

Aerobic exercise has an anxiolytic effect on streptozotocin-induced diabetic rats

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Diabetes is a metabolic disorder characterized by hyperglycemia and impaired insulin secretion or action. Psychological comorbidities, such as depression and anxiety, are more common in people with diabetes. Exercise results in anxiolytic effects, as demonstrated in numerous studies. This study aims to evaluate potential anxiolytic effects of aerobic exercise in streptozotocin (STZ)-induced diabetes. Male Wistar albino rats (n=40) were randomly divided into four groups of control, exercise, diabetes, and diabetes + exercise. Diabetes was induced with a single i.p. injection of STZ. The incremental load test was applied to exercise groups to determine maximal exercise capacity. Rats exercised on a treadmill at 70% of their maximal capacity for 45 min, five days per week for 12 weeks. On the day after the last exercise session the open field test and elevated plus maze test were carried out. Diabetes caused an increase in anxiety level, reflected in stretch-attend posture, self-grooming behaviors, and freezing time, with no significant changes for other behavioral parameters. Training normalized diabetes-induced deteriorations and also induced a significant anxiolytic effect both on diabetic and non-diabetic rats. This effect was observed for all behavioral parameters. The results of the open field test and elevated plus maze were consistent. The current results demonstrated a slight increase in anxiety with diabetes and a prominent anxiolytic effect of aerobic exercise. Considering the conflicting results in exercise-anxiety studies, this study highlights the importance of individually designed exercise protocols.

Key words: exercise, diabetes, anxiety, open field test, elevated plus maze

INTRODUCTION

Diabetes mellitus is a metabolic disorder that manifests itself with chronic hyperglycemia and partial or complete loss of insulin secretion (American Diabetes Association, 2010). Today, diabetes has spiked to an epidemic level worldwide (Tabish, 2007). Wild et al. (2004) reported that the prevalence of diabetes was 2.8% in 2000 and is expected to rise to 4.4% by 2030. Therefore, the current 171 million diabetics around the globe may increase to 366 million by the year 2030 (Wild, 2004).

Diabetes has many complications including cardiomyopathy, retinopathy, neuropathy, nephropathy (American Diabetes Association, 2010), as well as affecting the mental status of the patients. Various

psychological comorbidities, such as depression and anxiety, are associated with diabetes (Ducat et al., 2014) and the prognosis is worse in patients with poor glycemic control (Lustman et al., 2000). In addition to clinical studies, behavioral alterations were demonstrated in experimental models in different species. Streptozotocin (STZ) is the most common drug used in experimental diabetes (Radenkovic et al., 2016). STZ-induced diabetes aggravates anxiety-like behaviors (ALB) (Thorre et al., 1997; Aksu et al., 2012; Tang et al., 2015; Rajashree et al., 2017; Aswar et al., 2017; Pereira et al., 2018; Rahmani et al., 2018; Rajabi et al., 2018), depression-like behaviors (DLB) (da Silva Haeser et al., 2007; Gupta et al., 2014; Aswar et al., 2017; Rebollo-Solleiro and Fernandez-Guasti, 2018), and cognitive deficits (CD) (Zhou et al., 2015; Sun et al., 2017;

Gocmez et al., 2019) in rodents. Hilakivi-Clarke et al. (1990) reported that male (Swiss NIH) diabetic mice exhibited less social interaction and more aggressive behaviors in the resident-intruder test. It was also shown that diabetic mice displayed increased repetitive and compulsive-like behaviors in the marble-burying test (Phadnis et al., 2018). Our group recently reported that eight weeks of STZ-induced diabetes in female Wistar rats induced anxiety-like behavior in some behaviors (Caliskan et al., 2019).

Exercise has numerous beneficial effects on human health (Farrell et al., 2011), including a positive effect on the mood of the individual (Taylor et al., 1985). Ligtenberg et al. (1998) demonstrated that aerobic training reduced anxiety measures in patients with type 2 diabetes. This notion has also been supported by various behavioral studies in animals, which reported training protocols that induced an anxiolytic effect in rodents (Ke et al., 2011; Tchekalarova et al., 2015; Ghodrati-Jaldbakhan et al., 2017). On the other hand, some studies failed to demonstrate the anxiolytic effect of exercise training (Chaouloff, 1994; Hoffman et al., 2015; Georgieva et al., 2017), as well as Fuss et al. (2010a; b), which reported a contrary result suggesting an anxiogenic effect of exercise training. These contradictory results primarily originate from differences in type, intensity, and duration of exercise between protocols. Thus, we placed particular emphasis on determining the maximal exercise capacity of each animal and tailoring the individual training program according to the percentage of their maximal exercise capacity.

In the current study, we hypothesized that STZ-induced diabetes will increase ALB and exercise training will ameliorate the anxiogenic effect of STZ-induced diabetes. There are few studies in the literature about diabetes and exercise, and our study is the first that uses an individual training program in diabetic animals.

METHODS

Animals and ethical approval

Adult (10 weeks old) male Wistar albino (*Rattus norvegicus*) rats (n=40) were procured from Ankara University Experimental Animals Breeding and Research Laboratory. Rats were adapted to our laboratory for one week before the start of the experiments. All rats had access to *ad libitum* chow and tap water and were kept on a 12 h light/dark cycle, at constant temperature (21–25°C) and humidity (50–55%). All the rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National

Institutes of Health (NIH publication No. 85-23, revised 1996). The protocol is approved by the Committee on the Local Ethics of Animal Experiments of Ankara University (No: 2017-04-25).

Experimental design and induction of type I diabetes

Rats were randomly divided into four groups of Control (C; n=10), Exercise (EX; n=10), Diabetes (DM; n=10), and Diabetes + Exercise (DM+EX; n=10). At the end of 11th week, (STZ) (Sigma-Aldrich, Missouri, USA, S0130-1G) dissolved in 0.1 M citrate buffer (pH 4.5) was administered i.p. (50 mg/kg) to rats in DM and DM+EX groups. Blood glucose levels were measured 24 hours and seven days after injection and levels ≥ 300 mg/dl (16.6 mmol/L) was considered diabetic. Animal body weights and blood glucose levels were monitored regularly throughout the study. Blood glucose was measured from the tail vein with a glucometer (On Call Plus, ACON Laboratories). Non-diabetic rats' blood glucose levels were also measured initially to prove that they were non-diabetic (data not shown).

