Impaired neurogenic activity in the Zucker Diabetic Sprague Dawley (ZDSD) rat hippocampus

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SUMMARY

Diabetes prevalence is increasing worldwide, with type 2 diabetes (T2D), characterized by hyperglycemia and hyperinsulinemia being most common. Past studies associate diabetes with cognitive decline attributed to hippocampal neurodegeneration emanating from oxidative stress. However, findings from animal models are debatable as most involve a disturbance in the leptin signaling pathway. Therefore, we investigated hippocampal neurodegeneration associated with oxidative stress in the Zucker Diabetic-Sprague Dawley (ZDSD) as it maintains an intact leptin pathway. We used 15-week-old, male ZDSD rats (n=5) and Sprague Dawley rats (n=5) that served as controls. Adult hippocampal neurogenesis (AHN) was investigated by immunolabeling the hippocampal dentate gyrus for Ki 67 and doublecortin (DCX) for assessment of proliferating and differentiating neuroblasts, respectively. We quantified plasma malondialdehyde (MDA) concentration to evaluate oxidative stress.

There were fewer proliferating and differentiating neuroblasts, respectively, in the ZDSD rat dentate gyrus. Additionally, the ZDSD rat dentate gyrus had fewer dendritic extensions to the molecular layer compared to that of the SD controls. Oxidative stress was greater in the ZDSD group. Our findings show that the ZDSD exhibits neurodegeneration similar to what is observed in other rodent models of diabetes and in humans. Decreased neuronal proliferation coupled with fewer dendritic extensions in the molecular layer of the dentate gyrus are characteristic of features exhibited in the ZDSD. These results complement the explanations given in scientific literature about the involvement of oxidative stress in the degeneration of hippocampal dentate gyrus neurons observed in type 2 diabetes.

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INTRODUCTION

The global diabetes prevalence in 2021 was estimated to be 10.5%, rising to 12.2% by 2045, which would account for 783.2 million (Sun et al., 2022). Type 2 diabetes (T2D) is the most common and is characterized by insulin insensitivity in peripheral tissues resulting in hyperglycemia and hyperinsulinemia. In advanced stages, several complications may occur such as nephropathy, non-alcoholic fatty liver disease, cardiomyopathy, neuropathy, cognitive decline and dementia (Forbes and Cooper, 2013; Kim and Feldman, 2012; Peterson et al., 2015; Williamson et al., 2012; Wrighten et al., 2009).

Suitability of the Zucker Diabetic-Sprague Dawley (ZDSD) rat as a translational model of type 2 diabetes has been recently reviewed (Wang et al., 2022). However, the review refers to atrophy of the corpus callosum and no information on the neurogenesis in the hippocampus. Hippocampal neurogenesis is impaired in rodent models of type 2 diabetes (Bonds et al., 2020; Johnson et al., 2022). Adult hippocampal neurogenesis (AHN) is crucial in learning and memory, long-term potentiation and cognition (Ho et al., 2013; Wang et al., 2009; Zhang et al., 2008). Hyperglycaemia and insulin resistance affect cognition (Wang et al., 2009), however, there are no reports on diabetes effects on hippocampal neurogenesis in the ZDSD rat.

Abnormal glucose metabolism is characteristic of diabetes (Li et al., 2019). This has an impact on cognitive dysfunction through disturbances in glucose transport and a reduction in glucose metabolism (Zhang et al., 2023). In nonglycemic conditions, insulin regulates glucose transport and metabolism. On the contrary, insulin resistance, as observed in diabetes is associated with impaired glucose metabolism in the brain (Zhang et al., 2023). Diabetes reduces the expression and translocation of the insulin-sensitive glucose transporter type 4 (GLUT4) in hippocampal neurons, leading to cognitive impairment (Yonamine et al., 2023).

Regarding structural effects, diabetes causes a reduction in the volume of the hippocampus due to a decrease in dendritic branching (Ho et al 2013). Previous studies have established that the sub-granular zone of the hippocampal dentate gyrus (Johnson et al., 2022), CA1, CA3 (Artola et al., 2002; Dorsemans et al., 2017), CA4, and the subiculum (Monereo-Sánchez et al., 2023) have fewer neurons in type 2 diabetes.

Several T2D models such as the Zuker Diabetic fatty rats (Hwang et al., 2008; Wang et al., 2009; Yi et al., 2009), Goto-Kakizaki rats (Lang et al., 2009), and db/db mice (Ramos-Rodriguez et al., 2014; Stranahan et al., 2008) report impaired neurogenic activity. However, Beauquis et al., (2010) reported increased cell proliferation in the hippocampal dentate gyrus of the Goto-Kakizaki rats. The Zucker Diabetic Fatty rats and db/db mice have a monogenetic mutation affecting leptin signaling (Wang et al., 2014), which does not happen in humans (Peterson et al., 2015). Additionally, Goto-Kazikaki rats exhibit elevated corticosterone levels (Beauquis et al., 2010), which in themselves reduce hippocampal neurogenesis. Therefore, the observed alterations in hippocampal neurogenic activity may not be solely due to diabetes (Ramos-Rodriguez et al., 2014; Stranahan et al., 2008). Thus, better translational models are required.

