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Full Length Research Paper

The effect of long–term exercise training on the blood glucose level and weight in alloxan administered mice

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This study was to evaluate the effect of long-term exercise training on blood fasting glucose (BFG) level and weight in alloxan administered mice. Forty mice, *Mus musculus* species was alloxan administered by an intraperitoneal (i.p.) injection 3 times in two weeks intervals (200 mg/kg b.w.), and training program was administered by treadmill for 10 min 5 day/week and exercise period was prolonged for 2.5 min in a week. In the 8th week it was observed that sedentary male group BFG levels (172.3 mg/dl) were statistically significant higher than other groups ($p < 0.05$). Measurements at the end of the 10th week were showed that untrained sedentary group's BFG were found higher with statistically significant levels than trained groups ($p < 0.05$). Sedentary male group weight values were observed to be higher than other groups ($p < 0.05$). Long-term physical training has protective effects against chemically induced diabetes in mice.

Key words: Alloxan, diabetes mellitus, training exercise.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, heart, and blood vessels (Richard, 2003; Tannenbaum, 1981; Lim et al., 2004). Therefore, diabetes leads to reducing patients' quality of life and life expectancy (Eizirik, 1995; Kelly et al., 2003). Physical inactivity and poor physical fitness have been associated with increased mortality among persons with established diabetes (Wei et al., 2000). High blood glucose levels are toxic, causing serious microvascular and macrovascular damages (Rossini, 2004).

In literature, experimental studies on diabetes have usually been carried out on animals (Rossini, 2004). In most of animal model studies related to diabetes and exercises, animals are previously made diabetes by metabolic agents then exercise programs are applied (Rossini, 2004; Villanueva et al., 2003). Unlike this, diabetes mellitus is a chronic disease, continues many years and the affected systems e.g. endocrine, immune, hematological and musculoskeletal cause many different adaptations opposite to diabetes. In this study we focus on adaptations and protective effects of musculoskeletal system on diabetes. Alloxan as a chemical agent causing diabetes and exercise training counteracted diabetes complications are used together to be able to see the possible effects on diabetes. The aim of the study is to evaluate the effects of long-term exercise training on blood fasting glucose levels and weight in alloxan administered mice.

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Table 1. Training program for exercising group.

Week (m)	Distance	Time (min)	Frequencies (day/week)	Alloxan administered (mg/kg b.w)
1st	85.6	10	5	-
2nd	128.4	12.5	5	200
3rd	171.2	15	5	-
4th	214	17.5	5	200
5th	256.8	20	5	-
6th	299.6	22.5	5	200
7th	342.4	25	5	-
8th	385.2	27.5	5	-
9th	428	30	5	-
10th	470.8	32.5	5	-

METHODS

Chemicals and instruments

Alloxan monohydrate was produced by Sigma, USA. Treadmill for training exercises and electronic glicometer for blood glucose tests were used.

Experimental animals

All experimental animals were approved by the local animal Ethic Committee at Dumlupinar University, Turkey. Healthy, adult male and female mice (Mus musculus) of approximately 2 months in age, weighing 20 to 40 g were used for the study. Mice were housed in a room kept under controlled conditions with temperature maintained at 22 to 23°C on a 12 h light: 12 h dark cycle and were fed balanced mice feed obtained from the Department of Biology of the Dumlupinar University, Turkey.

Sample collection

Blood samples were collected by tail nipping and assessed for glucose level on an electronic glucometer (Vats et al., 2002).

Study design

Alloxan was administered by an i.p. injection 3 times in two weeks intervals (200 mg/kg b.w.; Sigma in 0.09 solutions) (Table 1). In the experiment, a total of 40 mice were used. All the animals were randomly divided into the following 8 groups with 5 mice in each group as Sedentary Control Male (SCM), Sedentary Control Female (SCF), Trained Control Male (TCM), Trained Control Female (TCF), Sedentary Alloxan Administered Male (SAAM), Sedentary Alloxan Administered Female (SAAF), Trained Alloxan Administered Male (TAAM) and Trained Alloxan Administered Female (TAAF). Training program was administered by treadmill for 5 days in a week. In the first week they run for 10 min. (85.6 m, stable speed 17 m/min) in a day. Each week exercise period was prolonged for 2.5 min. The body mass and blood glucose level were measured (blood sample was taken from tail vein of mice) between 2 week periods. Training and alloxan administering program is presented in Table 1.

