Additive Benefits of Environmental Enrichment and Voluntary Exercise on Cognition and Motor Coordination in Diabetic Mice

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Objective: To evaluate the effects of voluntary daily exercise and environmental enrichment on spatial memory and learning as well as motor coordination and learning, in diabetic mice.

Setting: College of Medicine and Medical Sciences, Arabian Gulf University, Bahrain.

Design: An Experimental Animal Study.

Method: BALB/C mice (20 g to 25 g) received 55 mg/kg streptozotocin IP daily for five days. Diabetic mice were randomly assigned to one of the following groups for 12 weeks' duration: (1) social isolation; (2) an environmental enrichment; (3) environmental enrichment and voluntary daily exercise. The fourth group consisted of normal controls. At the end of 12 weeks, the mice were assessed by the Morris Water Maze and the Rotarod for cognitive and motor performance respectively.

Result: All diabetic mice showed hyperglycemia. In water maze testing, exercise and environmental enrichment groups showed better learning as evidenced by reductions in time (escape latency) and distance swum to reach a submerged platform compared to diabetic isolated mice. In diabetic mice, exercise itself did not have a significant additional benefit on learning and memory compared to environmental enrichment alone. In rotarod test, motor learning was impaired with isolation but enhanced with environmental enrichment and exercise.

Conclusion: Environmental enrichment and exercise confer significant benefits on cognition and motor performance in diabetic mice.

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Long-term diabetes leads to discrete structural and functional disorders of the central nervous system including impaired synaptic plasticity, decreased dendritic complexity and decline of hippocampal adult neurogenesis, as well as moderate impairment of learning and memory in the middle-aged and increased risk of dementia or Alzheimer's disease in the elderly[1-4]. In rodents, streptozotocin (STZ) induces a diabetic-like state with a similar pattern of disorders in central and peripheral nervous system[5]. The animal model has been widely used to study disease progression as well as treatment[5,6].

Physical activity improves cognitive function, increases cell proliferation and survival in the hippocampus, ameliorates motor deficits, enhances brain neurotrophin expression and increases angiogenesis[7-11]. However, little information is available concerning exercise effects on learning and memory as well as neuropathy[12].

Simple enrichment has beneficial effects on hippocampal synaptic plasticity and spatial learning and memory processes in adult mice[13,14]. The benefits of enrichment on learning and memory have not been explored in diabetes. The effect of environmental enrichment in adult and elderly mice are mediated through key factors involved in long-term synaptic plasticity including NMDA receptors, intracellular kinases, transcription and epigenetic factors, hippocampal neurogenesis,

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increased dendritic arborization and spine density in CA1 pyramidal neurons, as well as vascularization[15,16].

The aim of this study was to evaluate the effect of voluntary daily exercise and environmental enrichment on spatial memory and learning, as well as motor coordination and learning in STZ-induced diabetic mice.

METHOD

Diabetes mellitus (DM) was induced in male BALB/C mice of 5 to 7 weeks' age (20 g to 25 g) by daily intraperitoneal injection of 55 mg/kg STZ (S-0130, Sigma, U.K.; dissolved in sodium citrate; pH4.5) (n=39) for a total of five days. One week following the final dose of STZ hyperglycemia was confirmed by measuring the glucose level in blood obtained by tail prick taken from each mouse. Streptozotocin-injected mice with a blood glucose of <15 mmol/L (280 mg/dL) were excluded.

Diabetic mice were randomly assigned to one of the following three treatment groups for 12 weeks duration: (1) social isolation (DI) for which the mice were individually housed (n=14); (2) environmental enrichment (DEN) which involved groups of 3 to 4 mice being housed in large colourful cages containing objects of shapes, textures, and sizes (n=13); (3) environmental enrichment and voluntary daily exercise (DENX) which included the above enrichment as well as free access to running wheels in the cages at all times (n=12). A fourth group consisted of the age-matched littermate controls (CONT; normal BALB/C mice) (n=13) which were housed under the same conditions as the DENX group. At the end of 12 weeks, the mice were assessed daily for a total of 5 days in the Morris Water Maze for spatial memory and learning, and on the Rotarod for motor coordination and learning.

