Int J Diabetes & Metabolism (2009) 17:93-98

Effect of exercise on spatial learning and memory in male diabetic rats

Rafah Sami Ayoub
Department of Basic Medical Sciences, College of Dentistry,University of Mosul, Mosul, Iraq

Abstract
The aim of the present study was to evaluate the effect of regular exercise at 2, 4, 6 weeks intervals on spatial learning and memory in Morris water maze and in passive avoidance test in male diabetic rats. For this purpose, male Wistar rats were divided into four groups, two groups of control rats, one sedentary control and one swimming exercise-trained control and two groups of diabetic rats. One sedentary diabetic, and the other swimming exercise-trained group. The diabetic sedentary group exhibited a significant increase in latency to reach platform as compared to control (sedentary). Physical activity produced a significant enhancement in spatial learning with a significant decrease in latency to reach platform. At the end of 2, 4, 6 weeks of regular exercise, the mean initial latency (IL) showed a significant increase in diabetic (sedentary) group compared to control (sedentary), while exercised diabetic group showed a significant increase in IL compared to control exercised and significant decrease as compared to diabetic (sedentary). In addition, step-through latency (STL) was significantly reduced in diabetic exercised group compared to control exercised, but at the end of 6th week showed enhancement in time as compared to control exercised and diabetic (sedentary) groups. Tissue malondialdehyde of kidney, liver and heart showed significant increase in diabetic (sedentary) and declined in diabetic exercised groups. The present results indicate that regular exercise enhanced learning and memory in diabetic rats and this may be mediated through the activation of the antioxidant system.

Keywords: *Learning and memory, exercise, passive avoidance, diabetic rat, streptozotocin, cognition.*

Introduction

The therapeutic use of physical exercise for diabetes treatment has been promoted since 600 B.C. before the discovery of insulin in 1922. Some investigators highlighted the interaction between this hormone and regular physical activity, with possible beneficial results in diabetes treatment.[1]

Diabetes mellitus is a common metabolic disorder characterized by hyperglycemia due to an absolute or relative insulin deficiency.[2] Diabetes mellitus is known to be associated with neurological complications in both the peripheral and central nervous systems.[3] In rats with experimentally induced diabetes using streptozotocin (STZ), the nerve damage was similar to the nerve degeneration observed in human diabetic neuropathy.[4] Recently, pathological studies have suggested that diabetes is one of the risk factors for Alzheimer's disease,[5] from a functional view point, diabetes mellitus is reported to specifically impair the memory function in experimental animals with strong involvement of the hippocampus and cerebral cortex. Interactions of glucose and cognitive function have been reported, both in the presence of elevated arterial blood glucose levels and with decreased cerebral glucose metabolism. This finding may indicate disturbed acquisition and/or consolidation of memory.[6] Enhanced physical activity in laboratory mice and rats has been reported to facilitate memory acquisition and retention in various behavioral tasks, which test different forms of learning. For instance, after one month of voluntary running, mice mastered the Morris water maze task faster than sedentary control.[7,8] Rats that had been housed with a running wheel for (4-8) weeks showed increased freezing behavior in the contextual fear conditioning task[9] and exercise also significantly improved retention in the passive shock avoidance task in mice.[10] Exercise also improves spatial memory in rodents.[11] Moreover, physical activity is associated with a variety of changes that may enhance synaptic plasticity independently from neurogenesis such as increase acetylcholine, opiate and monoamine neuro-transmitters, the transcription factor c-fos, insulin-like growth factor and fibroblast growth factor. Exercise may also increase the level of brain-derived neutrophic factor (BDNF) in the hippocampus and stimulate hippocampal neurogenesis.[12]

This study was undertaken to evaluate the relationship between exercise, learning and memory and diabetes mellitus

Received on: 4/10/2008
Accepted on: 24/10/2009

Correspondence to: Rafah Sami Ayoub, College of Dentistry, Department of Basic Medical Sciences, Mosul Uuniversity, Mosul. Iraq. E mail: dr-rafahsami@yahoo.com

Materials and Methods

Animals

Male albino Wistar rats weighing, 200 - 250g (about 3

months old), were housed in air-conditioned room (20 - 22 °C) and supplied with standard pelleted diet and tap water *ad libitum*. The animals were randomly divided into 4 groups (n=6 each). Two groups of control rats: one sedentary control and one swimming exercise-trained control and two groups of diabetic rats. One sedentary diabetic and the other swimming exercise-trained group.

