The effects of regular aerobic exercise on renal functions in streptozotocin induced diabetic rats

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Abstract

Diabetic nephropathy is a feared complication of diabetes since it can lead to end-stage renal failure and also it is a risk factor of cardiovascular disease. The important clinical problems caused by diabetic nephropathy are proteinuria and decreased renal function. Exercise is a cornerstone of diabetes management, along with diet and medication. Since acute exercise causes proteinuria and decreases glomerular filtration rate, the effect of exercise on diabetic nephropathy is controversial. The aim of this study was to investigate the effect of regular aerobic exercise on microalbuminuria and glomerular filtration rate in diabetic rats. Moderate diabetes was induced by streptozotocin (45 mg/kg IV) in rats and an aerobic exercise-training program on a treadmill was carried out for 8 weeks. Four groups of rats: control sedentary (CS), control exercise (CE), diabetic sedentary (DS) and diabetic exercise (DE) were included in the study. Blood glucose levels were determined from the plasma samples taken at the end of 4 weeks of stabilization period and 8 weeks of training program. Creatinine clearance (C\text{cr}) and microalbuminuria (MA) levels were determined to evaluate renal functions. The analyzed data revealed that regular aerobic exercise: 1) significantly decreased the plasma glucose level of the DE group compared to the DS group (p < 0.05), 2) significantly decreased the microalbuminuria level of the DE group compared to those of DS group (p < 0.01), 3) significantly decreased the creatinine clearance levels of the DE and CE groups compared to those of CS group (p < 0.05). The results of this study suggest that despite of decreasing creatinine clearance, regular sub-maximal aerobic exercise has a preventive effect on development of microalbuminuria and thus may retard nephropathy in diabetic rats.

Key words: Aerobic exercise, microalbuminuria, nephropathy, diabetes mellitus.

Introduction

Diabetic nephropathy affects 40 % of type 1 or type 2 diabetic patients and is the leading cause of end-stage renal disease (Gross et al., 2005). It increases the risk of death mainly from cardiovascular causes. The earliest clinical evidence of nephropathy is microalbuminuria. Without specific interventions, microalbuminuria may progress to overt nephropathy in years. Hyperglycemia, increased blood pressure levels, and genetic predisposition are the main risk factors for the development of diabetic nephropathy. High blood glucose level can start series of complicated pathophysiological processes. Accumulation of advanced glycosylation end products and changes in glomerular mesangium structure may contribute to renal damage. Therefore, regulation of blood glucose may ameliorate the progression of diabetic nephropathy. The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS) have definitely shown that intensive blood glucose control can significantly reduce the risk of the development of microalbuminuria and overt nephropathy in people with diabetes mellitus (American Diabetes Association, 2004).

For decades, exercise has been considered as a cornerstone of diabetes management, along with diet and medication. Nowadays, it is well known that aerobic exercise improves glycemic control and decreases mortality from cardiovascular diseases in diabetic patients (Sigal et al., 2004). However, exercise induces profound changes in renal hemodynamic and protein excretion. It reduces renal blood flow and glomerular filtration rate. While acute exercise increases urinary protein excretion (Poortmans and Vanderstraeten, 1994), the overall effect of exercise on diabetic nephropathy is controversial. Exercise increases glomerular permeability by influencing renal hemodynamic and by depleting negative charges on the glomerular capillary wall (Ala-Houhala, 1990). Exercise-induced oxidative stress may also contribute to the occurrence of post-exercise proteinuria (Gunduz and Senturk, 2003). Moreover, acute exercise-induced microalbuminuria has been used as a provocative test for the early detection of diabetic nephropathy in patients without microalbuminuria (Felt-Ramussen, 1985; O’Brien et al., 1995). Although some studies showed that physical activity accelerates diabetic nephropathy progression (Matsuoka et al., 1991), several randomized trials in diabetic animals with proteinuria showed that aerobic exercise training decreased urine protein excretion (Ward et al., 1994; Chisera et al., 2000). It has been also shown that the regular aerobic training had a preventive and therapeutic effect on development of microalbuminuria (Calle-Pascual et al., 1993; Lazarevic et al., 2007) and reduces the glomerular ultrastructural lesions in diabetes mellitus (Ward et al., 1994). However, in few studies it is shown that the intensity and duration of exercise seem to influence renal responses (Virvidakis et al., 1986; Poortmans et al., 1996). Thus, we aimed to investigate the effect of regular aerobic exercise on renal functions as measured with microalbuminuria and creatinine clearance (C\text{cr}) in streptozotocin (STZ)-induced diabetic rats in this study.
Methods