Statistical analysis

SPSS 13.0 version for Windows was used for all statistical analyses. Statistical evaluation of the data was performed by ANOVA. If the P value of coefficient is less than 0.05, that coefficient is accepted as statistically significant.

RESULTS

At the end of the first 6 weeks there was no significant difference in blood fasting glucose levels among the eight groups ($p > 0.05$). In the 8th week it was observed that SAAM group blood fasting glucose levels (172.3 mg/dl) were statistically higher than other groups ($p < 0.05$). Additionally, in the 8th week in SCF group was measured to be lower blood fasting glucose (65.2 mg/dl) levels than others ($p < 0.05$). Measurements made at the end of the 10th week showed that TAAF, SAAM and SAAF groups' blood fasting glucose levels were found statistically higher than other groups ($p < 0.05$) (Table 2). Body mass results were found similar in all groups except SCM group. SCM group mass values were observed to be higher than other groups at the beginning and at the end of the study ($p < 0.05$). TCM group mass results increased during the first 4 weeks training program ($p < 0.05$) (Table 3).

DISCUSSION

Recent studies showed that exercise can and should be used in the prevention and treatment of diabetes. It also covers the possible mechanisms by which exercise helps in diabetes – increased insulin sensitivity and reduction in obesity, as well as the other health benefits of exercise such as improvement in lipid profile, reduction of blood pressure, improved physical fitness and cardiovascular function. Our data provide strong evidence for a protective effect of long-term training exercise program against chemically-induced diabetes in mice. De oliveira et al. (2005) analyzed the effects of aerobic exercise on the metabolic effects of alloxan. They found out that on the 60th day, there was a reduction in blood glucose levels but in the 90th day, blood glucose levels increased.

Table 2. Presentation of glucose results in groups.

Glucose tests*	SCM	SCF	TAAM	TAAF	TCM	TCF	SAAM	SAAF
Initial	107.0 ± 33.6a	109.8 ± 20.4a	121.2 ± 13.1a	123.4 ± 23.0a	115.6 ± 16.0a	117.8 ± 1.8a	112.2 ± 13.8a	104.8 ± 24.8a
(Min.-max)	(70 - 147)	(94 - 145)	(107 - 139)	(100 - 155)	(98 - 132)	(116 - 120)	(94 - 132)	(77 - 130)
1st test (2nd w)	85.0 ± 9.3a	106.4 ± 27.7a	96.4 ± 22.8a	104.8 ± 21.8a	88.0 ± 11.2a	76.6 ± 9.3a	102.6 ± 4.7a	106.8 ± 21.1a
(Min.-max.)	(77 - 101)	(81 - 144)	(72 - 125)	(81 - 129)	(70 - 100)	(65 - 87)	(100 - 111)	(79 - 135)
2nd test (4th w)	117.4 ± 9.5a	100.4 ± 11.6a	98.4 ± 9.5a	116.8 ± 48.8a	81.6 ± 16.2a	112.5 ± 22.2a	114.0 ± 7.5a	97.0 ± 15.2a
(Min – max)	(110 - 134)	(84 - 114)	(88 - 108)	(63 - 160)	(58 - 105)	(84 - 137)	(107 - 124)	(71 - 111)
3td test (6th w)	111.6 ± 4.7 a	98.2 ± 19.6a	95.0 ± 12.5a	111.0 ± 17.5a	82.8 ± 17.8a	107.7 ± 6.3a	113.6 ± 19.0a	100.6 ± 19.0a
(Min.-max.)	(106 - 117)	(77 - 118)	(80 - 112)	(94 - 132)	(65 - 112)	(103 - 117)	(76 - 124)	(76 - 124)
4th test (8th w)	118.0 ± 23.7a	65.2 ± 15.2b	102.2 ± 17.1a	71.5 ± 7.7 a	109.6 ± 27.2a	79.3 ± 14.2a	172.3 ± 51.9c	101.8 ± 36.2a
(Min.-max.)	(99 - 152)	(42 - 78)	(80 - 118)	(63 - 79)	(69 - 130)	(59 - 91)	(105 - 227)	(65 - 161)
5th test (10th w)	118.0 ± 13.4a	79.0 ± 7.4a	108.4 ± 16.3a	145.3 ± 15.5b	102.8 ± 19.0a	85.8 ± 6.4a	222.8 ± 21.5c	144.2 ± 21.5b
(Min.-max.)	(105 - 138)	(70 - 84)	(87 - 128)	(124 - 161)	(71 - 118)	(77 - 91)	(125 - 275)	(70 - 248)