Diabetes was induced by a single intraperitoneal injection of streptozotocin (60 mg/kg) dissolved in cold 0.9% saline solution immediately before use. The glycemia was evaluated seven days after streptozotocin administration. Blood was obtained through a puncture in the rat tail and placed on glycol tape (One Touch Ultra, LifeScan, Johnson and Johnson, UK). Diabetes was characterized by glycemia indices higher than (200 mg/dl). Rats with this parameter were selected for this study.[13]

Exercise protocol

Swimming was done in a cylindrical tank, 120 cm in diameter and 80 cm in height, with 20 cm warm water (30 - 32 ^{o}C). The animals were placed in the tank every day at the same hour (10.00 - 11.00 am) and the training was monitored by the same person. The rats initially swam (10 min/day) five days/week which allowed the animals to adapt to water environment without determining physical condition through swimming practice[14] with progressive increase of 10 minutes per day up to the maximal time of 60 minutes. The 60 minutes per day time schedule was kept until the end of the experimental period of (6 weeks).

Swimming program was performed according to Volpato.[15] In brief, rats were adaptation to water for 20 minutes for 5 days. Swimming was increased progressively at 10 minutes/day for 5 day. Rats were made to swim for 60 minutes per day for 4 weeks.

Spatial learning

Control and test groups of rats were trained on a Morris water maze[16] with either two or four trials per days for 7 days. The platform was hidden 1cm below the surface of water. It was made opaque with white non-toxic paint. The starting points were changed every day. Each trial lasted either until the rat found the platform or for a maximum of 40 seconds. At the end of each trial, the rats were allowed to rest on the platform for 10 seconds. The time to reach platform (latency) was calculated.

Single trial passive avoidance test

This test was conducted 2-3 days after Morris water maze. The apparatus consisted of an illuminated chamber connected to a dark chamber by a guillotine door. Electric shocks were delivered to the grid floor by an isolated stimulator on the first and second days of the test. Each rat was placed on the apparatus and left for 5 minutes to habituate to the apparatus. On the third day an acquisition trial was performed. Rats were individually placed in the illuminated chamber. After a habituation period of 2 minutes, the guillotine door was opened and the door was closed after the rat entered the dark chamber. An inescapable scrambled electric shock (1 mA, 2 second once) was delivered, in this trial, the initial latency (IL) of entrance into the dark chamber was recorded and rats with IL greater than 60 seconds were excluded from the study. Twenty four hours later, each rat was placed in the illuminated chamber for retention. The interval between the placement in the illuminated chamber and the entry into the dark chamber was measured as step-through latency (STL), up to a maximum of 600 seconds as cut-off.

Tissue sample collection

At the end of memory and spatial learning experiments, the rats were subjected to intraperitoneal injection of ketamine hydrochloride and xylazine at doses of 100 mg and 5 mg per kilogram of body weight, respectively. Liver, kidney and heart were dissected out immediately and transferred into cold, physiological saline. Tissue homogenates from heart, liver and kidney were prepared for the measurement of malondialdehyde using thiobarbituric acid (TBA) reaction.[17]

Statistical analysis

All results were expressed as mean ± S.E.M using one way analysis of variance (ANOVA) and group differences were determined using Duncan multiple range test was used, in all calculations, a difference at $p<0.05$ was regarded as significant.[18]

Results

Figure 1 shows the time latency to reach platform in Morris water maze learning in control and diabetic (sedentary) and control and diabetic exercised after 2, 4, 6 weeks of exercise intervals. The diabetic sedentary group showed a significant $p < 0.05$ increase in time latency as compared with control sedentary while physical exercise produced a significant ($p < 0.05$) enhancement in diabetic rats as decrease in time to reach platform as compared with diabetic sedentary and significant ($p < 0.05$) increase as compared with control (exercised).