Animals
In this study, Wistar strain albino male rats whose beginning weights ranged between 150-300 g were used. According to the aim of the study, the four different groups of rats were set up: control-sedentary (CS; n = 10), control-exercise (CE; n = 10), diabetic-sedentary (DS; n = 11) and diabetic-exercise (DE; n = 14). At most 4 rats were kept in steel cages. They were fed and watered ad libitum and housed in a room maintained at 23±2°C with a 12-h dark-light cycle. The Committee of Animal Care and Use of the Çukurova University approved all procedures.

Experimental protocols

Inducing diabetes
Moderate diabetes was induced by single IV injection of streptozotocin (STZ) (Sigma, S-0130) with a dose of 45 mg/kg (Mythili et al., 2004; Danda et al., 2005) via the jugular vein. The animals in the control group, under the same conditions, had the same amount of 0.1 M citrate buffer injections as well. After 48 - 72 h of STZ injection, the inducement of diabetes was checked by evaluating the urine glucose existence via urine stick (Glukotest-No: 184047). Following the injection of STZ, animals were observed during a period of four weeks for stabilization.

Exercise protocol
Following the stabilization period, the animals in the exercise groups were performed aerobic exercise on a treadmill for a period of eight weeks. Before the training, the rats were adapted to the treadmill by placing them inside the treadmill for 30 minutes, twice a day for two days. The exercise protocol was performed in an 8º inclined treadmill twice a day during five days a week. The exercise protocol was arranged as follows: in the first two weeks animals run with a speed of 12 m·min⁻¹ for 10 minutes, in the following 3 weeks running speed was increased to 22-23 m·min⁻¹ for 40 minutes and in the last 3 weeks, treadmill speed was adjusted to 23-25 m·min⁻¹ for one hour. The related studies have shown that the exercise with this intensity is appropriate for approximately 75% constraining in the capacity of rats (Brooks and White, 1978; Lawler et al., 1993). The exercise sessions were performed during the same hours of the day and approximately 4 hours of resting period was given between training sessions. The animals in the sedentary groups were kept in their cages until the final experiment for 12 weeks.

Blood glucose measurements
The blood glucose levels of control and exercise group of animals were measured by using the samples taken from their tail veins at the end of four weeks of stabilization period and before the final experiment. The plasma glucose levels were determined by means of an enzymatic colorimetric method (Isotec 7130 Glucose).

Urine albumin and creatinine levels
At the end of 8 weeks of the training period, animals were placed in an individual metabolic cages and 24 hours urine was collected twelve to twenty four hours after the last exercise session to eliminate the acute effect of exercise. All samples were stored at −70°C until the day of assay. Urine creatinine level was measured by Jaffé method (Husdan and Rapoport, 1968). Albuminuria was measured using Diasis Diagnostic Systems (Microalbumin Reagent Systems).

Plasma creatinine levels
All animals were taken to a final experiment after the 24 hours urine collection period. In the final experiment, intraperitoneal pentobarbital sodium (50-75 mg·kg⁻¹) anesthesia was performed. Endotracheal tube was inserted into trachea and connected to ventilator (Harvard Rodent Ventilator - Model 683). Carotid artery was catheterized and connected to pressure transducer (Grass, PT 300) and blood pressure was recorded (Grass Polygraph, Model 7). At the end of the experiment blood samples were collected from the carotid artery and stored at −70°C until the day of creatinine assay. Plasma creatinine level was measured by Jaffé method (Husdan and Rapoport, 1968).

Creatinine clearance
Creatinine clearance was calculated as a ratio of urine creatinine concentration (mg·ml⁻¹) multiplied by urine volume (ml·min⁻¹) to plasma creatinine concentration (mg·ml⁻¹).

Statistical analysis
The data were analyzed by the SPSS 11.5 statistical program. The comparison was performed by one-way ANOVA. The difference between groups was evaluated with Duncan test. p < 0.05 were accepted as statistically significant, and confidence interval was chosen as 95 %. Data were given as mean ± SEM.

