrenaline), D-glucose and sodium pentobarbital (Sigma Chemical Co., St Louis, MO, USA).

### Statistical analysis

The results were expressed as mean  $\pm$  SEM. Comparisons between the different groups were made with one-way ANOVA while repeated measures ANOVA was used to test within-group variations with time. When an overall difference was detected by ANOVA, the Student–Newman–Keuls test was used to localize the statistically significant differences. Differences with two-sided  $P$  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, UK).

## RESULTS

### Exercise training efficacy

The ExT rabbits were able to run longer than SED ones, achieving exhaustion at  $1050 \pm 146$  s, when compared with  $522 \pm 32$  s for SED animals ( $n = 6$ ,  $P < 0.05$ ), thus confirming that the training program employed was effective in increasing exercise capacity.

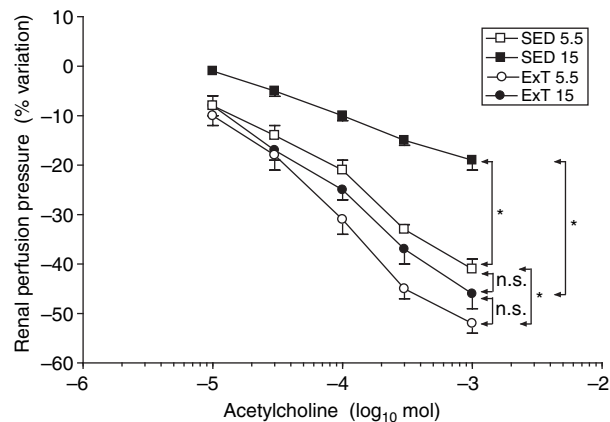
### Effects of high glucose on the rabbit renal vasculature

Table I shows the effects of exercise training on the alterations of vascular reactivity of the isolated rabbit renal circulation induced by high glucose concentrations. There were no significant differences concerning the mean basal values of perfusion pressure between the different experimental groups as shown by ANOVA ( $P > 0.05$ ). Figure 1 shows the relative changes in renal vasodilation between the four experimental groups. In SED 5.5 group, ACh induced dose-related endothelial-dependent vasodilator responses, the reduction in perfusion pressure reaching the maximum of  $41 \pm 2\%$  ( $P < 0.05$ ). However, in the kidneys perfused with high concentrations of glucose (SED 15), endothelium-dependent vasodilation was significantly blunted. Maximal relaxation in the presence of 15 mM glucose was of  $19 \pm 2\%$ , which was significantly different from the SED 5.5 group ( $41 \pm 2\%$ ,  $P < 0.01$ ). In the ExT 5.5 group, ACh-induced vasodilation was significantly enhanced when compared with the SED 5.5 group, reaching the

Group	Acetylcholine ( $\mu\text{mol}$ )					
	Baseline	10	30	100	300	1000
SED 5.5 ( $n = 10$ )	151 $\pm$ 9	139 $\pm$ 8	130 $\pm$ 8	119 $\pm$ 8*	101 $\pm$ 7*	92 $\pm$ 8*
SED 15 ( $n = 10$ )	142 $\pm$ 6	140 $\pm$ 6	135 $\pm$ 6	128 $\pm$ 6#	121 $\pm$ 6*#	118 $\pm$ 5*#
ExT 5.5 ( $n = 10$ )	144 $\pm$ 6	130 $\pm$ 8	111 $\pm$ 8	99 $\pm$ 6*#	82 $\pm$ 5*#	70 $\pm$ 5*#
ExT 15 ( $n = 6$ )	123 $\pm$ 6	114 $\pm$ 7	103 $\pm$ 6†	93 $\pm$ 6*†	78 $\pm$ 7*†	67 $\pm$ 6*†

Values represent mean  $\pm$  SEM of  $n$  experiments. The renal circulation was sub-maximally pre-contracted with NE (see Materials and methods).

\* $P < 0.05$  vs. baseline values; # $P < 0.05$  vs. SED 5.5 group; † $P < 0.05$  vs. SED 15 group.