Figure 2 shows the initial latency of control and diabetic (sedentary) and control and diabetic exercised groups in male rats in a single trial passive avoidance test. Diabetic (sedentary) group produced a significant $p < 0.05$ increase in time latency compared to control (sedentary) after 4 and 6 weeks of experiment. Diabetic exercised group showed a significant ($p < 0.05$) increase in time latency as compared to control exercised through 2, 4, 6 weeks of exercise interval and significant ($p < 0.05$) decrease in time latency as compared to diabetic (sedentary) group through 4 and 6 weeks of exercise intervals.

Figure 3 shows step through latency of control and diabetic (sedentary) groups and control and diabetic exercised groups there was a significant ($p < 0.05$) reduction in this parameter after 4 and 6 weeks of exercise. Diabetes clearly decreased consolidation and recall of passive avoidance response in diabetic (sedentary) as compared to control (sedentary).

The diabetic exercised group showed a significant ($p < 0.05$) increase in STL through 6 weeks duration of exercise compared to diabetic (sedentary).

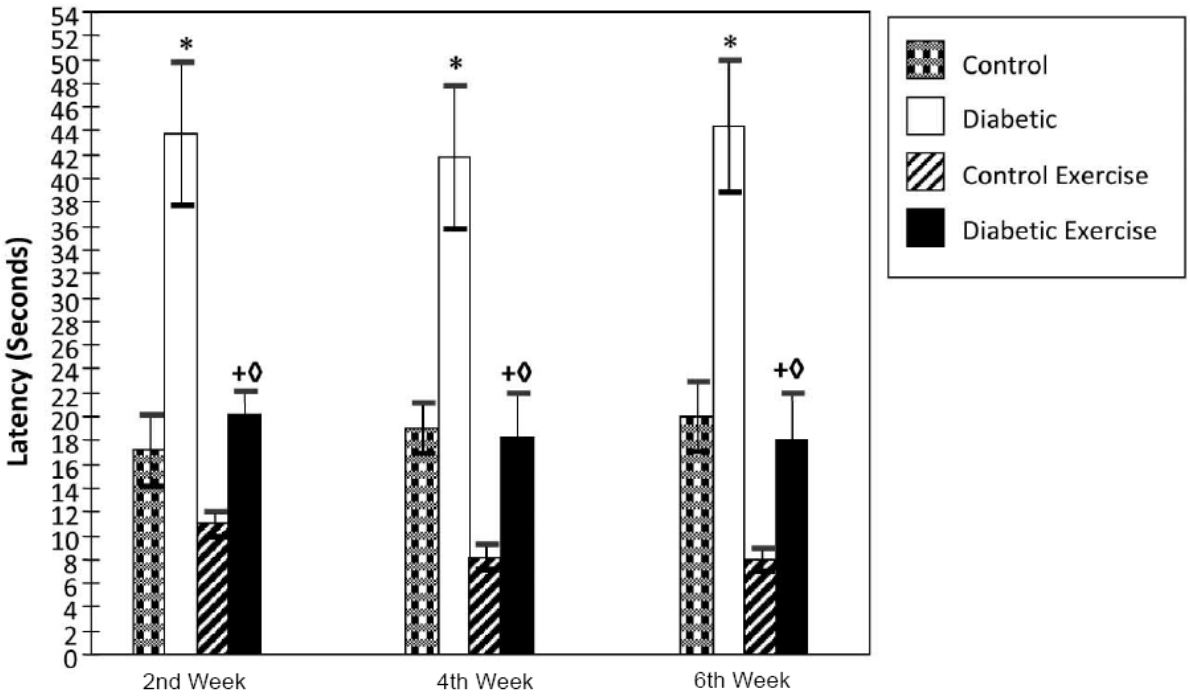

Figure 1: Morris water maze learning in control and diabetic sedentary groups and control and diabetic exercised groups in male rats to reach platform (latency). n = 6 animals per group, values are mean ± S.E.M, * statistical difference from control (sedentary) value $p < 0.05$, ◊ statistical difference from control (exercised) value $p < 0.05$, + statistical difference from diabetic (sedentary) value $p < 0.05$.