Each mouse was given five acquisition trials per day for five consecutive days to learn the position of a hidden 'escape' platform, submerged 2 cm below the water surface, at a fixed location inside a circular swimming pool[5,17]. The mice were given a maximum of two minutes to find the platform and were allowed to remain on the platform for 30 seconds. Mice that failed to locate the disc were put onto it by the experimenter. The position and movement of the mice, in the pool, was captured and analyzed every 0.2 s, using a video-camera computer system, and ANY-maze video tracking system (Stoelting Co, U.S.A). The outcome measures were the speed of swimming, latency time and distance swam to reach the platform. On the sixth day of water maze testing, the platform was removed, and each mouse was allowed to swim for 60 seconds. In this probe trial, the selective search strategy was indicated if the mice performed significantly above chance (25%).

Each mouse was placed in a lane on the Rotarod (Model: RR-01, Rotarod apparatus, Orchid Scientific, India) while it was rotating at a low speed for habituation. Each mouse was tested later with two rotarod speeds (4.5 and 6 m/min). The trial ended if the mouse either fell off the rotarod or gripped it and completed a full passive rotation without attempting to run. Each mouse attempted three such trials with a 15-minute interval between trials.

Data was expressed as mean ± SEM. Within groups, paired t-test was used to compare the performances of day 1 and day five measurements in the water-maze test. Same was used in the rotarod experiments. Comparison between the groups in water maze experiments was made using one-way ANOVA with post hoc Scheffe test. All statistical tests were performed with IBM SPSS version 22.

RESULT

Diabetes was confirmed in STZ-treated mice by non-fasting blood glucose measurements (22.1±0.2 mM) which were significantly higher than the control mice (8.1±0.0mM; $P=3.6x10^{-11}$, t=2.064, two-tail t-test). At week 12, non-fasting blood glucose was higher ($F=142.286$, $P=2.39x10^{-22}$; ANOVA test) in all the diabetic groups (DI mice, 30.9±0.2mM, $P=1.40x10^{-16}$, t=2.074; DEN mice, 31.9±0.2 mM, $P=3.57x10^{-11}$, t=2.145; DENX mice, 30.2±0.2mM, $P=2.17x10^{-9}$, t=2.179; two-tail tests), compared to controls, see table 1.

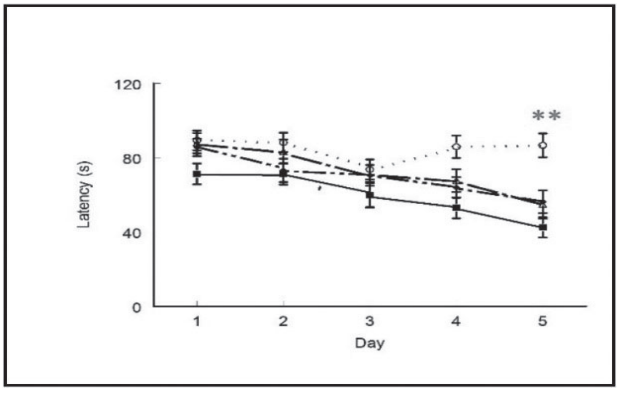
Table 1: Non-Fasted Blood Glucose Measurements at Start and End of Study

Group	Blood Glucose (mmol/l)	
	Week 0 (n=14)	Week 12 (n=13)
Control	8.1±0.0	9.5±0.1‡
Diabetic (n=14)	22.1±0.2*	
Diabetic Isolated (n=14)		30.9±0.2** +
Diabetic+Environmental Enrichment (n=13)		31.9±0.2** +
Diabetic+Environmental Enrichment+Exercise (n=12)		30.2±0.2**

* = significantly different from control group at week 0, P<0.01
**= significantly different from control group at week 12
+ = significantly different from diabetic group at week 0, P<0.01

There was no difference in spatial memory between the groups on day one based on comparisons of latency and distance swam to reach the platform (latency: ANOVA f=1.05742, p=0.956; distance: ANOVA f=1.5435, p=0.2063, f_{crit}=3.35235).

