Original Article

The effect of Aerobic Training on Serotonin and Tryptophan Hydroxylase of Prefrontal Cortex in type 2 Diabetic Rats

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Abstract

Background & Aims: Type 2 diabetes (T2D) is a self-management disease and depression is a common problem related to it. One of the causes of depression is serotonin (5-HT) depleted. The enzyme tryptophan hydroxylase (TPH) is known as limiting enzyme in the production of 5-HT in the brain. Aerobic exercise also has proven benefits in treating and reducing the incidence of chronic diseases such as diabetes. Thus, in this study, we examine the effect of aerobic training on 5-HT and TPHof prefrontal cortex in type 2 diabetic rats.

Materials & Methods: This study is experimental and post-test. 30 rats were randomly divided into 3 groups: 1- healthy control 2- diabetic control and 3- exercise diabetic. Groups 2 and 3 received streptozotocin (37mg/kg) by intraperitoneal injection two weeks after the high-fat diet Diabetic training 5 times a week for 8 weeks run on a treadmill with duration and intensity that in the final weeks were 55 min / d and 26 m / min , respectively. 24 hours after the last exercise the prefrontal cortex of mice tissue samples of all groups were extracted and 5-HT (µg/g) and TPHconcentration was measured respectively by Elisa and Western Blotting from prefrontal cortex tissue samples. To evaluate the differences between the group of design, analysis of variance (ANOVA) and Tukey post hoc test at the significance level was less than 0.05 were used.

Results: Statistical analysis showed that 5-HT levels in the diabetic group were significantly lower than the healthy control group (P=0.001) and exercise diabetic (P=0.009) and average 5-HT between control group and exercise diabetic has no significant difference. TPH results show that the average diabetic groups were significantly lower than the healthy control group (P=0.000). The results showed that the amount of TPH in the exercise diabetic group was significantly higher than the diabetic control group (P=0.000).

***Conclusion*:** In this study, diabetes reduces 5-HT in the prefrontal cortex. Some studies have shown that inflammation in type 2 increases the cytokines IL-6 and TNF-α, and these cytokines by increasing the activity of indolamine 2, 3 dioxygenase in the brain alters the metabolism of tryptophan and reduces the production of 5-HT. Chronic activity reduces systemic and tissue inflammation, thus increasing 5-HT in the brain. The reduction of TPH due to diabetes can also be the factors that affect in the decrease of prefrontal 5-HT.

KeyWords: Aerobic Training, Serotonin, Tryptophan hydroxylase, Prefrontal Cortex, Diabetes

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Introduction

Type 2 diabetes (T2D) is a self-care disease that affects over 350 million individuals in the world (1). Diabetes is characterized by hyperglycemia due to failure in insulin secretion or insulin effect caused by insulin resistance (2) and has complications such as cardiovascular disease, nephropathy, retinopathy, and widespread disease of both the peripheral and central nervous systems. Diseases of the nervous system could have debilitating side effects on sensitive areas of the brain like the prefrontal cortex (3). On the other hand, according to studies Asghar S et al (2007)(4), Khamesh M E et al (2007)(5) and Li C et al. (2009)(6) depression is a common problem among type 1 and 2 diabetic patients. The risk of depressive symptoms among diabetic patients is twice more than other people without diabetes independent of gender and type of diabetes and how to measure (7). The main mechanisms of the link between diabetes and depression are altered in insulin signaling in the brain, activation of inflammatory pathways and up-regulation of hormone systems such as the glucocorticoid. Another mechanism is the effect of lifestyle (8). Zaki and his colleagues (2013) have shown that a reduction in brain serotonin (5-HT) causes depression symptons in the rats (9) and Kim and colleagues (2015) showed that impose conditions causing depression reduces brain 5-HT in rats (10). Depletion of 5-HT in the etiology of depression is the neurobiologic factor (11). Various factors cause impaired in 5-HT system, which the researchers mentioned associated factors such as decreased plasm a tryptophan (12), and reduced production of 5-HT (13). Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in 5-HT biosynthesis (14) and so affect the amount of 5-HT in the brain.