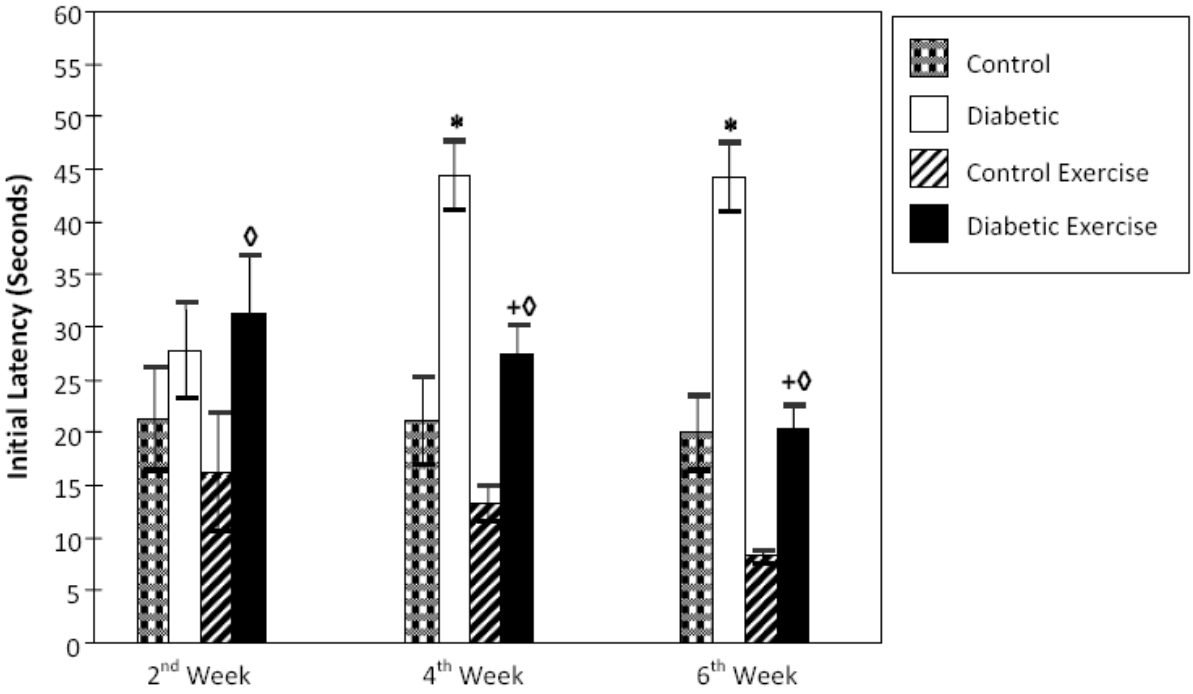

Figure 2: Initial latency of control and diabetic (sedentary) and control and diabetic (exercised) groups in male rats in a single, trial passive avoidance test. Values are mean ± S.E.M, n = 6 animals per group, *statistical difference from control (sedentary) value $p < 0.05$, ◊statistical difference from control (exercised)value $p < 0.05$, +statistical difference from diabetic (sedentary) value $p < 0.05$

