Effects of Endurance Training on Lipid Metabolism and Glycosylated Hemoglobin Levels in Streptozotocin-induced Type 2 Diabetic Rats on a High-fat Diet

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Abstract. [Purpose] Exercise has been recognized as a simple and economical therapeutic modality that effectively benefits patients with diabetes, for instance, increasing insulin sensitivity in type 2 diabetes. However, thus far, no studies have examined the effect of endurance training exercises on type 2 diabetes. Therefore, this study examined the effect of endurance training exercise regimens on body weight, glucose and insulin levels, lipid profiles, and HbA_{1c} levels in STZ-induced type 2 diabetic rats on a high-fat diet. HbA_{1c} was considered an indicator of glucose control during endurance training. [Methods] A total of 36 rats were included in this study. Diabetes was induced by administering STZ to 2 groups of 12 rats each, and, the remaining 12 rats were classified as the normal group. Biochemical parameters were measured 28 days later, and included: serum total cholesterol, triglyceride, high-density lipoprotein, glycosylated hemoglobin, glucose, and insulin levels. [Results] A significant decrease in serum TC and TG levels, and an increase in HDL cholesterol level were observed in the endurance training group. Moreover, blood glucose and HbA_{1c} levels after 28 days of exercising were significantly lower in the endurance training group than in the control group (p<0.05). [Conclusion] These results indicate that endurance training affects body weight and, lipid profiles, as well as fasting blood glucose, HbA_{1c}, and insulin levels, in STZ-induced type 2 diabetic rats on a high- fat diet. We suggest that endurance training exercises may exhibit therapeutic, preventative, and protective effects against diabetes mellitus through improving lipid metabolism, glycemic control, and HbA_{1c} levels.
Key words: Diabetic mellitus, Endurance training, Glycosylated hemoglobin

INTRODUCTION

Diabetes mellitus is a very common chronic disorder characterized by hyperglycemia. Over 90% of diabetic mellitus cases are classified as type 2 diabetic mellitus (T2DM), a condition indicated by reduced sensitivity to the insulin hormone, and subsequently, diminished insulin activation of the glucose transporter (GLUT) system in skeletal muscles[1]). In T2DM, individuals have endogenous insulin production, but insulin activity at the cellular level is ineffective due to insulin resistance[2]).

The development of T2DM is commonly associated with obesity, lipid metabolic dysfunction, and hypertension, all of which are factors that may influence a patient's quality of life[3, 4]). Adipose tissue plays an important role in controlling body glucose homeostasis in both normal and disease states[5]). Increased triglyceride accumulation, especially in visceral fat or subcutaneous adipose tissues, results in large adipocytes that are resistant to insulin-dependent inhibition

of lipolysis, leading to an increase in glycerol and free fatty acid release[6]). Consequently, hyperglycemia and hyperlipidemia, which are considered significant stress factors in the body that have also been shown to cause cellular inflammation and contribute to the pathogenesis of T2DM[7]).

T2DM patients may not require insulin treatment for survival and mostly need other types of therapy to prevent hyperglycemia and its subsequent complications. Type 2 diabetes belongs to a group of diseases (cardiovascular diseases, Alzheimer's disease, cancer, etc.) that are defined by their increased risk of development in association with physical inactivity, and hence, it is important to understand the mechanisms underlying the beneficial effects of exercise training[8–10]). Physical inactivity has been shown to play a key role in the development of insulin resistance and pancreatic β-cell dysfunction[11]). Moreover, regular aerobic exercise has been increasingly viewed as an effective therapeutic approach for the management of T2DM[12, 13]). Indeed, regular aerobic exercise reportedly improves metabolic imbalance and insulin sensitivity, reducing the risk of diabetes complications such as kidney failure, blindness, and cardiovascular disease[14]).

In the present study, we examined the hypothesis that

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Table 1. Experimental group design

Groups (Total N=36)	Treatment conditions
Group I (n=12)	Rats were fed standard diet. T2DM was not induced.
Group II (n=12)	Rats were fed HFD (40% of calories as fat) for 2 weeks and then injected with streptozotocin (50 mg/kg, IP).
Group III (n=12)	Rats were fed high fat diet (40% of calories as fat) for 2 weeks, injected with streptozotocin (50 mg/kg, IP), and then performed endurance exercise (20 m/min on a 0% incline, 30 min/day).

endurance training influences the lipid profile as well as glucose, insulin, and hemoglobin levels in diabetes. Due to lack of sufficient data with regard to this topic, our study was aimed at assessing the beneficial effects of endurance training on the lipid profile and glucose, insulin, and glycosylated hemoglobin (HbA_{1c}) levels in streptozotocin (STZ)-induced type 2 diabetic rats on a high-fat diet.