The Zucker Diabetic-Sprague Dawley (ZDSD) rat is a translational model that spontaneously develops T2D. The model is a cross breed of homozygous Zucker Diabetic Fatty (ZDF) rats, expressing beta cell failure with the $Lepr^{fa}$ / $Lepr^{fa}$genotype, and Diet Induced Obese (DIO) rats, derived from the CD (SD) strain, exhibiting polygenetic obesity and insulin resistance (Peterson et al., 2015). The ZDSD model is polygenetic, without monogenetic leptin mutations, therefore, resembles T2D characteristics in humans (Peterson et al., 2015).

Considering the ZDSD's intact leptin signaling, we sought to assess neurogenic activity in the hippocampal dentate gyrus to establish that the previously reported impairments in neurogenesis are solely due to diabetes. This study investigated AHN by immunohistochemistry for Ki 67, a protein expressed in all cell cycle stages (Cooper-Kuhn and Georg Kuhn, 2002), and doublecor-

tin (DCX), a protein expressed transiently in migrating and differentiating neuroblasts (Brown et al., 2003).

MATERIALS AND METHODS

Animals

We used 8-week-old, male ZDSD rats (n=5) acquired from PreClinOmics (Indianapolis, USA) and Sprague Dawley rats (n=5) acquired from the Central Animals Services Unit (University of the Witwatersrand in South Africa). Before they could be used for any experiments, all rats were given a two-week acclimatization period and handled according to supplier specifications (Peterson et al., 2015). The rats were housed individually in acrylic cages lined with wood shavings. Ambient temperatures were maintained at 22 ± 2°C and a 12-hour light/dark cycle was followed. All rats received food (Purina 5008 rodent chow; LabDiet, St Louis, Missouri, USA) and water *ad libitum.* Animals were treated in accordance with Guide for the Care and Use of Laboratory Animals (8th edition, National Academies Press) and the Animal Ethics and Screening Committee of the University of the Witwatersrand approved the protocol and procedures (Ethics clearance number 2015/07/28C).

Experimental procedures

Body mass, fasting glucose, cholesterol, and triglycerides

Body mass was measured once weekly from 10 to 20 weeks of age, using an electronic scale (Diamond, US; accurate to 1 g). We measured fasting levels of blood glucose and triglycerides once every fortnight. Rats were fasted overnight (12 hours) after which tail vein blood was collected using the pin prick method. Circulating levels of glucose were measured using a glucometer (Accutrend®Plus, Roche Diagnostics, Germany) and levels of triglycerides were measured using a GCT meter (Accutrend®Plus, Roche Diagnostics, Germany). Permanent onset of diabetes in the experimental animals was confirmed by two subsequent fasting glucose readings above 13.9 mmol/L or 250 mg/dL at the ages of 18 and 20 weeks (Peterson et al., 2015). We monitored glucose handling through oral glucose tolerance tests, which were performed once in every four weeks. Rats were fasted for 12 hours overnight, and their fasting glucose levels recorded (T_0) before they received an oral glucose load (2 g/kg). Glucose levels were then measured from blood collected from tail vein blood at 15, 30, 60 and 120 min after the glucose load.

Terminal procedures

At 20 weeks of age, rats were euthanized by terminal anesthesia of 1 ml sodium pentobarbital (Euthanase, 200 mg/ml; Kyron Laboratories (Pty) Ltd, South Africa). Blood was collected by cardiac puncture into serum separating tubes, centrifuged at 500 RPM (4°C for 10 minutes) and stored at -20°C for biochemical analyses. The rats were transcardially perfused using ice cold saline (0.9%) for 2 min and brains were collected and preserved in 10% phosphate-buffered formalin for histopathological analyses.

Following perfusion, brains were carefully removed from the skull, immersion-fixed overnight in 4% paraformaldehyde in 0.1M PB, weighed before being transferred into 30% sucrose in 0.1M PB until equilibration. The brains were sectioned in a coronal plane at 50 µm using a cryostat (Shandon Cryotome E, Thermo Fischer scientific, UK) at -24 °C and repeated series of 5 sections were collected free-floating into 24 multi-well plates containing 0.1 M PB. The first series of sections was mounted on gelatin- coated slides before staining with 1% cresyl violet for anatomical orientation. The second and third series were used for Ki 67 and DCX immunohistochemistry respectively. The remaining two series were stored in a cryoprotectant solution at -20 °C for future use.