Incremental load test and exercise protocol

The rats in the exercise groups (EX and DM+EX) were adapted to treadmill running for a week. The DM+EX group was introduced to the training program after diagnosis of diabetes (7th day after STZ injection), according to their incremental load test (previously described in Teixeira et al., 2012). Briefly, rats started to exercise on the treadmill with a low workload (5 m/min and 0°). Speed was increased by 3 m/min, or slope was increased by 2° every 3 minutes. The workload at which the animal was not able to continue further, even in the presence of electrical or mechanical stimulation, was accepted as the animal's maximal workload.

Rats began training at 70% of their maximal workload. The incremental load test was repeated at the third, sixth, and ninth weeks and the training program was modified according to these results to ensure a 70% intensity throughout the training period. In order to alter exercise intensity, the running speed of the treadmill was increased or decreased while the slope was fixed to 10° for all rats throughout the training program.

Rats were exercised on the treadmill for 45 minutes at the specified workload five days a week for 12 weeks. A 7.5-minute warm-up period in which the speed and slope were gradually increased was applied before reaching the target speed and slope. A 7.5-minute

cool-down period was also applied after the completion of the exercise session.

Behavioral tests

Both behavioral tests were carried out in the early morning (09:00–12:00) on the day after the final exercise session. The open field test (OFT) always preceded elevated plus maze (EPM), and there was no break between tests. The test room was kept silent under consistent light (110 lux, warm light). The observer remained outside during the test session. A hidden camera recorded the entire test, and the tapes were analyzed blindly. Test setups were cleaned with 70% etha-

nol after each animal to prevent an effect of odors left by another animal.

Open field test

The OFT was carried out as previously described (Caliskan et al., 2019). Briefly, we performed the test in a 100 × 100 × 40 cm hypethral box. The base was divided into 25 equal squares. The squares adjacent to the edges of the box were defined as the peripheral region, and the squares in the middle of the box were defined as the central region. Rats were always started in the central region. The first 5 min of the recording was used for the analysis. The behavioral parameters

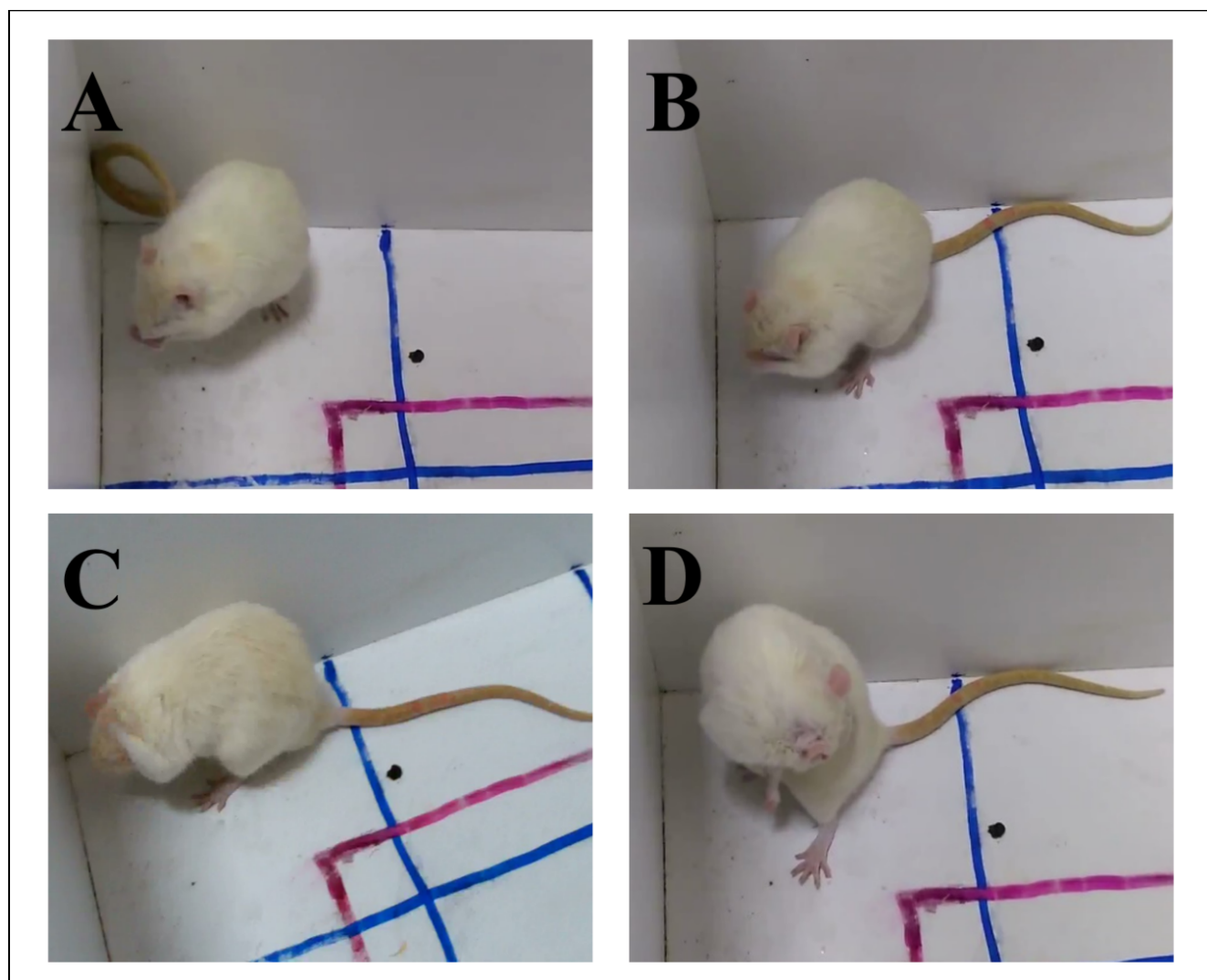


Fig. 1. Cephalocaudal transition of self-grooming behavior in rats. (A) Phase 1: elliptical bilateral paw strokes made near the nose (paw and nose grooming) (B) Phase 2: series of unilateral strokes (each made by one paw) from whiskers to below the eye (face grooming) (C) Phase 3: bilateral strokes backwards and upwards made by both paws simultaneously (head grooming) (D) Phase 4: body licking (body grooming) (Kalueff et al., 2016).