Each group performance was evaluated by comparing the time spent to reach the platform (latency) in the first day of training to the latency measured on the fifth day. During acquisition, all groups except the DI mice, showed improvement in their performance over the five–day period of training as evidenced by reductions in time (escape latency) (ANOVA CONT: f=4.2892, p=0.00223; DEN f=4.77074, p=0.001, f_{crit}=2.41287; DENX f=4.00482, p=0.00352) and distance swum (ANOVA CONT: f=3.35235, p=0.01068; DEN f=4.73775, p=0.0011, DENX f=2.6547, p=0.03327), to reach the platform, see figure 1 (A and B).



O= DI Δ = DEN ♦=DENX ■= CONT

Figure 1 (A): Gradual Decrease in Escape Latency Across the Five Days for All Groups Except DI Mice. On Day 5, DI Mice Had a Higher Latency Compared to All Other Mice (p<0.01)**

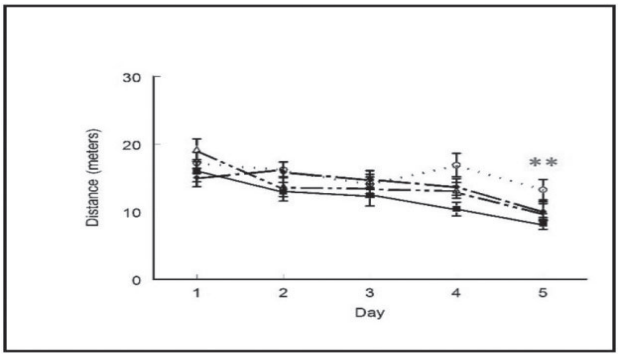


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Figure 1 (B): A Shorter Swimming Distance On Day 5 in CONT (p<0.01) Compared to DI**

The DI group of mice, on the other hand, showed no significant enhancement in their performance (day one latency; 89.3 ± 5.3 s, day five; 86.6 ± 6.3 s). Similar results are presented by calculating the distance swam by the mice to reach the platform, see figure 1 (B). Groups analysis showed that on day five of training, the improvements in escape latency were greater (ANOVA f=8.4376, p=3.1 x 10^{-5}) in the CONT (42.5 ± 5.4 s, p=8.897 x 10^{-7}), DEN (54.7 ± 7.6 s, p=0.00185) and DENX (56.3 ± 6.1 s, p=0.00116) compared to DI (86.6 ± 6.3 s, p> 0.05) mice. These improvements were also mirrored by a shorter swimming distance on day 5. There was no difference in latency or swimming distance between the DEN and DENX groups on day 5.

There were no differences within-group for swimming speed across the five days, except for the DEN group (ANOVA f=2.9506; p=0.021), see figure 1C.

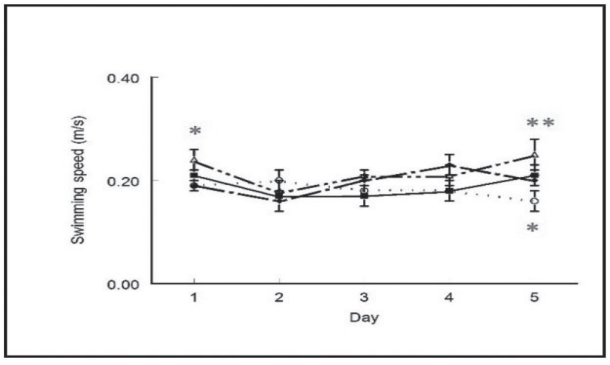

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Figure 1 (C): DEN Mice Swam Faster Compared to DENX and DI (*p<0.05) Mice on Day One. DI Mice Swam at Lower Speed Compared to CONT (*p<0.05) and DEN (p<0.01) Mice on Day Five**

The mice which spent more than 25% of the time in the target quadrant when the platform was removed were considered as having learned the test. The probe trial showed that all groups spent significantly more time in the target (training) quadrant than would be expected by chance (DEN: 61.2±1.6%, P<0.00001, t=22.68; DENX: 60.8±2.0%, P<0.00001, t=17.92; CONT: 71.0±1.5%, P<0.00001, t=30.67; two tail, one sample t-test), except the DI mice which spent less time (DI: 16.5±1.1%, P <0.00001, t=7.63), see figure 1 (D).