SUBJECTS AND METHODS

In this study, we used 36 male 5-week-old Sprague–Dawley rats (Orient, Seoul, Korea), each weighing 150–160 g. Animals were reared at a temperature of 22 ± 2 °C and 55 ± 5% humidity with a 12/12-h light/dark cycle. Rats were acclimatized for 1 week before experiments were performed. All procedures involving rats were conducted in strict compliance with the animal welfare act, public health services policy, and guidelines established by the university institutional animal care and use committee. A total of 36 rats were randomly divided into 3 groups of 12 rats each (Table 1).

The high-fat diet (HFD) fed, STZ-treated rats provides a novel animal model for T2DM that simulates the human syndrome and is suitable for testing antidiabetic compounds[15]. Animals were divided into 3 groups: group I (control group) rats were fed a standard diet (12% of calories as fat); group II (HFD/STZ group) rats were fed a HFD (40% of calories as fat) for 2 weeks, and then STZ (50 mg/kg of body weight, Sigma, USA) was administered through intraperitoneal (IP) injection; and group III (HFD/STZ + endurance training) rats were fed a HFD (40% of calories as fat) for 2 weeks before being injected with STZ (50 mg/kg of body weight), and treadmill exercise was performed 5 days a week, for 4 weeks. Before the STZ injection, blood glucose levels in experimental and control rats were measured and compared. Animals with fasting glucose levels ≥ 150 mg/dL after the STZ injection were considered to resemble those observed in T2DM in humans.

Diabetes was induced in rats by a single IP injection of STZ (50 mg/kg of body weight) freshly dissolved in a 0.1 M citrate buffer (pH 4.5). Control rats were injected with citrate buffer only. Diabetes was confirmed in the STZ-injected rats by measuring fasting blood glucose levels 72 h post injection. The endurance training group followed a 4-week constant treadmill exercise regimen (20 m/min on a 0% incline) starting at 30 min/day, and gradually increasing the duration to 1 h/day by the end of the second week. Exercise sessions were conducted for each experimental

group 5 times a week, between 10.00 a.m. and 11.00 a.m. and involved a 5-min warm-up phase with slowly increasing speed. Body weight was measured at the end of the experiment.

After 4 weeks of exercise, rats were fasted (for 12 h overnight) and then sacrificed; blood samples were obtained. Rats did not exercise during the 24-h prior to sample collection. Approximately 1 mL of blood was withdrawn from a prominent superficial vein, using a clean venipuncture with minimal stasis; blood was collected in a tube containing ethylenediaminetetraacetic acid (EDTA), centrifuged at 3,500 rpm for 10 min, and then frozen. Serum glucose, insulin, HbA_{1c}, total cholesterol, triglyceride, and HDL levels were determined. Fasting serum glucose levels were determined using Glucotrend Plus Glucose Test Strips (Roche Diagnostics GmbH, Mannheim, Germany). Serum insulin was assayed using an enzyme-linked immunosorbent assay kit (ELISA, Boehringer Mannheim, Germany). HbA_{1c} levels were measured using a DCA 2000 analyzer (Siemens, Munich, Germany). Serum levels of total cholesterol (TC), triglycerides (TG), and high-density lipoprotein (HDL) cholesterol were estimated using commercial diagnostic reagents (Bayer, Pittsburgh, PA, USA) and a biochemical analyser (RM 2060-18, Eltec. Co., Italy).

All statistical analysis was performed using SPSS version 18.0 for Windows (Statistical Package for the Social Sciences). All values are expressed as means ± standard deviation. Comparisons of body weight as well as glucose, HbA1c, insulin, TC, TG, and HDL levels between the 3 groups were performed using the Kruskal-Wallis test, a nonparametric counterpart to the ANOVA test. The relationship between pre-experimental and post-experimental values was assessed using the Wilcoxon test, a nonparametric test. A p value < 0.05 was considered statistically significant.

RESULTS

Table 2 shows the effect of endurance training on body weight and visceral fat weight. Body weight and visceral fat levels significantly differed between the experimental groups ($p < 0.05$).

Table 3 shows the effect of endurance training on TC, TG, and HDL serum levels. A significant decline in TC and TG ($p < 0.05$) levels, and a significant increase in HDL ($p < 0.05$) level were observed in the endurance training group (group III). The TC level increased to 75.2 ± 9.60 mg/dL in the T2DM group (group II), while it decreased to

Table 2. Effect of endurance exercise on body weight and visceral fat level in a type 2 diabetes mellitus rat model (Unit: g)

	Group I	Group II	Group III
Body weight (g)	228.50±10.5	265.80±12.0*	235.60±14.6**
Visceral fat (g)	3.24±0.35	4.80±0.26*	3.4±0.42**

All values are shown as means ± SD. *p<0.05 as compared with group I. **p<0.05 as compared with group II.