assessed were total time in the central region, i.e., central time; the time spent before leaving the central region, i.e., central latency time; the number of entries into the central region, i.e., central region entries; freezing time; total distance traveled; unsupported rearing behavior; and total (unsupported + supported) rearing behavior.

Additionally, we evaluated the self-grooming behaviors of the rats in the OFT (Fig. 1). Self-grooming in rats shows a high level of behavioral complexity and organization, which involves a series of highly stereotyped patterns (Kalueff et al., 2016). Acute stressors (for example, exposure to a new environment) increase the frequency and duration of self-grooming behavior (Delprato et al., 2017; Zhang et al., 2019). Additionally, stressors result in a disorganized grooming pattern (i.e., incorrect transition) (Spruijt et al., 1987; 1992), whereas anxiolytic treatments tend to reduce rodent self-grooming activity and normalize its sequential organization (Kalueff and Tuohimaa, 2005; Nin et al., 2012). To evaluate self-grooming behavior, we assessed total grooming time and incorrect transition parameters.

Elevated plus maze

EPM was carried out as previously described (Caliskan et al., 2019). Briefly, we performed the test in a plus-shaped maze which consisted of two open and two closed arms at a specific height (70 cm). Rats were started in the middle of the open arm. The first 5 min of the recording was used for analysis. The behavioral parameters analyzed were time spent on the open arms (total and distal portion: distal half of the open arm), the number of entries to the open arms, freezing time,

and the number of total head-dipping behaviors and stretch-attend postures (SAP).

Statistical analysis

The statistical analyses were performed using GraphPad Prism for Windows v5 2007 (GraphPad Software Inc.) software. Shapiro-Wilk test was applied to all groups for all parameters to determine normal distribution. Data sets were distributed normally and other parametric test assumptions were met so a parametric test was chosen for statistical analysis. We used two-way ANOVA in a 2×2 (sedentary/exercise \times diabetic/non-diabetic) factorial design. Bonferroni test was preferred for *post-hoc* comparisons. The values were presented as mean \pm SE. For all comparisons, $p < 0.05$ was considered to be significant.

RESULTS

Animal-follow parameters

The body weight and blood glucose levels of the rats were measured before sacrificing (data shown in Fig. 2). Terminal body weight and blood glucose levels rats did not differ significantly between sedentary and exercising rats ($F_{(1,25)}=2.59$, $p > 0.05$; $F_{(1,24)}=0.90$, $p > 0.05$, respectively). The body weight of diabetic rats was significantly lower and the blood glucose of the diabetic rats was significantly higher compared to their non-diabetic matches ($F_{(1,25)}=63.50$, $p < 0.001$; $F_{(1,24)}=430.50$, $p < 0.001$, respectively). This difference was observed both in sedentary and exercising pairs ($p < 0.01$). The interac-

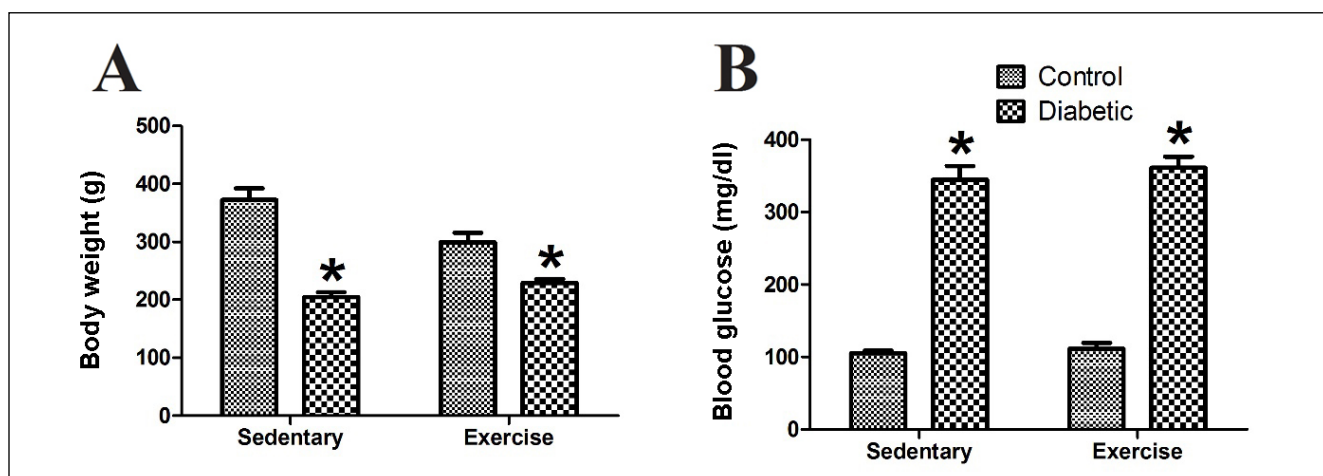


Fig. 2. Animal-follow parameters. (A) Terminal body weight (C=7; EX=9; DM=6; DM+EX=7). (B) Terminal blood glucose level of rats (C=7; EX=9; DM=6; DM+EX=6). (*: $p < 0.05$; $\bar{x} \pm SE$).

tion between diabetes and exercise was significant for body weight ($F_{(1,25)}=10.62$, $p<0.01$) but not significant for blood glucose ($F_{(1,24)}=0.18$, $p>0.05$).