Prefrontal cortex (PFC) regulates emotion and participated actively in the pathophysiology of psychiatric disturbance. It is well established that 5-HT neurons in the PFC are projected from both the dorsal raphe nuclei (DRN) and the median raphe nuclei (MRN) and thus is very sensitive to serotonergic stimulations (15). It seems that the reduction of plasma tryptophan decreases the production of 5-HT in the brain of diabetic rats because the brain tryptophan compared to other amino acids is more sensitive to decrease in plasma tryptophan (16). Studies also show that inflammatory cytokines associated with type 2 such as IL-6 and TNF-α increase activity in the brain indoleamine 2,3-dioxygenase (IDO) which change the tryptophan metabolism, reduce the production of 5-HT and the increase in production of Kynurenine (KYN) in the brain. Stress can also activate the enzyme tryptophan 2,3-dioxygenase (TDO) and is another way to reduce the synthesis of 5-HT in the brain. Decrease in 5-HT and its metabolites lead to consequences such as disruption of biological rhythms of sleep and wakefulness and neurological disorders observed in depression. Up regulation of KYN make oxidative and cognitive disorders that typically affect on depression (17).

Health benefits of regular physical activity of the brain, such as the treatment of mental illness, brain injury and degenerative diseases of the nervous system are improved. Adjust the factors related nerves, factors related to vascular, inflammatory mediators and neurotransmitters are involved in the effect of regular physical activity in the brain function (18). Among these effects, release of neurotransmitter, especially monoamines relates to adaptation to regular physical activity. Some studies have shown the reduction of the amount of 5-HT in some brain areas due to adaptation resulting from endurance training. Reduce in the 5-HT ratio of tryptophan in the trained rats suggest that a decrease in brain 5-HT, due to endurance training, may be due to reduced expression of TPH. Previous findings showed that TPH in cerebral cortex and striatum of regular trained rats was reduced. This reduction of TPH can be caused by decreased production or increased protein breakdown (19). Studies on the impact of regular

exercise on the serotonin system in the diabetic subjects were not observed and studies in this object are essential.

Some studies on the impact of physical activity on the serotonin system in depression conditions are available. For example Wang J et al (2013) showed that stress-induced reduction in hippocampus serotonin was compensated with physical exercise (20). Liu W et al (2013) represented that swimming training increases PFC serotonin in rats exposed to stress (21). Kim T et al (2015) indicated that aerobic exercise improved depression and increased brain serotonin (10). But Lee H et al (2013) showed that despite improvements in depression, there was no change in brain serotonin of trained rats (22). It seems that factors such as the type of exercise, the duration of exercise and the environmental factors impact on results (18).

The possible mechanism of anti-inflammatory effects of regular exercise on diabetes, are include reducing the percentage of body fat and macrophage accumulation in adipose tissue. Regular exercise also reduces inflammation by reducing the inflammatory mediators such as C-reactive protein (CRP), IL-6 and TNF-a and increases anti-inflammatory cytokines such as IL-4 and IL-10. In addition, regular exercise may reduce cells that produce proinflammatory cytokines by chronic reduction of oxidative stress. Cells in response to stimulation through physical exercise, activate pathways relieve stress, including increased expression of enzymes such as superoxide dismutase (SOD) and Glutathione peroxidase (GPx). Reducing oxidative stress in patients with type 2 through regular physical exercise can prevent leakage of blood-brain barrier (BBB) and prevent the infiltration of inflammation into the brain (23). Therefore, presumably regular exercise with reduce inflammation and oxidative stress can enhance brain serotonin of patients with type 2. But the direct impact of exercise on the serotonin system in diabetic subjects was not observed. There are many uncertainties about impact of regular exercise on the brain of patients with type 2 especially serotonin system. In this study, we investigated the effect of eight weeks aerobic training on serotonin and TPH in PFC of rats with type 2.