Results
In this study, body weights of CS, CE and DE animals increased significantly at the end of 12 weeks period (p < 0.001) (Table 1). However, there was no significant difference between beginning and final body weights of DS animals (220.5 ± 8.2 and 225.2 ± 7.3 gram).

After four weeks of STZ injection, the blood glucose levels were measured. The plasma glucose levels of diabetic animals were found to be statistically higher than the control citrate buffer infused animals (p < 0.05). In addition, plasma glucose levels of diabetic animals were also found significantly higher than those of the control animals before the final experiment (p < 0.05). However,

| Table 1. Body weight (gram) changes before and after the 12 weeks of period. Data were given as mean (± SEM). |
|---|---|---|---|---|
| | CS (n = 10) | CE (n = 10) | DS (n = 11) | DE (n = 14) |
| Initial | 249.7 (9.9) | 256.7 ± 8.9 | 220.5 (8.2) | 229.5 (6.2) |
| Final | 295.7 (41.3) *** | 301.4 (19.5) ** | 225.2 (7.3) # | 308.0 (10.7) **** |

** p < 0.01 , *** p < 0.001 compared to initial value in the same group. * p < 0.05 compared to the final values of other groups.
Table 2. Changes of blood glucose (mg/dl) level before and after regular aerobic training. Data were given as mean (± SEM).

<table>
<thead>
<tr>
<th></th>
<th>CS (n = 10)</th>
<th>CE (n = 10)</th>
<th>DS (n = 11)</th>
<th>DE (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>training</td>
<td>153.6 (12.3)</td>
<td>166.6 (4.6)</td>
<td>265.9 (4.6) *</td>
<td>250.2 (6.4) *</td>
</tr>
<tr>
<td>After</td>
<td>151.1 (8.9)</td>
<td>164.7 (6.6)</td>
<td>264.6 (5.4) *</td>
<td>231.5 (10.8)*#</td>
</tr>
</tbody>
</table>

* p < 0.05 compared to both CS and CE groups. # p < 0.05 compared to before and after training values of DS group.

following 8 weeks of training period, blood glucose levels of exercised diabetic animals were significantly lower when compared with the sedentary diabetic animals (p < 0.05) (Table 2).

There was no significant difference among the mean blood pressure values of the CS, CE, DS and DE groups (137.1 ± 8.9; 117.7 ± 7.5; 108 ± 9.05; 111.1 ± 5.6 mmHg, respectively). All blood pressure values of rats were in line with literature (Pannani and Overback, 1976; Özaykan and Doğan, 1999).

The microalbuminuria values showed no significant difference among DE, CE and CS groups (301.1 ± 45; 131.7 ± 21.3; 159.8 ± 24.4 µg·day⁻¹, respectively). On the other hand, the microalbuminuria levels of DS group (594.1 ± 154.6 µg·day⁻¹) was found significantly higher than the others (p < 0.01) (Figure 1).

Creatinine clearance (Ccr) values of the DS group was significantly lower than the CS group (616.8 ± 124.9, 1412 ± 312 µl·min⁻¹, respectively, p < 0.05). Ccr values of the DE group was higher than the DS group and the difference between two groups was not significant (926.5 ± 147.8, 616.8 ± 124.9; µl·min⁻¹, respectively). Even though, creatinine clearance (Ccr) values of the CE group (814 ± 111 µl·min⁻¹) was significantly lower than the CS group (p < 0.05), we did not find any significant difference between this group and diabetic groups (Figure 2).

Discussion

Exercise is a cornerstone of diabetes management, along with diet and medication but acute exercise induces profound changes in renal hemodynamics and protein excretion. While it reduces renal blood flow and glomerular filtration rate, it increases urinary protein excretion; the overall effect of exercise on diabetic nephropathy is controversial. In this study, the effects of regular aerobic exercise on renal functions were assessed in streptozotocin (STZ)-induced diabetic rats.

The final plasma glucose levels of exercised diabetic animals were significantly lower than the beginning, in contrast to the sedentary diabetic animals. Furthermore, animals in DE and CS groups significantly gained weight, but those in DS group did not. These results indicate that regular aerobic exercise improves glycemic control and ameliorates the catabolic process in diabetic individuals. The effects of the exercise on glycemic control in diabetic individuals have been studied in many trials. The meta-analysis which was undertaken by Boule' et al. (2001) showed that exercise training programs had statistically and clinically significant beneficial effects on glycemic control and this effect was not mediated primarily by weight loss.