The initial exercise capacity of the rats did not differ among groups ($p>0.05$). Training increased the exercise capacity of both diabetic and non-diabetic rats, but this increase was not significant ($p>0.05$). The running speed of the diabetic rats was lower compared to non-diabetics but did not reach significance ($p>0.05$).

Open field test

Conventional OFT parameters are shown in Fig. 3. According to statistical analysis, the conventional OFT parameters of central time, central latency time, and central region entries were significantly higher in exercising rats compared to sedentary rats ($F_{(1,36)}=106.10$, $p<0.001$; $F_{(1,36)}=27.58$, $p<0.001$; $F_{(1,36)}=172.80$, $p<0.001$, respectively). The effect of diabetes on these same parameters also varied ($F_{(1,36)}=1.29$, $p>0.05$; $F_{(1,36)}=0.38$,

$p>0.05$; $F_{(1,36)}=6.53$, $p<0.05$, respectively). Although the effect of diabetes was significant for central the entry parameter, no significant differences were observed in all pairwise comparisons (C vs. DM+EX). Finally, the interaction between exercise and diabetes was not significant for central time, central latency time, or central region entries ($F_{(1,36)}=0.36$, $p>0.05$; $F_{(1,36)}=0.01$, $p>0.05$; $F_{(1,36)}=0.53$, $p>0.05$, respectively).

Parameters that evaluated the quiescence and locomotion of the rats in the OFT are shown in Fig. 4. Two-way ANOVA analysis revealed that unsupported rearing behavior, total rearing behavior, and distance traveled were markedly higher in exercising rats compared to sedentary rats ($F_{(1,36)}=82.13$, $p<0.001$; $F_{(1,36)}=65.62$, $p<0.001$; $F_{(1,36)}=75.99$, $p<0.001$, respectively). Furthermore, freezing was significantly lower in exercising rats compared to sedentary rats ($F_{(1,36)}=140.70$, $p<0.001$). There was a significant effect of diabetes on unsupported rearing behavior, total rearing behavior, and distance traveled ($F_{(1,36)}=9.13$, $p<0.01$; $F_{(1,36)}=7.14$, $p<0.05$; $F_{(1,36)}=20.80$, $p<0.001$; $F_{(1,36)}=152.80$, $p<0.001$, re-

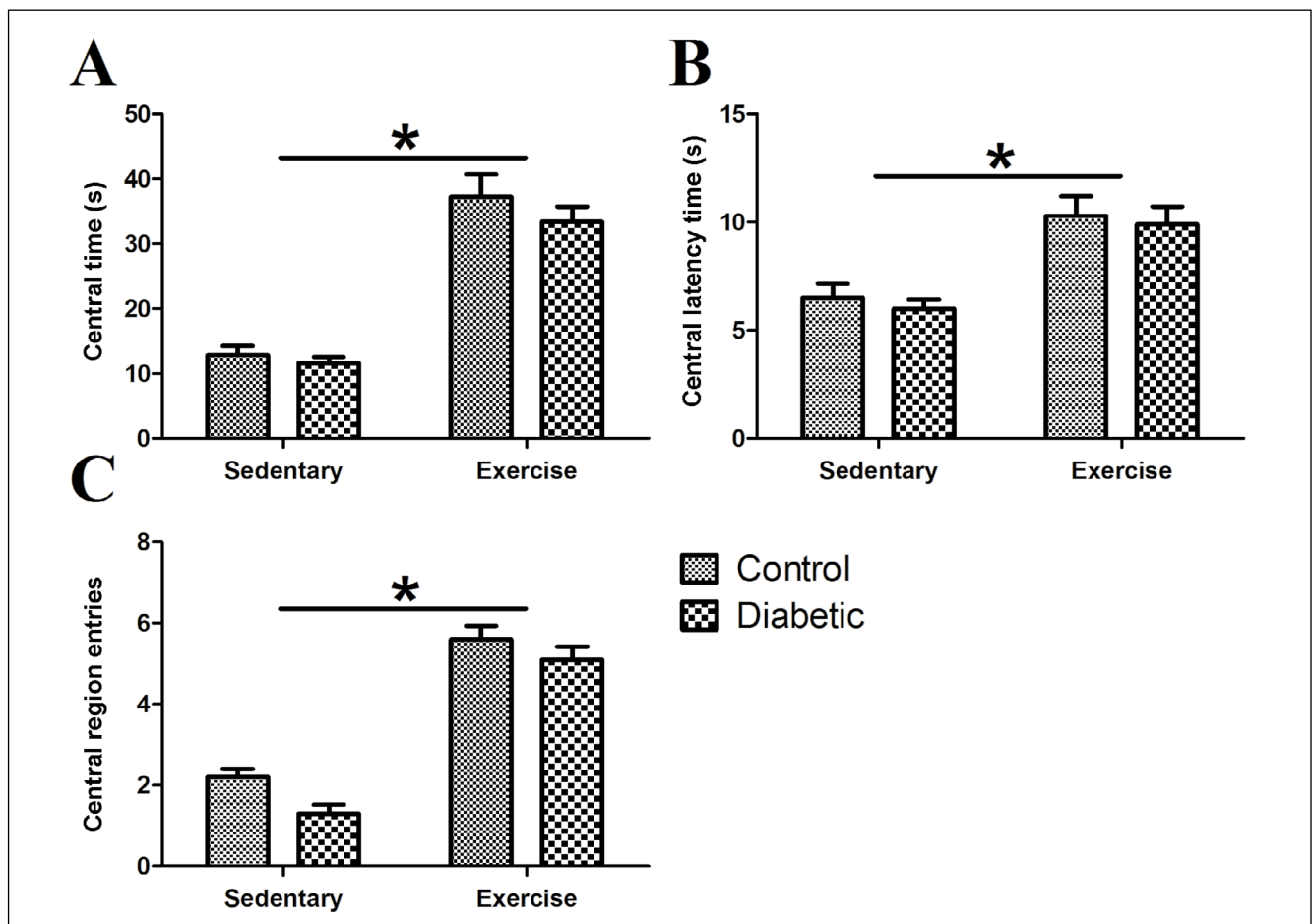


Fig. 3. Conventional OFT parameters. (A) Time spent in central region. (B) Time spent before the first entry to central region. (C) Total number of entries to the central region. (*: $p<0.05$; $X\pm SE$).

spectively). Unsupported rearing behavior was significantly lower in sedentary diabetic rats compared to their non-diabetic pairs ($p < 0.05$). Total rearing behavior and distance traveled were significantly lower in exercising diabetic rats compared to non-diabetics ($p < 0.05$; $p < 0.001$, respectively). Freezing time was higher both in exercising and sedentary diabetic rats compared to their matched controls ($p < 0.001$).