Materials and Methods

This study is experimental and post-test with control group. In this study 30 male Wistar rats (n=30) age about 6 months and weight between 225- 300 g purchased from Pasteur Institute (Tehran, Iran) were used. Rats were randomly and equally divided into three groups: 1-healthy control (HC) 2-diabetic control (DC) 3-diabetic training (DT). Each rat was housed alone in one regular cage and was maintained with 12 hours light/dark cycles in a quiet environment and with 50% humidity and 22 ± 1°C temperature. The animals were fed standard lab rat chow dieting water ad libitum. The compliance of all steps of this experiment with the National Institutes of Health publication (revised in 1996) was approved by the Animal Care and Use Committee of Tabriz University of Medical Sciences (Tabriz, Iran).

Induction of diabetes:

In order to adapt to environmental, all interventions were done at least two weeks after lodgment of animals and those rats were used that in normal conditions without fasting their serum glucose level were less than 250 mg /dl. DC and DT groups receiving fat rich diet including carbohydrates (25%), proteins (25%) and lipids (50%) for two weeks before intra-peritoneal receiving 37 mg/kg streptozotocin citrate buffer (0.1M, pH 4.5). The HC group was injected the buffer. 72 hours after injection, glucose of blood samples from the tail vein were tested using a portable glucometer and higher glucose concentration higher than 300 mg/dl as the diabetic rats were interred in this study. After injection of streptozotocin three rats died so were replaced with new rats.

Aerobic training:

Rats of DT group after diabetes ran five sessions a week for eight weeks on a treadmill. Firstly rats began their training for ten minutes a day, speed ten m/min and with slope of 6º(10%). Speed and duration of the training were increased gradually during the next three weeks until the final weeks duration and intensity of training was 55 minutes a day and 26 m/min respectively. According to previous studies, factors of central fatigue have been reported in training intensity at 19 meters per minute for one hour at eight weeks (24) or began with 16 meters per minute and finally raised to 28 meters per minute in six weeks (24,25). During aerobic training some rats were not completed training protocol, so the sound stimuli in addition to electric stimuli on the treadmill are also used to stimulate the animals.

Samples collection and measurements:

24 hours after the last training session, rats in all groups by intra-peritoneal injection of ketamine (90 mg/kg) and xylazine (10 mg/kg) anesthetized and then underwent surgery and their PFC tissue samples immediately extracted and nitrogen -80ºC were frozen and stored for further analysis. Due to the small size of the rat brain, the PFC is sampled **as a** unit and taking of the sample from subunits of PFC was avoided. By using a syringe, blood samples were obtained from the heart and plasma and serum samples were extracted. Measurement of serum glucose was performed with mg/dL sensitivity by the enzyme glucose oxidase method (Bio-Chemistry).

PFC samples were homogenized and for 25 minutes at 15000 g (4ºC) was centrifuged. Then supernatant was collected and the concentration of serotonin was measured by ELISA kit (Ng/ml sensitivity). In addition, the plasma insulin concentration was measured by ELISA using an insulin kit (µg/L sensitivity). Serotonin and insulin kits were prepared for *Crystal Day Biotech Company*.

Western blotting for TPH:

Protein (30 µg) homogenized samples were isolated by gel electrophoresis Sodium dodecyl sulfate (SDS) or by gels Solver % 5.5 (ACCphospho). Isolated proteins were electrotransferred from the SDS polyacrylamide gel to polyvinylidene fluoride membrane. The membrane was incubated overnight at 4ºC with monoclonal murine anti-TpH. The TPH- antibody complex was incubated for 1 hour at room temperature with goat-anti mouse IgG and then emerged with luminescence quantity method and the use of X-ray film. Band densities were measured by Image J software and then normalized versus beta-actin band obtain the amount of TPH. The sensitivity of this method is in the range of pg/ml. TPH antibodies were prepared by *Santa Cruz Company*.