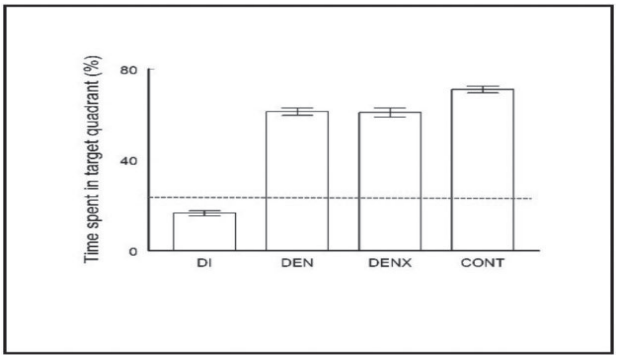

Figure 1 (D): Percentage of Time Spent in the Target Quadrant During Probe Trial

Figure 2 (A) shows the result obtained on the fifth day when the rotation speed was 4.5 m/min. The mice of the CONT (22.5 ± 3.9 s, p=0.039) and DENX (29.4 ± 4.1 s, p=0.0023) groups stayed significantly longer on the rotating rod than the DI (13.9 ±2.1 s). There was also a higher latency to fall in DENX mice compared to DEN (16.7 ± 2 s, p=0.0069) mice.

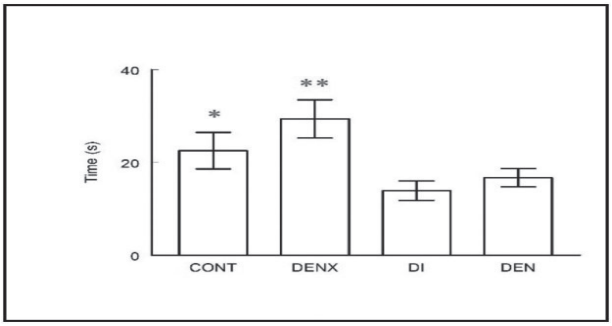

Figure 2 (A): Day 5 Latency to Fall when Rod Rotation Was 4.5 m/min. There Was a Higher Latency to Fall in CONT (*p<0.05) and DENX (p<0.01) Compared to DI and DEN**

Comparing the results of day five testing with a rod speed of 6 m/min, the data showed no significant differences in the performance of the CONT (19.3 ± 2.8 s), DEN (16.2 ± 2.3 s) and DENX (15.9 ± 1.4 s) groups. However, there was a higher latency to fall (ANOVA f=3.3557, p=0.0258) in the CONT (p=0.0095), DENX (p=0.0030) and DEN groups (p=0.0233) compared to DI mice, see figure 2 (B).

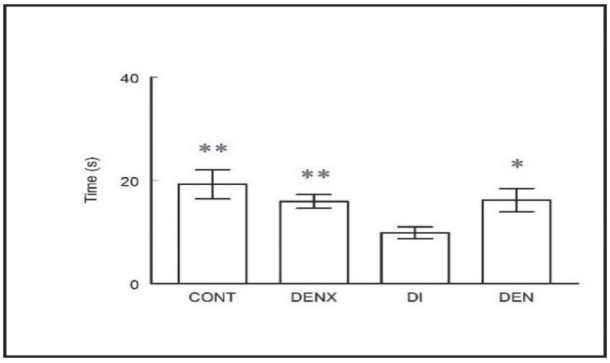

Figure 2 (B): Day 5 Latency to Fall When the Rod Rotation Speed was 6 m/min. All Groups Showed Significantly Higher Latency to Fall Compared to the DI Group (p<0.01; *p<0.05)**

When the rod was made to rotate at higher speed (6 m/min) on day one, the CONT mice stayed on it significantly more time before falling (11.6 ± 1.9 s) than the DI (7.5 ± 1.1 s) and DEN (7.6 ± 0.9 s) groups. No significant difference was noticed between the CONT and DENX (10 ± 1.1 s), see figure 2 (C). Performance of all the groups except the DI mice, in the five days of testing when the rod rotation speed was 6 m/min, demonstrated increased time of staying on the rotating rod before falling, indicating that they developed specific strategy to stay more balanced (ANOVA f=4.2180, p=0.029).