The interaction of diabetes and exercise was not significant for unsupported and total rearing behaviors ($F_{(1,36)} = 0.11$, $p > 0.05$; $F_{(1,36)} = 0.56$, $p > 0.05$, respectively) but was significant for distance traveled and freezing parameters ($F_{(1,36)} = 5.48$, $p < 0.05$; $F_{(1,36)} = 23.86$, $p < 0.001$).

Self-grooming parameters are shown in Fig. 5. All self-grooming parameters (total grooming time and incorrect transition time) were markedly lower in exercising rats compared to sedentary rats ($F_{(1,36)} = 77.67$,

$p < 0.001$; $F_{(1,36)} = 98.46$, $p < 0.001$, respectively) and higher in diabetic rats ($F_{(1,36)} = 97.13$, $p < 0.001$; $F_{(1,36)} = 145.60$, $p < 0.001$, respectively) compared to non-diabetic rats. The interaction between diabetes and exercise was not significant for total grooming time ($F_{(1,36)} = 3.96$, $p > 0.05$) but was significant for incorrect transition time ($F_{(1,36)} = 25.78$, $p < 0.001$).

Elevated plus maze

Conventional EPM parameters are shown in Fig. 6. Conventional EPM parameters of total open arm time, distal open arm time, and open arm entries were dramatically higher in exercising rats compared to sedentary rats ($F_{(1,36)} = 96.55$, $p < 0.001$; $F_{(1,36)} = 146.30$, $p < 0.001$; $F_{(1,36)} = 80.38$, $p < 0.001$, respectively). Diabetes effected

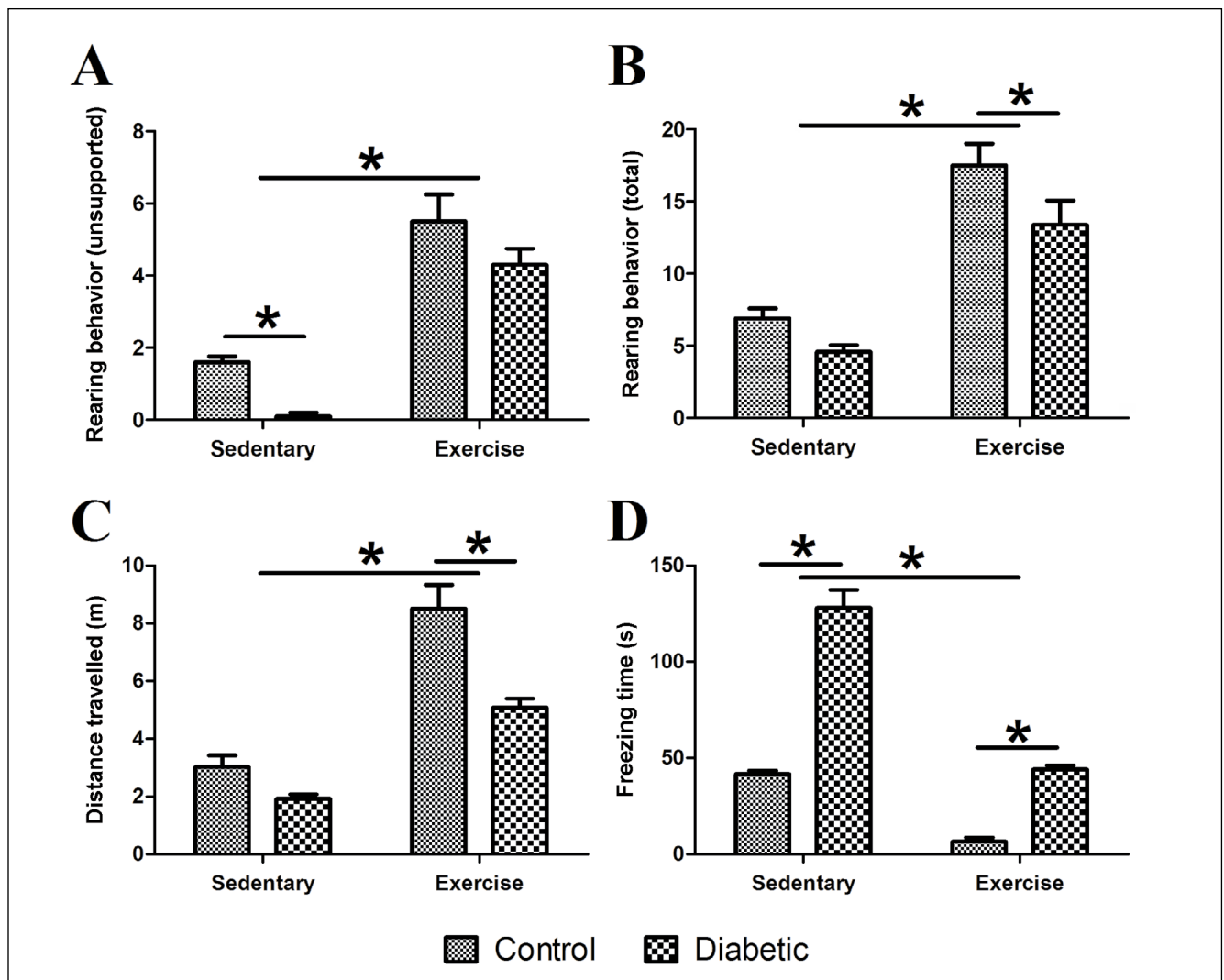


Fig. 4. Parameters that evaluate the quiescence and locomotion of animal in OFT. (A) Unsupported rearing behavior. (B) Total rearing behavior. (C) Distance travelled. (D) Freezing time. (*: $p < 0.05$; $X \pm SE$).