Lipid peroxidation quantification

A lipid peroxidation assay kit obtained from Sigma- Aldrich (MAK085) was used to quantify plasma malondialdehyde (MDA) concentration as a marker for oxidative stress according to the manufacturer instructions. The colorimetric assay method was used to measure the absorbance at 530 nm (A_{530}) using a pate reader (Athnos® 2010).

Immunohistochemistry

Ki 67 and doublecortin immunohistochemistry was done according to Nkomozepi et al. (2019). Briefly, brain sections were pre-treated for 30 min at room temperature under gentle shaking with an endogenous peroxidase inhibitor solution [49.2% 0.1 M PB, 49.2% methanol, 1.66% H_2O_2]. Sections were subsequently pre-incubated in a blocking buffer solution [3% normal goat serum, 2% bovine serum albumin, 0.25% Triton X-100 in 0.1 M PB] for 2h under gentle shaking at room temperature to prevent non-specific binding. Sections were then transferred into a primary antibody solution [1:1000, rabbit anti-Ki 67 (AB15580, Abcam, UK) or 1: 2000, rabbit anti-DCX (AB18723, Abcam, UK), in the blocking buffer solution] and were incubated for 48h at 4 °C under gentle shaking. Sections were then incubated in secondary antibody solution [1,000 dilution of biotinylated anti-rabbit IgG (BA-5000; Vector Laboratories, USA) in blocking buffer solution] for 2 h at room temperature under gentle shaking before incubation in an avidin-biotin solution [1: 125 A and 1: 125 B (Vector Laboratories) in 0.1 M PB] for 1 h. The sections were then transferred into a solution containing 0.05% 3, 3 Di-amino-benzidine tetrachloride (DAB) in 0.1 M PB for 5 min. To each 1 ml of this solution, 3.3 µl of 30% H_2O_2 were added, and chromatic precipitation was visually monitored under a low power stereomicroscope.

Development was subsequently stopped by placing the sections in 0.1 M PB, followed by a final 10-min rinse in 0.1 M PB. Sections were mounted on 0.5% gelatinized slides, left to dry overnight, dehydrated in a graded series of alcohols, cleared in xylene and cover slipped with Depex. To ensure that non-specific staining was not affecting the results, control sections taken at random were processed in the same manner, but either the primary or the secondary antibody was omitted. No labelled cells were observed in either case.

Volume of the dentate gyrus

The volume of the dentate gyrus was measured on photomicrographs of the DG (stained with Cresyl violet), using ImageJ (version 1.47) plugin, VOLUMEST (Schneider et al., 2012).

Pyknotic cell count

Cresyl violet stained sections were used for identification of Pyknotic cells based on their neuronal nuclear morphology. These cells were counted in the granular cell layer (GCL) of all sections of the brain containing the dentate gyrus (DG) that were 250 µm apart, using a Leica IC550 HD microscope at 100X magnification.

Ki 67 and DCX quantification

Ki 67 and DCX quantification was carried out by one of the authors who was blinded to the animal treatments. Quantification was carried out in 6 sections per animal containing the hippocampus that were 250 µm apart corresponding to anatomical landmarks extending from Bregma -3.00 to -4.08 mm of rat brain atlas (Paxinos and Watson, 2007). Sections were digitized using an Olympus CXR10 HD digital camera linked to an Olympus BH2 RFCA microscope (40X objective) and immunoreactive cells in the subgranular (SGZ) were manually counted using the Cell Counter function of ImageJ (Schneider et al., 2012). Immunoreactive cell counts for each section were obtained by averaging the counts from the sections taken from each animal.

Representative photomicrographs of the hippocampal dentate gyrus were prepared using Adobe Photoshop 2024 software (Adobe Systems, Mountain View, CA). No pixelation adjustment or manipulation of the captured images was undertaken, except for the adjustment of contrast and brightness.

Statistical analysis

All data are expressed as mean and standard deviation (SD) and were analyzed using GraphPad Prism (version 7, GraphPad Software Inc, USA). Body mass was plotted against time and analyzed using a repeated measures (RM) two-way Analysis of Variance (ANOVA) with treatment and time as main effects. Bonferroni's test was used to identify differences whenever the ANOVA detected significant main effects or interactions. Fasting levels of glucose, cholesterol and TGs were analyzed using a RM two-way ANOVA with treatment and time as main effects followed by Bonferroni *post hoc* test. The number of Ki-

67-ir and DCX-ir cells in the hippocampal dentate gyrus were compared by the Mann-Whitney U test with a level of significance of $p< 0.05$.

RESULTS

Body mass

Fig. 1A shows growth curves of ZDSD and Control SD rats over a 10-week period. The main effects of time ($F_{(10,80)} = 54.23$, $P < 0.0001$), treatment ($F_{(1,8)} = 15.18$, $P = 0.0046$) and their interaction ($F_{(10,80)} = 21.20$, $P < 0.0001$) were such that the body mass of Control SD rats increased progressively from week 10 to 20 but the body mass of ZDSD rats increased from week 10 to 16 then significantly decreased after the onset of diabetes.