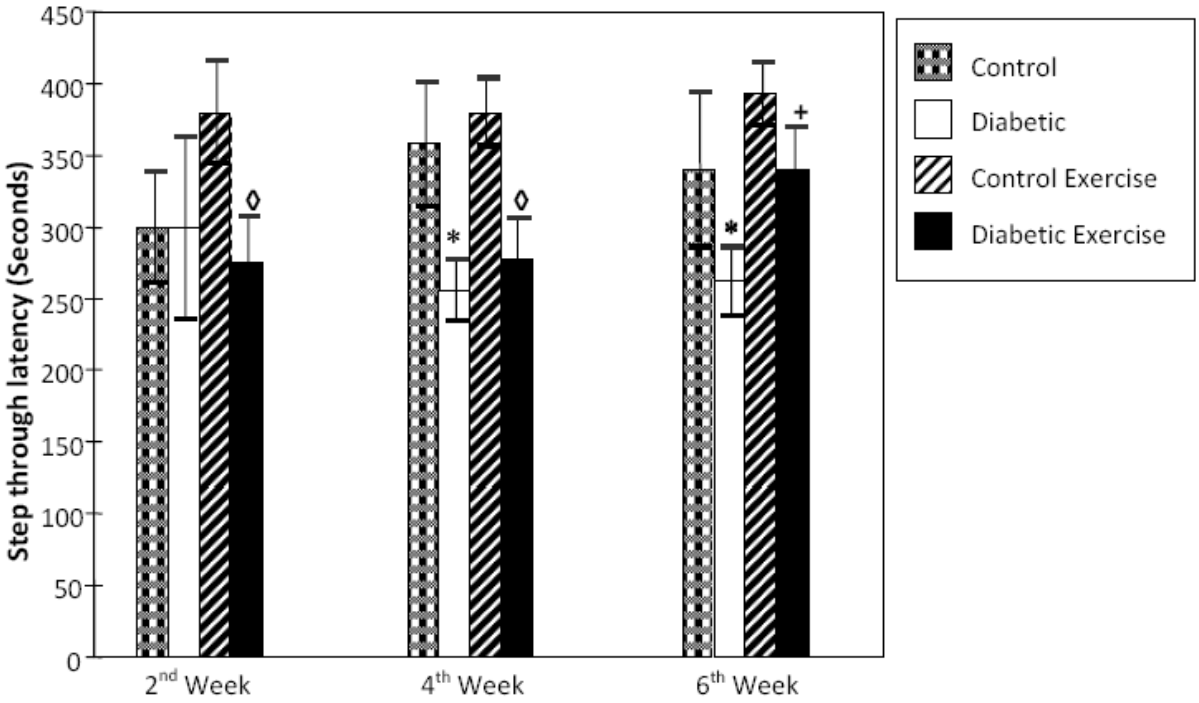

Figure 3: Step through latency of control and diabetic (sedentary) groups and control and diabetic (exercised) groups of male rats in single-passive avoidance test. Values are mean ± S.E.M, n = 6 animals per group, *statistical difference from control (sedentary) value, $p < 0.05$; ◊statistical difference from control (exercised) value, $p < 0.05$; + statistical difference from diabetic (sedentary) value, $p < 0.05$.

Table 1: Effect of exercise on malondialdelyde contents (MDA mmol/g) of wet weight of kidney, liver, heart of control and diabetic (sedentary) and control and diabetic (exercised)

Experimental groups	Kidney	Liver	Heart
Control (sedentary)	356.00 ± 12.40	370.30 ± 17.30	700.10 ± 15.06
Diabetic (sedentary)	540.00 ± 22.10	570.40 ± 11.06	900.50 ± 45.06
Control (exercised)	289.38 ± 30*	390.50 ± 11.30*	550.00 ± 50.20*
Diabetic (exercised)	426.40 ± 48*	410.50 ± 25.00*	500.20 ± 50.30*

Values are mean ± S.E.M, * = $p < 0.05$ compared to controls

Table 1 shows the effect of exercise on MDA contents expressed as MDA mmol/g of wet weight of kidney, liver, heart of control and diabetic (sedentary) and control and diabetic (exercised).