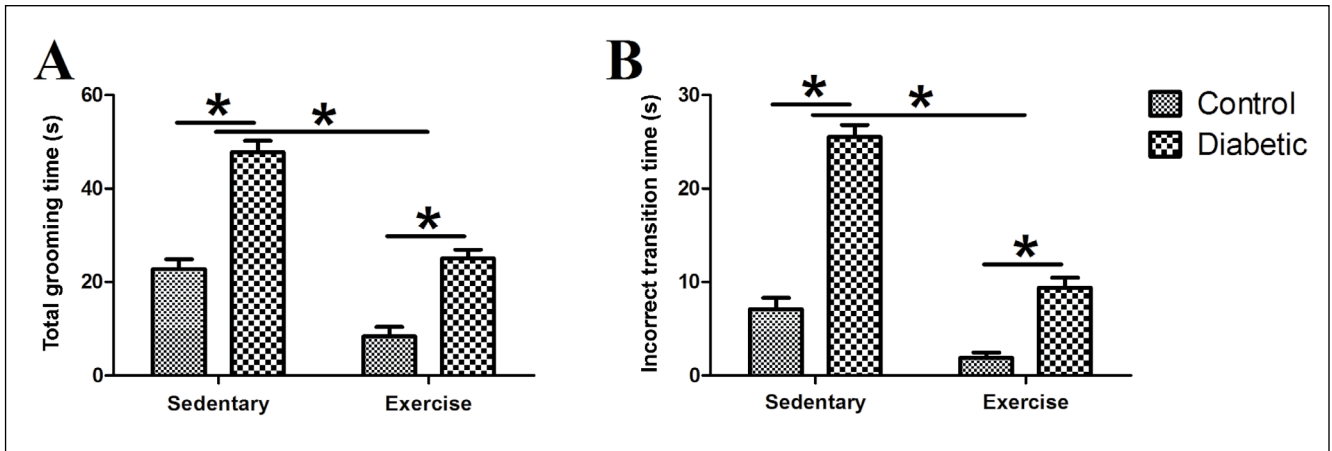


Fig. 5. Self-grooming parameters in OFT. (A) Total grooming time. (B) Incorrect transition time (*: $p < 0.05$ vs. its non-diabetic pair; (*: $p < 0.05$; $X \pm SE$)).

distal open arm time markedly ($F_{(1,36)}=18.74$, $p < 0.001$) but did not affect total open arm time and open arm entries ($F_{(1,36)}=2.90$, $p > 0.05$; $F_{(1,36)}=0.55$, $p > 0.05$, respectively). Distal open arm time was significantly lower

in diabetic exercising rats compared to their non-diabetic pair ($p < 0.001$). The interaction between exercise and diabetes was significant for distal open arm time ($F_{(1,36)}=4.63$, $p < 0.05$) but was not significant for total arm