**Figure 1** Effects of 3-h infusions of normal (5.5 mM) or high (15 mM) concentrations of glucose on the dose-response curves of vasodilation induced by acetylcholine in the isolated perfused rabbit kidney of sedentary (SED) and exercise-trained (ExT) animals. The renal circulation was sub-maximally pre-contracted with norepinephrine. Percentage variation in renal perfusion pressure was calculated as percentage of basal perfusion pressure. Values represent mean  $\pm$  SEM of 6–10 experiments. \* $P < 0.05$ .

maximum of (52  $\pm$  2%,  $P < 0.05$ ). In addition, renal vasodilation induced by ACh was similar ( $P > 0.05$ ) in exercise-trained animals exposed either to normal (ExT 5.5: 52  $\pm$  2%) or high (ExT 15: 46  $\pm$  3%) glucose. Finally, exercise training prevented the deleterious effects of high glucose on endothelial-dependent renal vasodilation (SED 15: 19  $\pm$  2% vs. ExT 15: 46  $\pm$  3%;  $P < 0.05$ ).

## DISCUSSION

The results of the present study demonstrate that exercise training leads to a protective effect on endothelial dysfunction of the non-diabetic rabbit kidney circulation observed after acute exposure to high glucose concentrations corresponding to postprandial levels in patients with type 2 diabetes. Accordingly, enhanced

**Table I** Mean absolute values of renal perfusion pressure (mmHg).

endothelium-dependent renal vasodilator reserve elicited by exercise training turns out to be a response that protects the kidney from the deleterious effects of glycemic peaks, at least in the experimental model of the isolated perfused rabbit kidney.

Endothelial dysfunction caused by impaired bioavailability of endothelial NO is associated with most cardiovascular disease risk factors including hypertension [21] and diabetes [7]. In this context, a substantial body of experimental and clinical evidence indicates that the physiology of the vascular endothelium is altered in DM2 [3,4]. It also is noteworthy that chronic vascular complications still represent the main cause of morbidity and mortality in diabetic patients [6]. In fact, hyperglycemia is known to induce endothelial dysfunction in diabetes by decreasing the production of NO and/or by inactivating it by oxygen-derived free radicals [6]. Moreover, hyperglycemia-induced endothelial dysfunction in the renal circulation is involved in the pathophysiology of diabetic nephropathy [7], which represents nowadays the leading cause of end-stage renal disease [22]. However, studies relative to the acute effects of hyperglycemia on normal vascular reactivity are rather scarce. We have recently demonstrated that acute hyperglycemia corresponding to postprandial levels in patients with DM2 under regular treatment, as well as those used to establish the diagnosis of diabetes, induces endothelial dysfunction of conduit vessels as well as of the renal circulation of non-diabetic rabbits [11]. Even if we consider that these results were obtained with an in vitro experimental model, which presents several obvious differences from the clinical conditions, these results suggest that an acute challenge with moderately elevated glucose concentrations is able to alter the vascular reactivity of the renal circulation. The main mechanism involved in this phenomenon is considered to be the high glucose-induced increase in oxidative stress, which is related to endothelial generation of reactive oxygen species, particularly superoxide anion ( $\text{O}_2^-$ ), and the

consequent activation of protein kinase C [6]. This enzyme is able to induce a de novo synthesis of NAD(P)H oxidase, which significantly contributes to the production of an excess of  $O_2^-$ , resulting in the inactivation of constitutive nitric oxide synthase (NOS), thus reducing NO bioavailability [6]. Moreover, the excess of  $O_2^-$  can also favor an increased generation of the strong oxidant peroxynitrite, which in turn avidly oxidizes tetrahydrobiopterin inducing an uncoupled state of the enzyme NOS, the final effect being an increased production of  $O_2^-$  rather than NO [6].

It is well known that exercise training improves endothelial vasodilator function in patients with DM2, probably eliciting an increase of NO bioavailability [18,23]. In this context, extensive experimental evidence show that chronic aerobic exercise enhances vascular reactivity in territories that undergo an increase in blood flow during acute exercise such as the coronary and skeletal muscle circulations [14]. Notwithstanding, we recently demonstrated that exercise training also enhances renal vascular reactivity, a visceral circulation where the blood flow actually diminishes during acute exercise. Using the experimental model of the isolated perfused rabbit kidney, we showed that exercise training of moderate intensity increases vasodilation of the whole renal circulation elicited by endothelium-dependent and -independent vasodilating agents [20]. The potentiation of endothelial-dependent vasodilation induced by exercise training appeared to be related to an increased NO bioavailability as this effect was completely blunted in the presence of an inhibitor of NO synthesis [20]. Thus, we hypothesized that long-term aerobic exercise training could elicit a protective effect against endothelial dysfunction resulting from acute hyperglycemia in the kidney vasculature. We used the *ex vivo* experimental model of the isolated perfused kidney already mentioned above, which represents the renal vascular function as a whole and is classically used in order to investigate renal vascular reactivity in physiological situations as well as in different disease states [24]. This model allows the accurate control of regional hemodynamic variables such as perfusion pressure and flow intensity, as well as the elimination of neuro-humoral and blood cell influences on renal vascular function [25]. It is also noteworthy that in these experimental conditions endothelial function was not influenced by several risk factors that are present in human studies, such as dyslipidemia, thus allowing the individual investigation of the acute vascular effects of high glucose on the normal endothelium. Endothelium-dependent vasodilation was assessed