Although it is known that acute exercise has hazardous effects on renal functions, the effects of regular exercise on renal functions are different (Zambraski, 2006). Although some studies showed that physical activity accelerates diabetic nephropathy progression (Matsumaki, 1991), several randomized trials in diabetic animals with proteinuria showed that aerobic exercise training decreased urine protein excretion. Ward et al. (1994) and Chiasera et al. (2000) reported that exercise training significantly improved metabolic control and reduced albuminuria in diabetic rats. In contrast, Albright et al. (1995) reported that treadmill exercise did not cause any change in proteinuria. Recently, Lazarevic et al. (2007) also showed that prevalence of microalbuminuria tended
to decrease after six months of aerobic exercise in type 2 diabetic patients.

In our study, the presence of high microalbuminuria level in DS group is the evidence for the development of diabetic nephropathy. The microalbuminuria levels showed no significant difference among the DE, CE and CS groups. These data are compatible with the previous studies and suggest that submaximal regular aerobic exercise may prevent the development of diabetic nephropathy and does not cause significant proteinuria in healthy subjects. Because of DS animals were normotensive; we can eliminate hypertension induced nephropathy.

This renoprotective effect of aerobic exercise may be related to its regulatory effect on hyperglycemia. The Diabetes Control and Complications Trial (DCCT) and the United Kingdom Prospective Diabetes Study (UKPDS), which are the major studies in diabetes, showed that a strict glycemic control significantly reduce the risk of microalbuminuria (American Diabetes Association, 2004). Also, De Moraes et al. (2005) showed that exercise training prevented the deleterious effects of high glucose. Therefore, in spite of the possible hemodynamic changes on kidney, the aerobic training may ameliorate the progression of diabetic nephropathy with regulatory effect on hyperglycemia. On the other hand, it is shown that 8 weeks of endurance training had decreased thiobarbituric acid reactive substances levels in kidney at rest and after exercise in streptozotocin induced diabetic rats. Therefore, beneficial effects of aerobic exercise on exercise-induced oxidative stress might have also taken part in this renal protective effect (Gul et al., 2002).

At the end of this study, creatinine clearance (Ccr) levels of the DS group were significantly lower than the CS group (p < 0.05). These unexpectedly decrease in Ccr levels of the CE group suggest that regular exercise may also result in adverse effects on renal functions. This result may be explained with renal ischemia–reperfusion injury and / or exercise-induced oxidative stress. It is well known that strenuous exercise causes marked reductions in renal blood flow which is probably due to renal vasoconstriction (Hisanaga et al., 1999). Oh et al. (2006) showed that renal vasoconstriction and renal ischemia–reperfusion injury was probably the main pathophysiologic mechanism of acute renal failure induced by exercises. Repeated minor ischemic events may be the cause of this low level of Ccr in exercised animals. Also, exercise-induced oxidative stress may contribute to the occurrence of post-exercise decrease of Ccr in CE group (Gunduz and Senturk, 2003; Atalay and Laaksonen, 2002). On the other hand, the role of the intensity and duration of exercise program is an important factor in the renal response to exercise. Poortmans et al. (1996) showed that exercise in different duration and intensity affected different part of the kidney and postexercise proteinuria was directly related to the intensity of exercise rather than its duration. An increased susceptibility of the kidney to ischemic injury in diabetic rats has been previously shown by Melin et al (1997). However, in our study, the Ccr level of DE group was not significantly lower than CE group. It has been shown that hyperglycemia was the most probable contributing factor in the development of ischemic acute renal failure and metabolic control before renal ischemia–reperfusion injury protected kidneys from damage (Melin et al., 2003).

**Conclusion**

In conclusion, regular submaximal aerobic exercise can facilitate the control of blood glucose level, and has a preventive effect on development of microalbuminuria, despite decreasing creatinine clearance. Further investi-
ations are needed to estimate the optimal intensity and duration of physical activity for renal protection in diabetic persons.

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Key points

- Regular submaximal aerobic exercise can facilitate the control of blood glucose level in diabetic rats.
- Streptozotocin induced diabetes may cause microal-
buminuria and regular submaximal aerobic exercise may have a preventive effect on renal functions.

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