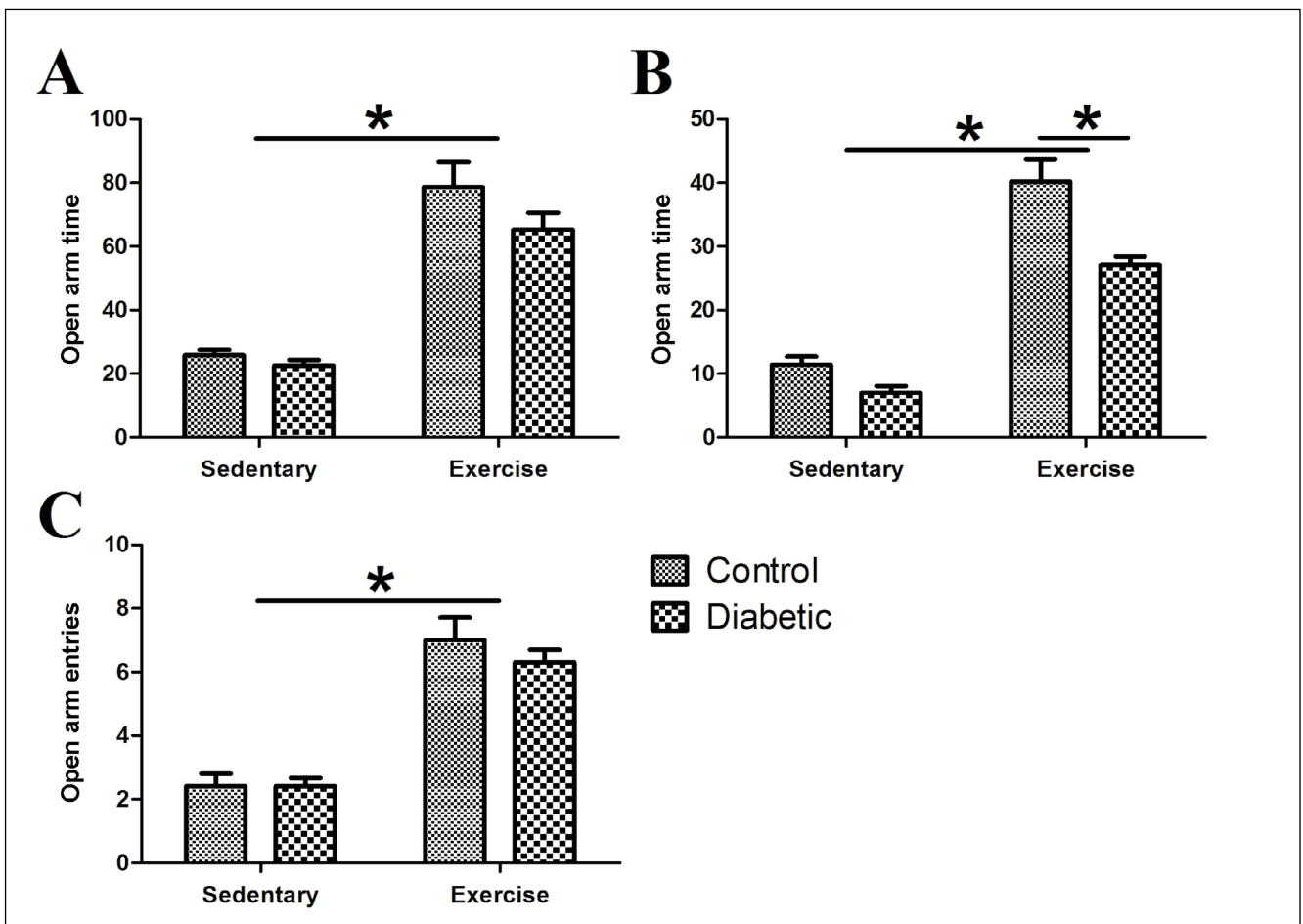


Fig. 6. Conventional EPM parameters. (A) Time spent in open arm. (B) Time spent in distal portion of the open arm. (C) Total entries to the open arm. (*: $p < 0.05$; $X \pm SE$).

time and open arm entries ($F_{(1,36)}=1.05$, $p>0.05$; $F_{(1,36)}=0.55$, $p>0.05$, respectively).

Additional EPM parameters are shown in Fig. 7. Exercising rats showed more head-dipping and less SAP behavior compared to sedentary rats ($F_{(1,28)}=154.50$, $p<0.001$; $F_{(1,28)}=60.20$, $p<0.001$, respectively). Diabetes markedly decreased head-dipping behavior ($F_{(1,28)}=4.73$, $p<0.05$) and markedly increased SAP ($F_{(1,28)}=44.57$, $p<0.001$). Although the effect of diabetes was significant for head-dipping behavior, no significant differences were observed in paired comparisons ($p>0.05$). Regarding SAP, there was a significant difference between both sedentary and exercising diabetics and their matched controls ($p<0.01$). The interaction between exercise and diabetes was significant neither for head-dipping nor SAP ($F_{(1,28)}=0.39$, $p>0.05$; $F_{(1,28)}=3.94$, $p>0.05$, respectively).

DISCUSSION

According to the results, twelve weeks of aerobic exercise evoked a potent anxiolytic effect on male Wistar rats. The anxiolytic effect was reflected in almost every behavioral parameter, both in diabetic and non-diabetic rats. On the other hand, STZ-induced diabetes caused a slight anxiogenic effect, which was only notable in SAP, freezing, and self-grooming parameters.

Our training regimen lowered the bodyweight of rats, but the effect was not significant. Weight loss is typical in exercise training because of increased energy expenditure; however, there was a significant interaction between diabetes and exercise, which suggests that diabetic rats lose less weight with training compared to non-diabetic runners. We speculate that diabetes had already created a severe weight loss effect in these rats; therefore, exercise training did not cause

any further weight loss. Blood glucose levels were significantly higher in both diabetic groups compared to their non-diabetic pairs, and training did not affect blood glucose levels in diabetic or non-diabetic rats.

STZ-induced experimental diabetes increases ALB in rodents. This phenomenon was demonstrated in various dose, strain, and diabetes duration models, including in both genders (Thorre et al., 1997; Aksu et al., 2012; Tang et al., 2015; Aswar et al., 2017; Rajashree et al., 2017; Rajabi et al., 2018; de Souza et al., 2019). Furthermore, our group recently reported a similar result. We used female Wistar albino rats, and the diabetes duration was six weeks. An anxiogenic effect was visible in head-dipping behavior and SAP, but other parameters did not differ compared to the control (Caliskan et al., 2019). In the current study, the duration of diabetes was longer, and we used male rats instead of females; however, we only observed a slight anxiogenic effect, which was reflected in behavioral parameters such as SAP and self-grooming behavior.

In general, stressors increase total grooming time and disrupt the sequential nature of self-grooming behavior (Kalueff et al., 2016). In rodents, there is a correct sequence (a stereotyped order of movements) of self-grooming behavior, which is called “cephalocaudal progression” (Fig. 1). Correct transition is defined as the total time of grooming that is executed in cephalocaudal progression; thus, the incorrect transition is the period of irregular self-grooming behavior (Kalueff et al., 2007). Anxiogenic stimuli increase total grooming time and impair the correct order of self-grooming behavior, manifested as an increase in incorrect transition time. Our data showed that both incorrect transition and total grooming time were increased significantly in the DM group. Taken together with the increase of SAP in diabetic rats, this may be interpreted as an increase in ALB. Furthermore, since diabetic animals seem to pre-