Statistical analysis:

The data were expressed as mean ± SEM and analyzed using SPSS (version 18). To compare between group differences we used of ANOVA and Tukey test as a post hoc test. $P < 0.05$ was considered as statistical significance.

Results

Statistical analysis showed that the difference between the three groups of serum glucose ($F_{(2,18)}$=65.386 and P=0.000), plasma insulin ($F_{(2,18)}$=6.883 and P=0.006) and PFC serotonin ($F_{(2,18)}$=13.195 and P=0.001) and TPH is significant ($F_{(2,18)}$=699.437 and P=0.000). Tukey post hoc tests showed that there is a significant difference between HC&DC groups(P=0.000) and DC&DT groups (P=0.000) for serum glucose and HC&DC groups (P=0.017) and HC&DT (P=0.011) groups for plasma insulin, HC&DC groups (P=0.001) and DC&DT groups (P=0.009) for PFC serotonin and HC&DC groups (P=0.000), HC&DT groups (P=0.000) and DC&DT groups (P=0.000) for TPH of PFC (Table 1). The results also showed that difference between HC&DT groups of serotonin is not significant (P>0.05). But the mean of serotonin in HC and DT groups is significantly higher than the DT group (P<0.05). TPH results showed that mean in DC and DT groups is

significantly lower than the HC group (P<0.05) and mean in DT group is significantly (P<0.05) higher than DC group (Figure 1)

Table 1. The levels of serum glucose, plasma insulin and PFC serotonin and TPH.

	Healthy Control (n=10)	**Diabetic Control (n=10)**	**Diabetic Training (n=10)**
Glucose (mg/ml)	124±15	392±56 *	175±57 #
Insulin (µIU/L)	5.10±0.40	3.44±0.54 *	3.60±1.36 *
Serotonin (µg/g)	0.246±0.013	0.198±0.013 *	0.234±0.020 #
TPH	1	0.732±0.006 *	0.888±0.016 *#

* $P < 0.05$ compared with a healthy control group. # $P < 0.05$ compared with the diabetic control group
TPH, Tryptophan Hydroxylase; PFC, Prefrontal Cortex.