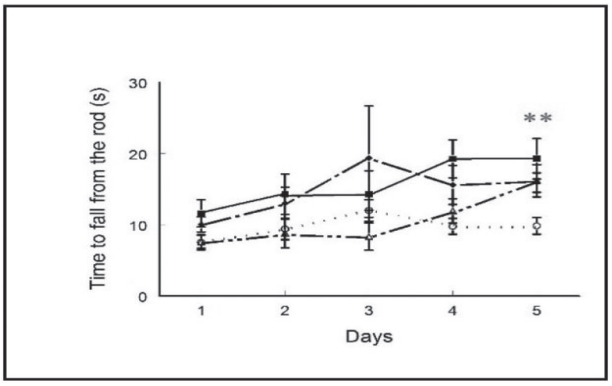

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Figure 2 (C): Motor Learning Throughout the Five-Day Trials with Rod Rotation Speed at 6 m/min. By Day Five, All Groups, Except DI, Demonstrated an Increase in Time to Fall (p<0.01 Compared to Day One)**

DISCUSSION

In this study, it has been shown that 12 weeks of environmental enrichment alone or with voluntary daily exercise (wheel running) resulted in an improvement in cognition and motor learning of STZ-induced diabetic mice compared to socially isolated diabetic mice.

In the water maze test, all groups except the DI mice, showed improvement in their performance over the 5-day period of training as evidenced by reductions in time (escape latency) and distance swum to reach the platform. These results support previous studies of memory impairment in diabetes and show that exercise itself did not have a significant additional benefit on learning and memory compared to environmental enrichment alone[3,4]. These results suggest that cognitive benefits can be achieved by enrichment alone, and this is similar to previous studies of memory decline in aging, dementia, and Alzheimer's disease[18-21]. Other studies compared exercise and enrichment had reported mixed findings for neurogenic and behavioral effects[22-24]. Whether or not these effects of enrichment are driven by hippocampal cell proliferation remains unclear.

No differences in motor coordination (latency to fall on day one) between the groups were found. However, at both speeds, there was evidence of impaired motor learning in the DI mice. Furthermore, there was an enhanced motor learning with enrichment and exercise similar to the CONT group. This result is similar to the study of Van Meeteren et al who showed that forced exercise improved motor function[25]. Recently, there is evidence from human studies to suggest that moderate aerobic exercise may have a role in ameliorating diabetic peripheral neuropathy[26]. The DENX group had enhanced motor learning compared to the DEN groups on day five at 4.5 meters/minutes (but not 6.0 meters/min) suggesting that motor learning is more evident at a lower speed only. At higher speeds, remaining on the rotarod is more demanding due to other more complex factors making the difference between the groups below our threshold for detection.

In the Arabian Gulf countries, the prevalence of diabetes are amongst the highest globally and the number of cases is expected almost to double by the year 2040[1]. This projected increase in diabetic patients would lead to increased financial burden on national health services. The effects of diabetes on higher functions of the brain such as learning and memory are underappreciated in clinical practice.

Diabetic patients are faced with two challenges, both of which deteriorate cognitive power: the effect of aging and the effect of diabetes on the brain[1]. In this study, we have demonstrated that environmental enrichment could reverse the effects of diabetes on learning and memory. However, a limitation of our study is the lack of investigation of intracellular markers of hippocampal function. In rats, diabetes deteriorates learning and memory as well as hippocampal long-term potentiation (LTP) and depression (LTD) which are forms of synaptic plasticity[27,28]. In addition, the negative effects of behavioral stress on LTP and LTD can be reversed by environmental enrichment[29]. Further studies of cellular and molecular changes related to brain higher functions in diabetes should be addressed, and the role of environmental enrichment in reversing these changes should be investigated.

Long-term diabetes leads to increased risk of falls in the elderly as a result of neuropathy which affects gray matter volume, in the cerebellum and basal ganglia, as well as peripheral nerves[30-32]. In this study, we have demonstrated the value of environmental enrichment on motor performance in diabetes. Further studies may be warranted with consideration given to the enhanced motor learning.

CONCLUSION

This study shows that exercise itself does not have a significant additional benefit on learning and memory compared to environmental enrichment in diabetic mice. Motor learning is impaired in diabetic socially isolated mice and enhanced with environmetal enrichment and exercise. The effects of enrichment on motor learning (as measured by Rotard performance) are worthy of further investigation regarding the mechanism and the benefits to other motor activities.

Author Contribution: All authors share equal contribution towards: (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Yes.

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Competing Interest: None.

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