using acetylcholine, which is known to produce NO-dependent vascular relaxation and has been classically used to characterize endothelium function in different pathophysiological conditions both in animal and human studies [3,4].

Using this experimental set up, we demonstrated that endothelium-dependent vasodilation of the renal circulation, which is impaired upon acute exposure to high glucose concentrations in sedentary animals, is preserved in rabbits submitted to a chronic aerobic exercise program. These results may represent a novel protective action of exercise training on the deleterious effects of high glucose on the renal circulation, with potential clinical implications. In this context, it is well known that chronic aerobic exercise improves metabolic control in diabetes and metabolic syndrome [9]. If the renal vascular adaptations shown in the present study also occur in these conditions, they could represent another mechanism involved in the overall beneficial effects of exercise training. Nevertheless, future prospective clinical studies should be designed in order to specifically address this issue.

In addition to the hormonal and local biochemical alterations, the increased vascular wall shear stress associated with acute bouts of aerobic exercise, essentially determined by blood flow and viscosity, may represent the main stimulus for the vascular adaptations resulting from chronic aerobic exercise [15,16]. The exercise-induced increase in blood flow most likely elicits its beneficial effects through an increase in the availability of endothelial-derived vasodilating substances [12–14]. Even though renal blood flow is known to be reduced during acute exercise, flow velocity is probably increased rather than decreased, considering that cardiac output and aortic pressure are elevated and vasoconstriction is known to occur in renal arteries during exercise [26]. In spite of the current paradigm implying that shear stress in the renal circulation is reduced during exercise, our results suggest that it could actually be otherwise, considering that flow velocity and not bulk flow determines endothelial shear stress. Thus, renal vascular adaptations in trained animals observed with our experimental protocol could have resulted from repeatedly increased shear stress, which is known to be involved in the release of NO from the endothelial cell through an elevation of intracellular  $Ca^{2+}$  levels [18]. In fact, exercise training simultaneously increases the expression and activity of the enzymes NOS [12] and Cu/Zn superoxide dismutase (SOD-1) [27] in the endothelial cell, resulting respectively in increased synthesis

and reduced inactivation of NO by reactive oxygen species [16]. In this context, it has already been demonstrated that the alterations of endothelium-dependent vasodilation induced by hyperglycemia can most likely be related to an increased inactivation of NO by  $O_2^-$ , rather than to a reduced synthesis and release of NO [28,29]. Moreover, the protective effect against endothelial dysfunction induced by high glucose observed after exercise training could also be due to alterations of other endothelium-derived mediators such as prostaglandins (prostacyclin) and EDHF [14,13]. Alternatively, the upregulation of the principal myoendothelial gap junction protein of intercellular communication, connexin43, and the consequent spread of endothelial cell hyperpolarization to the vascular smooth muscle, may well result from shear stress-induced release of EDHF [30]. Considering that our previous studies had shown that exercise training enhances renal vascular reactivity through an increase in NO bioavailability [20], it is reasonable to speculate that a NO reserve in excess to the maximal renal vasodilating response could be involved in the protective effect of exercise training against glucose-induced endothelial dysfunction. However, the precise mechanisms involved in this phenomenon as well as its physiological significance and therapeutic implications deserve further investigation.

In summary, we demonstrated for the first time that exercise training protects the non-diabetic rabbit renal circulation against the deleterious effects elicited by acute exposure to moderately elevated glucose levels, corresponding to the postprandial glycemia of DM2-treated patients.

## ACKNOWLEDGEMENTS

This investigation was supported by grants of CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and FAPERJ (Fundação de Amparo à Pesquisa, Rio de Janeiro, Brazil) as well as FIOCRUZ (Fundação Oswaldo Cruz).

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