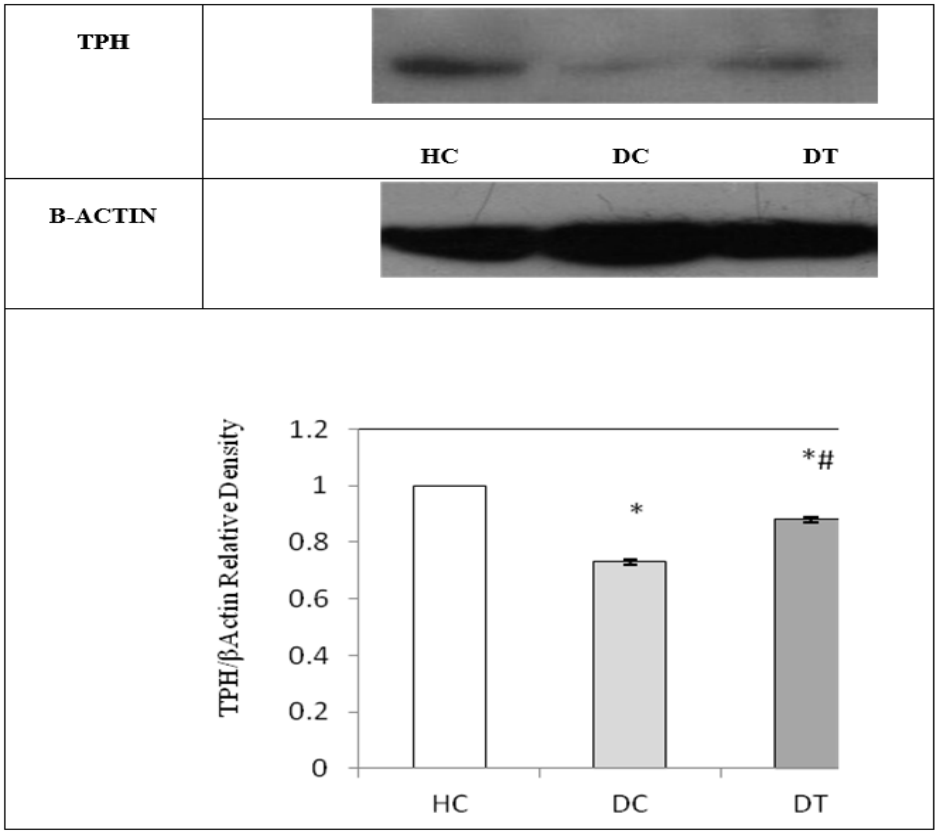

Figure1. Western blotting results for TPH. TPH, Tryptophan hydroxylase; PFC, Prefrontal Cortex; HC, healthy control; DC, diabetic control; DT, diabetic training. * $P < 0.05$ compared with a healthy control group. # $P < 0.05$ compared with the diabetic control group

Discussion

1- The results of study showed that glucose levels in DC and DT groups were higher than HC group and the difference in HC&DC was significant. This results point that increase in serum glucose was due to the induction of diabetes in rats. Plasma insulin levels in the HC group were significantly higher than DC and DT groups that indicates induction of type 2 reduces serum insulin as well.

Studies have shown that the use of low-dose streptozotocin injection and a high-fat diet, induced type 2 and along with hyperglycemia, insulin sensitivity reduces in insulin receptors (26). Type 2 is closely linked to obesity and most animal models of type II diabetes are obese. Streptozotocin injection can cause weight loss in animals to be studied (27). Streptozotocin one injection models of type 2 with normal diet cause a decline in plasma insulin but with high fat diet cause a normal plasma insulin (28). Therefore, in this study streptozotocin one injection with 37 mg/kg dose and high fat diet were used.

2- The results showed that serotonin levels of PFC in DC group were significantly lower than in HC group. The result of this study is in line with results of Trulson (1986) (29), Miyata (2007) (15) and Manjarrez (2015) (30) but is not in line with Hussein (2012) (31).

Miyata S et al showed that the release of serotonin after mental stress is reduced in diabetic rats and this decrease may be responsible for mental health problems in people with diabetes (15). Trulson M et al represented that streptozotocin-induced diabetes reduced brain serotonin in rats (29).In the study of Manjarrez G et al brain serotonin decreased due to the induction of diabetes (30). But Hussein et al showed that streptozotocin-induced diabetes increased the level of serotonin in the brain of rats (31). The studied brain regions and food intake of rats in Hussein J et al study was different with current study.

It seems that the reduction of plasma tryptophan (16) and an increase in inflammatory pathways (17) can induce the factors affecting on the decrease of serotonin is PFC. Studies have also shown that the amount of glucose in the brain affects on impaired serotonin activity in the PFC of diabetic rats. This glycemic control prevents serotonin system disorder caused by diabetes and behavior change. In clinical studies, the severity of symptoms of depression associated with glycemic control. Some results show that reduced serotonin responses to psychosocial stress in the PFC probably is involved in higher prevalence of depression in patients with diabetes (15).

3- The results of this study show that serotonin levels of PFC in DT group were significantly higher than DC group. Serotonin difference in DT group and HC group were not significant. The result of this study is in line with the results of Liu (2013) (21), Kim (2012) (10) and Wang (2013) (20) but is not in line with Langfort (2006) (25), Chen (2007) (32) and Lee (2013) (22).