*Values shown with different letter in the same horizontal and vertical column are significant (P<0.05), values shown with same letter are not significant (P>0.05), Data is presented as mean ± SD (Min – Max). W: week, SCM: Sedentary Control Male, SCF: Sedentary Control Female, TAAM: Trained Alloxan Administered Male, TAAF: Trained Alloxan Administered Female, TCM: Trained Control Male, TCF: Trained Control Female, SAAM: Sedentary Alloxan Administered Male, SAAF: Sedentary Alloxan Administered Female.

Table 3. Presentation of body mass changes in groups.

Body mass	SCM	SCF	TAAM	TAAF	TCM	TCF	SAAM	SAAF
Initial	41.2 ± 4.3a	31.2 ± 1.9b	34.3 ± 8.0b	31.1 ± 2.6b	23.5 ± 2.4c	31.4 ± 2.3b	26.6 ± 2.7 b	32.3 ± 1.9b
(Min. – max)	(35 - 46)	(29 - 34)	(26 - 45)	(28 - 34)	(20 - 26)	(29 - 35)	(23 - 29)	(29 - 34)
1st test (2nd w)	40.9 ± 3.7 a	30.1 ± 2.0b	32.0 ± 6.7b	29.5 ± 2.1b	24.2 ± 2.9c	28.0 ± 2.8b	28.4 ± 2.0b	32.8 ± 2.3b
(Min. – max)	(35 - 45)	(28 - 33)	(26 - 40)	(27 - 31)	(21 - 27)	(26.5 - 33)	(26 - 31)	(29 - 35)
2nd test (4th w)	43.1 ± 4.2a	29.8 ± 2.0b	34.3 ± 5.7 b	28.5 ± 3.7 b	27.4 ± 4.0b	29.4 ± 4.0b	32.4 ± 3..2b	33.2 ±3.3b
(Min – max)	(37 - 49)	(27 - 31)	(28 - 42)	(23 - 31)	(22 - 32)	(24 - 34)	(28 - 37)	(28 – 36.5)
3td test (6th w)	40.7 ± 2.9a	30.7 ± 2.9b	33.6 ± 5.1b	27.9 ± 3.5b	28.3 ± 3.9b	27.9 ± 4.0b	28.4 ± 2.0b	32.6 ± 2.8b
(Min – max)	(36 - 43)	(26 - 33)	(27 - 40)	(23 - 30)	(23 - 33)	(24 – 33.5)	(25 - 31)	(28 - 35)
4th test (8th w)	40.7 ± 2.8a	31.0 ± 3.5b	31.2 ± 7.7 b	27.8 ± 3.6b	25.2 ± 3.6b	30.2 ± 3.3b	31.2 ± 3.5b	31.8 ± 3.4b
(Min - max)	(37.5 - 45)	(26 - 34)	(23 - 41)	(22 - 30)	(20 - 29)	(27.5 - 35)	(26 - 35)	(27 - 35)
5th test (10th w)	41.1 ± 3.1a	31.5 ± 3.3b	31.6 ± 7.2b	28.1 ± 3.9b	25.3 ± 3.0b	30.9 ± 3.0b	32.2 ± 3.7 b	33.3 ± 3.2b
(Min – max)	(37.5 - 46)	(27 - 34)	(24 - 41)	(22 - 31)	(21 - 29)	(28 - 35)	(28 - 37)	(29 - 36)

*Values shown with different letter in the same horizontal and vertical column are significant (P<0.05), values shown with same letter are not significant (P >0.05), Data is presented as mean ± SD (Min – Max). W: week, SCM: Sedentary Control Male, SCF: Sedentary Control Female, TAAM: Trained Alloxan Administered Male, TAAF: Trained Alloxan Administered Female, TCM: Trained Control Male, TCF: Trained Control Female, SAAM: Sedentary Alloxan Administered Male, SAAF: Sedentary Alloxan Administered Female.