The MDA content of kidney, liver and heart in diabetic (sedentary) groups increased significantly ($p < 0.05$) compared to control (sedentary) and exercised diabetic groups produced a significant ($p < 0.05$) increase in MDA in kidney tissue as compared with control exercised. Physical exercise in diabetic groups caused a significant ($p < 0.05$) decrease in MDA content in kidney, liver, heart tissue as compared to diabetic sedentary groups.

The control (exercised) group showed a significant ($p < 0.05$) decrease in MDA content in heart tissue as compared to control (sedentary) groups.

Discussion

The results clearly demonstrated that diabetes is accompanied with disturbance in animal performance in Morris water maze learning. Diabetes produced a significant increase in time latency to reach platform as compared to control (sedentary), two, four and six weeks after the onset of diabetes. Previous studies have reported that diabetes mellitus is associated with neurological complications in both peripheral and central nervous systems. Impairment of learning and memory is also recognized as a complication of diabetes.[19]

Cognitive deficits in diabetes mellitus can result from metabolic impairment or cerebral vascular complications.[2] Our observations on spatial learning deficits in cognitive task in diabetic rats agree with those reported in literature.[4] There is a strong evidence for the involvement of micro-vascular dysfunction and oxidative stress due to excessive production of free radicals. Since the mammalian

hippocampus and cerebral cortex play a pivotal role in a diverse set of cognitive functions, such as novelty detection and memory, these areas are very vulnerable to oxidative damage in streptozotocin-induced diabetic animals.[20] Furthermore, streptozotocin- induced diabetes in rats results in the altered function of N-methyl-D- Aspartate (NMDA) and amino-hydroxy-propionic acid type glutamate receptors, which are implicated in learning and memory process.[21]

While physical exercise in adult rats and diabetic rats showed significant enhancement in spatial learning performance apparent with decreased latency finding the platform in Morris water maze, this is a novel finding, both running and living in an enriched environment double the number of surviving newborn cells and improve water maze performance.[22] It has been reported that running enhances neurogenesis level of brain-derived neurotrophic factor (BDNF) and other growth factors and neurotransmitters especially in the hippocampus.[16] In addition, hippocampal dependent learning may enhance survival of cells prior to spatial learning.[23] The observation that diabetic sedentary rats had significant decrease in initial latency time after four and six weeks of exercise indicates that locomotor and exploratory activities may have an influence on the regulation of some behavioral tests such as passive avoidance test.[6] There are some reports on the involvement of cholinergic system abnormality in the impaired acquisition and/or retention of passive avoidance learning. It has been postulated that the observed behavioral abnormalities may be a consequence of an impairment of cerebral glucose metabolism and may be suggestive of cholinergic dysfunction.[24] It has been reported that the level of neurotrophic, insulin-like growth factor (IGF) is reduced in diabetic patients and rodents. IGF is essential for learning/memory processes and a loss of IGF activity due to diabetes may contribute to cognitive disturbance.[25] Other findings postulate altered expansion of NCAM (neural cell adhesion molecule) in diabetes. Lesions of the brain regions involved in learning and memory may be important cause of learning and memory deficits in diabetes mellitus.[6] From a biochemical view point, cognitive impairment in diabetes mellitus has associated with hippocampus apoptotic neuronal loss. In this respect, impaired insulinomimetic action by C-peptide plays a prominent role in cognitive dysfunction and hippocampus apoptosis in type 1 diabetes.[26] It has been shown that physical activity enhances significantly step-through latency and initial latency through an increase in circulatory BDNF levels especially when exercise is performed voluntarily.[27] Exercise has also been shown to facilitate neurogenesis, plasticity and dendrite proliferation in the hippocampus.[7] The physiological benefits of exercise seen in the hippocampus correlate well with the research showing increased performance on learning and memory tests resulting from exercise in rodents.[28] Our results agree with literature reports that regular exercise plays a role in the reduction of oxidative stress and an increase in antioxidant defenses and reduction of MDA.[29]

Conclusion: Our results emphasize the role of physical activity in the promotion of learning and memory function in diabetic animals probably by enhancing antioxidant status of the body.

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