A study that examined the effect of exercise on brain serotonin in diabetic subjects was not observed, but studies in healthy subjects, such as reading Langfort J (25) and Chen (32) showed reducing in the levels of brain serotonin due to regular exercise. Some studies have also examined the effect of exercise in reducing the condition of brain serotonin (21,22).For example, in a study of Liu W et al, stress, stimulation of the immune system and increases in proinflammatory cytokines reduced serotonin in the PFC of rats but swimming training increased PFC serotonin in exposed to chronic stress rats (21). Lee H et al showed that regular exercise improved depression without effective change in brain serotonin (22). Kim and his colleagues showed that reducing the amount of serotonin in the dorsal raphe nucleus due to stress, be compensated with swimming training (10). Wang J et al also represented that reduction in hippocampus serotonin was compensated with regular exercise (20). Previous studies have shown

that in subjects with diabetes, chronic exercise reduced systemic and tissue inflammation and decreased IL-6 and TNF-α cytokines and oxidative stress (23). Therefore, probably aerobic training can increase serotonin in patients with T2D. Also, aerobic training in conditions such as chronic diseases that reduce serotonin in the PFC is a solution to increase the activity of the serotonin system.

4- The results showed that the TPH in PFC in DC group was significantly lower than HC group. This result of the current study is in line with the results of Herrera (2004) (33), Kim (2015) (10), Manjarrez (2015) (30). In the study of Herrera R et al diabetes reduces the activity of TPH in several regions of the brain (33). Kim M et al showed that stress reduced TPH level in the dorsal raphe (10). In the study of Manjarrez G and his colleagues diabetes induced with streptozotocin reduced brain levels of TPH (30). The reduction in the dependence of TPH to the substrate has been shown in the brain of diabetic rats and probably second messenger such as cAMP, inositol triphosphate (IP3) and diacylglycerol (DAG) involved in the activity of TPH. It has been shown T2D alters the activity of enzymes due to changes in cellular phosphorylation mechanism. The mechanism for reduce protein expression in THP is involved enhancement of oxidative stress and increasing in brain glucose. Chronic increases of brain glucose disturb signaling of PKA and PKC and reduce calcium calmodulin related protein kinases. These factors immediately affect the catalytic properties of TPH (30).

5- Results of this study showed that the TPH of PFC in DT group was significantly higher than DC group. A study that examined the effect of exercise on TPH in diabetic subjects was not observed. This result of the current study is in line with results of Kim (2015)(10), but is not in line with results of Langfort (2006)(25). In the study of Kim M et al shown that reduction of TPH in dorsal raphe caused by stress was compensated with swimming training (10). But Landfort showed that aerobic training reduces the amount of TPH in several brain regions of healthy rats (25). Probably the mechanism of tryptophan to serotonin in diabetic rats is different with healthy rats. In healthy rats, regular exercise reduces the amount serotonin with reducing in TPH. But in diabetic rats probably due to lower levels of serotonin, aerobic training increases the amount of TPH and serotonin. Reducing of blood glucose and reactive oxygen species (ROS) can increase TPH in training diabetic rats (30). Therefore aerobic training can be considered as a factor for enhancement of TPH and serotonin in the PFC.

Conclusion

Due to lack of brain samples from human subjects, male Wistar rats were used for this study. Therefore, caution should be considered referring these results to human. For induction of diabetes, as well as some other studies, low-dose streptozotocin injection and a high-fat diet was used and probably caused login factors different from normal conditions of diabetes. Numerous studies have examined the effect of aerobic training on prevention, treatment and reduction of diabetic complications, but there are few studies about the impact of training on the brain in subjects with T2D and its complication such as depression. The study was not found about of the effect of aerobic training on the serotonin system in diabetes condition. It seems that the mechanism of the effect of aerobic training on PFC serotonin in healthy people is different with people with T2D. Serotonin levels are normal in healthy subjects and increasing of serotonin cause inhibitory effect and feeling of fatigue and with aerobic training can reduce this inhibitory effect. But in diabetic subjects' serotonin levels is lower than needs and cause feelings of depression.