Table 3. Effect of endurance exercise on serum lipid profiles in a type 2 diabetes mellitus rat model (Unit: mg/dL)

	Group I	Group II	Group III
Total cholesterol	46.50±6.50	75.2±9.60*	95.37±13.10**
Triglycerides	56.44±7.90	84.80±12.2*	66.36±8.90**
HDL cholesterol	35.70±7.50	26.20±6.80*	32.90±5.00**

All values are shown as means ± SD. *p<0.05 as compared with group I. **p<0.05 as compared with group II.

Table 4. Effect of endurance exercise on insulin and HbA_{1c} serum levels in a type 2 diabetes mellitus rat model

	Group I	Group II	Group III
Glucose (mg/ml)	99.50±14.5	185.4±13.0*	115.45±12.5**
Insulin (µg/l)	1.8±0.03	1.5±0.03	1.7±0.06
HbA_{1c} (mg/g of Hb)	0.245±0.04	0.580±0.02*	0.295±0.02**

HbA_{1c}: glycosylated hemoglobin. Data are presented as means±SD. *p<0.05 as compared with normal group. **p<0.05 as compared with the placebo group.

95.37±13.1 mg/dL in the endurance training group. Moreover, the HDL cholesterol level decreased to 26.20 ± 6.80 mg/dL in the T2DM group, and to 32.90±5.00 mg/dL in the endurance training group.

Table 4 shows the effect of endurance training on serum glucose, insulin, and HbA_{1c} levels. The glucose level increased to 185.40 ± 13.0 mg/dL in the T2DM group, while it decreased to 115.45±12.5 mg/dL (p < 0.05) in the endurance training group.

The HbA_{1c} level was significantly decreased by 0.295 ± 0.02 mg/g of total hemoglobin (Hb) in the endurance training group compared with the T2DM group, which showed a decrease of 0.580 ± 0.02 mg/g of total Hb (p < 0.05). However, the difference in insulin level between the groups was not statistically significant (Table 4).

DISCUSSION

Insulin resistance is a common metabolic impairment that affects individuals with type 2 diabetes, which itself is reportedly associated with sedentary lifestyles, consumption of high-energy diet, obesity, and physical inactivity[16, 17]. The insulin hormone and receptor are expressed in metabolic organs such as the skeletal muscles, pancreas, liver, and fat tissue, which play an important role in glucose and lipid metabolism[18]. In the present study, we demonstrated that endurance training has a beneficial effect on body weight management, lipid profiles, and on glucose, insulin

hormone, and HbA1c levels in STZ-induced type 2 diabetic rats on a high-fat diet.

Exercise, which has been studied for its role in improving insulin resistance, is classified by type, intensity, and activity period[19, 20]. Of the different types of exercise, endurance training reflects prolonged and continuous periods of contractile activity against low resistance, whereas strength training involves short periods of contractile activity against a high resistance[11]. Our results showed that endurance training improved dyslipidemia in HFD/STZ rats. Dyslipidemia, which is associated with T2DM, is characterized by low levels of HDLs, high levels of TGs, and LDLs that are associated with very low-density lipoprotein (VLDL)[21]. High levels of TG represent a major risk factor for atherosclerosis and subsequent cardiovascular complications in type 2 diabetic patients[22]. Our results showed that endurance training improved lipid metabolism as well.

Several experimental and clinical studies, have examined the effect of regular exercise on metabolic disease and diabetes, as well as on cardiovascular disorders[13, 14, 23]. Insulin resistance in skeletal muscles is a critical, early defect that leads to the initial development of impaired glucose tolerance in prediabetes, and subsequently, to progression from prediabetes to overt type 2 diabetic mellitus[24]. Exercise has been shown to exhibit insulin-like activity by increasing muscle glucose uptake capacity via reduction of intramuscular fat reserves[25].

In this study, the blood glucose level was determined in

patients by measuring the HbA_{1c} level. Normal red blood cells contain below 5.8% HbA_{1c}, which increases during diabetes, reflecting the average glucose amount that red blood cells are carrying[26, 27]. In the present study, the high HbA_{1c} level in red blood cells reflected a high blood glucose level in the T2DM group that was decreased in endurance training group. It is possible that HbA_{1c} more than for the reduction of hyperglycemia, in determining the improvement of hyperglycemia might be supposed.

Our data clearly demonstrated that endurance training can act as a major homeostatic regulator of lipid metabolism and glucose and HbA_{1c} levels, which has important implications for diabetic mellitus management. Endurance training seems to play an important role in improving glucose homeostasis. Therefore, our data suggest that endurance exercise can potentially be helpful in the management of diabetes, especially in patients who experience adverse effects from current drug therapies and invasive treatments.

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