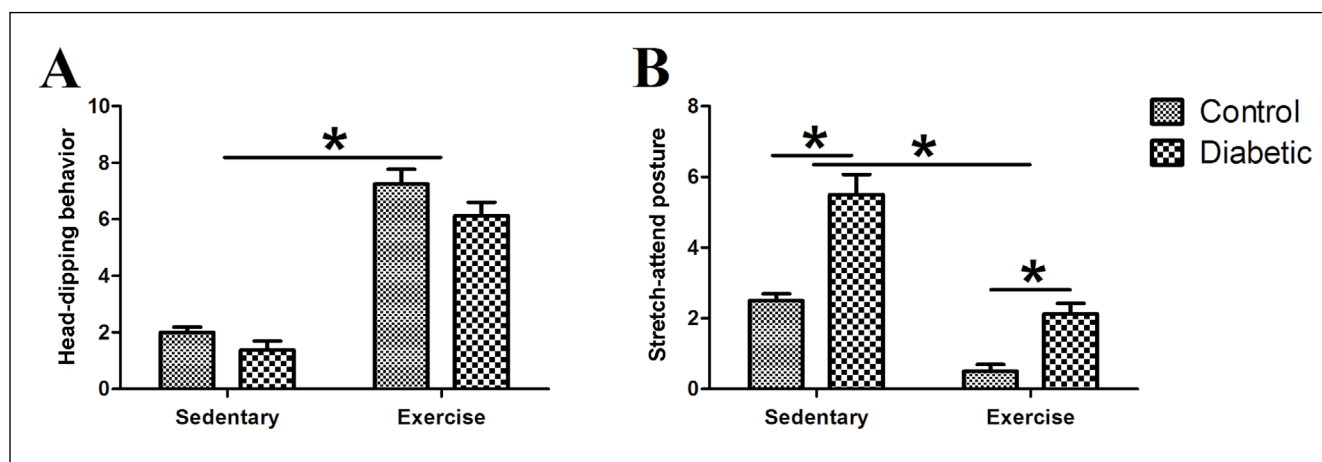


Fig. 7. Additional EPM parameters. (A) Head-dipping behavior. (B) Stretch-attend posture. (*: $p<0.05$; $X\pm SE$).

fer passive strategies to cope with anxiogenic stimuli (Rebolledo-Solleiro et al., 2016), due to their poorer physical conditions, freezing may be a better behavioral measure for assessing ALB. Additionally, freezing responses are associated with anxiety and may be etiologically related to several anxiety disorders (Riskind et al., 2016), and they have also been used to model anxiety disorders (Likhtik et al., 2005). Freezing time was significantly increased in the diabetic rats. Exercise training normalized freezing time in diabetic runners and also decreased it in non-diabetic ones.

Our aerobic exercise protocol induced an apparent anxiolytic effect, which was visible both in OFT and EPM results. In the literature, there are contradictory results regarding the effect of exercise. Some authors reported an anxiolytic effect with training (Ke et al., 2011; Tchekalarova et al., 2015; Ghodrati-Jaldbakhan et al., 2017), and some observed no differences (Chaouloff, 1994; Hoffman et al., 2015; Georgieva et al., 2017). Then, others reported an exercise-induced anxiogenic effect (Fuss et al., 2010a; 2010b). Protocol diversity, which is the biggest dilemma for all exercise studies, is the main reason for this contradiction. Sciolino et al. (2012) also emphasized the contradictory results of exercise and anxiety studies in animals and pointed out the diversity of training protocols. On the other hand, diabetes lowers the exercise capacity of the animal (Wahl et al., 2018), so applying the same protocol to both diabetics and non-diabetics constitutes a methodological problem. For these reasons, we chose to measure the maximal exercise capacity of animals and use an individualized training protocol.

Although it is hard to distinguish the anxiolytic effect from a locomotor activity increase because of the nature of exercise-anxiety studies, we suggest that training induced an anxiolytic effect based on several observations. First, unsupported rearing behavior, which is the anxiety-specific element of rearing behavior (Sturman et al., 2018), was significantly increased both in diabetic and non-diabetic runners. Secondly, the time spent in the distal portion of the open arm, which is the more anxiety-sensitive aspect of the total open arm time parameter, was increased in both running groups. Finally, measures of head-dipping, SAP, and self-grooming behaviors, which are not directly related to locomotion, shifted in favor of anxiolysis in exercising rats. Taken together, we argue that our aerobic exercise protocol has an apparent anxiolytic effect both in diabetic and non-diabetic rats. These data are consistent with our previous study.

Fuss et al. (2010a; b) reported an anxiogenic effect of exercise in contrast to our results. However, there was a fundamental methodological discrepancy. The authors stated that animals began the light-dark box

test in the dark zone. We argue that this approach causes misinterpretation of anxiety-like behavior and dampens the strength of the tests. A general principle of anxiety testing requires that the animal is initially placed into the anxiety-generating zone. The dark zone is considered safe for animals and does not induce anxiety. In a light-dark box test, the animal should be placed into the light zone, the risky or anxiety-generating region for the animal, at the outset (Hascoët and Bourin, 1998; Bourin and Hascoët, 2003; Serchov et al., 2016) otherwise the animal may spend more time than it would have in the dark zone and the results will present an erroneous tendency towards anxiety.

There are several considerations we have noted for future studies. First, the level of light intensity in the test room is an important variable. Low-intensity luminosity reduces open arm avoidance (Pereira et al., 2005). Leo and Pamplona (2014) argued that to observe an anxiogenic effect low-intensity lighting (5-30 lux) is preferred, whereas an anxiolytic effect should be analyzed under higher intensity lighting (200-400 lux or greater). Rebolledo-Sollerio et al. (2013) used a higher intensity of light and demonstrated prominent ALB in STZ-induced diabetes. Since the degree of lighting is closely correlated with the activity level of the animal, one may speculate that the activity level of our animals may have blunted the ALB in diabetic rats. Additionally, supplemental behavioral tests, such as light-dark box, may be useful to increase the depth of and further validate the observations.

CONCLUSIONS

The current study is the first that applies individually designed training programs to diabetic rats and observes ALB. Although the increase of ALB was not extremely intense in the diabetic rats, it was still discernable in the freezing, self-grooming, and SAP parameters. Additionally, we were able to demonstrate a potent anxiolytic effect of aerobic exercise. Considering the conflicting results in exercise-anxiety studies, our study emphasizes the importance of individually designed exercise protocols. Exercise is not beneficial, and can even be hazardous, unless its intensity is matched to the capacity of the individual. Finally, the use of supplemental behavioral tests such as light-dark box and the analysis of biochemical parameters such as cortisol and brain-derived neurotrophic factor would be useful and promising additions to forthcoming studies. Evaluating the effect of different STZ doses will also be crucial in future studies to observe for dose-dependent effects. These results underline the beneficial effects of exercise in the diabetic population and provide

valuable information for the design of the proper training method for diabetic patients.

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