In this study, diabetes was reduced serotonin in the PFC of DC group.TPH enzyme reduction due to diabetes can be one of the factors affecting in a decrease of serotonin in the PFC. According to the findings of this study,

aerobic exercise increases serotonin in the PFC of diabetic rats. An increase of TPH due to aerobic training may have the effect on the enhancement of serotonin in the PFC of diabetic rats. Therefore aerobic exercise in order to increase the activity of the serotonin system in the PFC of patients with T2D and is recommended. To clarify the mechanism of the effect of diabetes on the monoamine systems in different region of the brain, the interaction between systems, mental function as well as the impact of regular training on diabetic complications on the brain more studies are necessary.

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References

1. Oxenkrug GF. Increased Plasma Levels of Xanthurenic and Kynurenic Acids in Type 2 Diabetes. Mol Neurobiol 2015;52(2):805–10..
2. Lamb RE, Goldstein BJ. Modulating an oxidative-inflammatory cascade: potential new treatment strategy for improving glucose metabolism, insulin resistance, and vascular function. Int J Clin Pract 2008;62(7):1087–95.
3. Maiese K, Chong ZZ, Shang YC. Mechanistic insights into diabetes mellitus and oxidative stress. Curr Med Chem 2007;14: 1729-38.
4. Asghar S, Hussain A, Ali SMK, Khan AKA, Magnusson A. Prevalence of depression and diabetes: a population-based study from rural Bangladesh. Diabet Med 2007;24(8):872–7.
5. Khamseh M E, Baradaran H R, Rajabali H. Depression and diabetes in Iranian patients: a comparative study. Int J Psychiatry 2007; 37 (1): 81–6.
6. Li C, Ford E S, Zhao G, Ahluwalia I B, Pearson W S, Mokdad A H. Prevalence and correlates of undiagnosed depression among U.S. adults with diabetes: the Behavioral Risk Factor Surveillance System. Diabetes Res Clin Pract 2009; 83 (2): 268–79.
7. Anderson RJ, Freedland KE, Clouse RE, Lustman PJ. The prevalence of comorbid depression in adults with diabetes: a meta-analysis. Diabetes Care 2001;24(6):1069–78.
8. Haghighatdoost F, Azadbakht L. Dietary treatment options for depression among diabetic patient, focusing on macronutrients. J Diabetes Res 2013; 2013:421832.
9. Zaki HF, Rizk HA. Role of serotonergic and dopaminergic neurotransmission in the antidepressant effects of malt extract. Afr J Pharmacol 2013; 7(46): 2960-71.
10. Kim TW, Lim BV, Baek D, Ryu D-S, Seo JH. Stress-Induced Depression Is Alleviated by Aerobic Exercise Through Up-Regulation of 5-Hydroxytryptamine 1A Receptors in Rats. Int Neurourol J 2015;19(1):27–33.
11. Jans L a. W, Riedel WJ, Markus CR, Blokland A. Serotonergic vulnerability and depression: assumptions, experimental evidence and implications. Mol Psychiatry 2007;12(6):522–43.
12. Cowen PJ, Parry-Billings M, Newsholme EA. Decreased plasma tryptophan levels in major depression. J Affect Disord 1989;16(1):27–31.
13. Birkmayer W, Riederer P. Biochemical post-mortem findings in depressed patients. J Neural Transm 1975; 37: 95–109.
14. Boadle-Biber M C. Regulation of serotonin synthesis. Prog Biophys Mol Biol 1993; 60: 1–15.
15. Miyata S, Yamada N, Hirano S, Tanaka S, Kamei J. Diabetes attenuates psychological stress-elicited 5-HT secretion in the prefrontal cortex but not in the amygdala of mice. Brain Res 2007;1147:233–9.
16. Thorre K. Differential effects of restraint stress on hippocampal 5-HT metabolism and extracellular

levels of 5-HT in streptozotocin-diabetic rats . Brain Res 772. 1997; 209–16.