This study suggested that exercise intensity should be adjusted to the diabetic condition. In another study Barakat et al. (1987) exercised the rats for seven days 2 h/day at 20 m/min (0 grade) after induced diabetes by intravenous alloxan injection. They concluded that the exercises did not affect glucose levels in either the diabetic or nondiabetic groups. Keller et al. (1993) investigated the effect of two different exercises duration on blood glucose levels in diabetic and normoglisemic mice (5 h/day, 5 days/week for 3 week, exercised 2 h/day, 5 days/week, and 9 week). It was found that the running exercises are associated with decrease in blood glucose and the amount of voluntary exercise performed correlates with blood glucose in diabetic animals. Our results are parallel to the literature studies. A difference of our study is that, experimental animals were not diabetic in the beginning. Alloxan administration (200 mg/kg, 3 times) and training program are both applied to the mice. In this way, we can see the effect of resistance against induced diabetes.

Alloxan doses for induced diabetes are shown variation in literature studies. For intravenous application (Xu et al., 2006) 120 mg/kg b.w., (Kumar et al., 2008) 70 mg/kg b.w. were administered. In addition for intra peritoneal application (Zhang et al., 2007) and (Sheng et al., 2005) 200 mg/kg b.w., (Syiem et al., 2002) 150 – 650 mg/kg b.w. used. We can not say a standard alloxan dose for induced diabetes. In our opinion, that depends on experiment animals, laboratory environments and animal diets. In our laboratory diabetes- induction studies for single i.p injection minimum 400 mg/kg b.w. alloxan were used.

In present study, blood glucose level especially in male mice did not increase in training group. It is well known that physical activity improves glucose uptake from circulation due to some adaptations in skeletal muscles and the increase in enzymatic capacity, muscle capillaryzation, and insulin sensivity (Borghouts and Keizer, 2000; Lee et al., 2002) In addition activation of endothelial nitric oxide synthase has been to exert protective effects against the metabolic syndrome and induction of diabetes (Zhang et al., 2007). Previous research has also shown that nitric oxide facilitates glucose uptake by skeletal muscle and adipose tissue (Higaki et al., 2001). In present study, training group's glucose levels were observed lower than other groups. Activation of musculoskeletal system by training exercises increases the endothelial nitric oxide syntheses and so increases the glucose uptake. This mechanism can be explained by the protective effect of training exercises against on induced diabetes. Controversy to these studies, Bradley et al. (2007) reported that four weeks of exercise training improves insulin sensitivity without influencing skeletal muscle neuronal nitric oxide synthase μ protein expression. Because exercise training improves, it seems that glucose levels are affected from many mechanisms. Therefore, studies should be addressed to investigate the biochemical effect of training on diabetes. This is also our study limitation.

Diabetes mellitus is slightly more common in older women than men (Ligaray, 2007). Low testosterone levels or testosterone deficiencies were found independently associated with higher insulin resistance (Rabijewski et al., 2005). Insulin resistance has been affected worse in women whom estrogen levels are higher when compared with women with lower estrogen levels (Ryan et al., 2002). In current study, we found that the male mice had been showed better blood fasting glucose level than female mice in training groups. However, in sedentary group's blood fasting glucose level female mice had been showed better than male mice. The studies investigating the protective effects of training programs on diabetes in gender are rare in the literature.

Conclusion

Our results indicate that long-term physical training have protective effect against chemically induced diabetes in mice. Further studies are needed to investigate the biochemical mechanism of training effect on diabetes.

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