17. Oxenkrug G. Insulin resistance and dysregulation of tryptophan-kynurenine and kynurenine-nicotinamide adenine dinucleotide metabolic pathways. Mol Neurobiol 2013;48(2):294–301.
18. Lin T W, Kuo Y M. Exercise Benefits Brain Function: The Monoamine Connection. Brain Sci 2013; 3: 39-53.
19. Langfort J, Barańczuk E, Pawlak D, Chalimoniuk M, Lukacova N, Marsala J, et al. The effect of endurance training on regional serotonin metabolism in the brain during early stage of detraining period in the female rat. Cell Mol Neurobiol 2006;26(7–8):1327–42.
20. Wang J, Chen X W, An G H, Zhang N, Ma Q. Effects of Exercise on Stress-Induced Changes of Norepinephrine and Serotonin in Rat Hippocampus. Chinese J Physiol 2013; 56(5): 245-52.
21. Liu W, Sheng H, Xu Y, Liu Y, Lu J, Ni X. Swimming exercise ameliorates depression-like behavior in chronically stressed rats: Relevant to proinflammatory cytokines and IDO activation. Behav Brain Res 2013; 242: 110–6.
22. Lee H, Ohno M, Ohta S, Mikami T. Regular moderate or intense exercise prevents depression-like behavior without change of hippocampal tryptophan content in chronically tryptophan-deficient and stressed mice. PLoS ONE 2013;8(7):e66996.
23. Bertram S, Brixius K, Brinkmann C. Exercise for the diabetic brain: how physical training may help prevent dementia and Alzheimer's disease in T2DM patients. Endocrine 2016;53(2):350–63.
24. Kim MH , Leem YH. Chronic exercise improves repeated restraint stress-induced anxiety and depression through 5HT1A receptor and cAMP signaling in hippocampus. J Exerc Nutr Biochem 2014; 18(1): 97-104.
25. Langfort J, Barańczuk E, Pawlak D, Chalimoniuk M, Lukacova N, Marsala J, et al. The effect of endurance training on regional serotonin metabolism in the brain during early stage of detraining period in the female rat. Cell Mol Neurobiol 2006;26(7–8):1327–42.
26. Gilbert ER, Fu Z, Liu D. Development of a nongenetic mouse model of type 2 diabetes. Exp Diabetes Res 2011;2011:416254.
27. King A J. The use of animal models in diabetes research. Br J Pharmacol 2012;166877–94.
28. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, et al. A New Rat Model of Type 2 Diabetes: The Fat-Fed, Streptozotocin-Treated Rat. J Metab 2000; 49(11): 1390-4.
29. Trulson ME, Jacoby JH, MacKenzie RG. Streptozotocin-induced diabetes reduces brain serotonin synthesis in rats. J Neurochem 1986;46(4):1068–72.
30. Støving RK, Hangaard J, Pedersen KK, Hagen C. (Menstruation disorders in insulin-dependent diabetes mellitus--epidemiology and causes). Ugeskr Laeg 1994;156(42):6180–4.
31. Hussein J, El-Matty D, El-Khayat Z, ABDEL-LATIF Y. Brain neurotransmitters in diabetic rats treated with CO enzyme Q10. Int J Pharm Pharm Sci 2012;4:554–6.
32. Chen H-I, Lin L-C, Yu L, Liu Y-F, Kuo Y-M, Huang A-M, et al. Treadmill exercise enhances passive avoidance learning in rats: the role of down-regulated serotonin system in the limbic system. Neurobiol Learn Mem 2008;89(4):489–96.
33. Herrera R, Manjarrez G, Hernandez J. Inhibition and kinetic changes of brain tryptophan-5-hydroxylase during insulin-dependent diabetes mellitus in the rat. Nutr Neurosci 2005;8(1):57–62.