Oral glucose tolerance tests

ZDSD rats exhibited significant abnormalities in glucose handling (Fig. 1B) at 20 weeks (main effects of time ($F_{(4,32)} = 8.81$, $P < 0.0001$), treatment ($F_{(1,8)} = 357.1$, $P < 0.0001$) and their interaction ($F_{(4,32)} = 1.982$, $P = 0.1210$). The baseline glucose concentration was higher in ZDSD rats than in controls ($P < 0.0001$). A glucose challenge significantly increased glucose concentrations in ZDSD rats to peak at 27.94 ± 2.78 mmol/L after 30 minutes compared to a peak of 6.78 ± 1.09 mmol/L achieved by Control SD rats at 15 min ($P < 0.0001$). The 2-hour glucose concentration of ZDSD was similarly higher than in non-diabetic controls ($P < 0.0001$).

Fasting metabolites

The ZDSD rats were overtly diabetic with a mean fasting glucose concentration of 20.3 ± 4.68 mmol/L as compared to control SD rats (mean fasting glucose concentration 3.94 ± 0.55 mmol/L) (Fig. 2A). An independent t-test showed that fasting glucose concentrations in ZDSD rats were statistically higher than in the non-diabetic controls ($t = 19.84$; $P = 0.0321$). Circulating concentrations of triglycerides were not significantly different between the two treatments groups ($P = 0.4851$) (Fig. 2B) but cholesterol levels werehigher in ZDSD rats compared to controls ($t = 3.577$; $P = 0.072$) (Fig. 2C).

Oxidative stress

Malondialdehyde (MDA) concentration was significantly higher in the ZDSD group (19.21 ±6.62 nmol/µl) compared to SD controls (3.25 ±2.27 nmol/µl) ($t=5$, $df= 8$, $p=0.001$) (Fig. 3).

Dentate gyrus (DG) volume

The DG volume was significantly reduced in the ZDSD group (5.00 mm^3) compared to SD controls (5.54mm^3) ($t=5$, $df= 8$, $p=0.001$) (Fig. 4A).

Pyknotic cells

The quantity of pyknotic cells was significantly higher ZDSD group (7.3) compared to SD controls (3.4) ($t=15.43$, $df= 10$, $p<0.001$) (Figs. 4A, B and C).

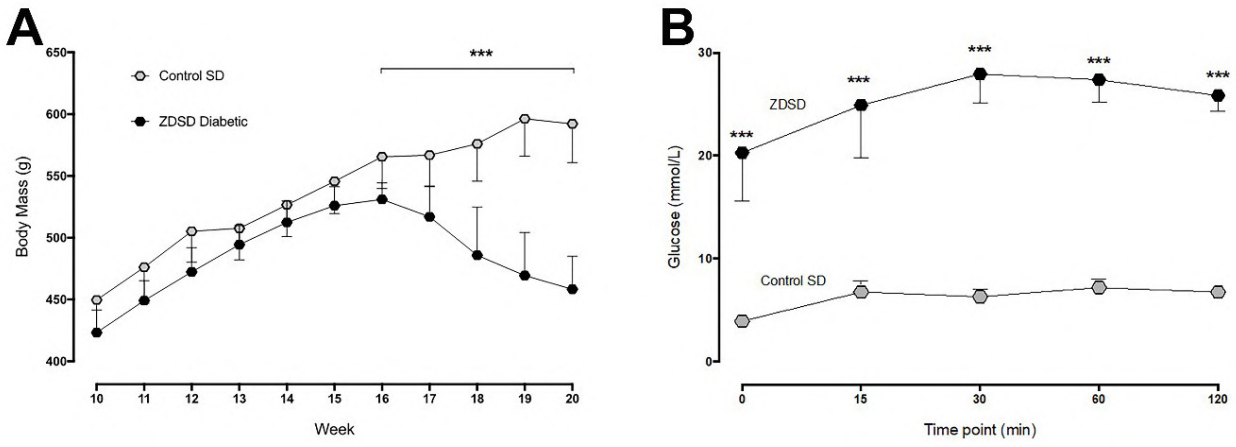

Fig. 1.- Body mass and oral glucose tolerance tests. **A.** Body mass (mean and SD) of Zucker Diabetic Sprague Dawley rats and normal Sprague Dawley rats (Control SD) recorded over a 10-week period. **B,** Glucose levels (mean and SD) from oral glucose tolerance tests performed in Zucker Diabetic Sprague Dawley (ZDSD) rats and normal Sprague Dawley rats (Control SD) at 20 weeks of age. *** indicates glucose concentration in ZDSD significantly dfferent from Control SD ($P < 0.0001$; Bonferroni).

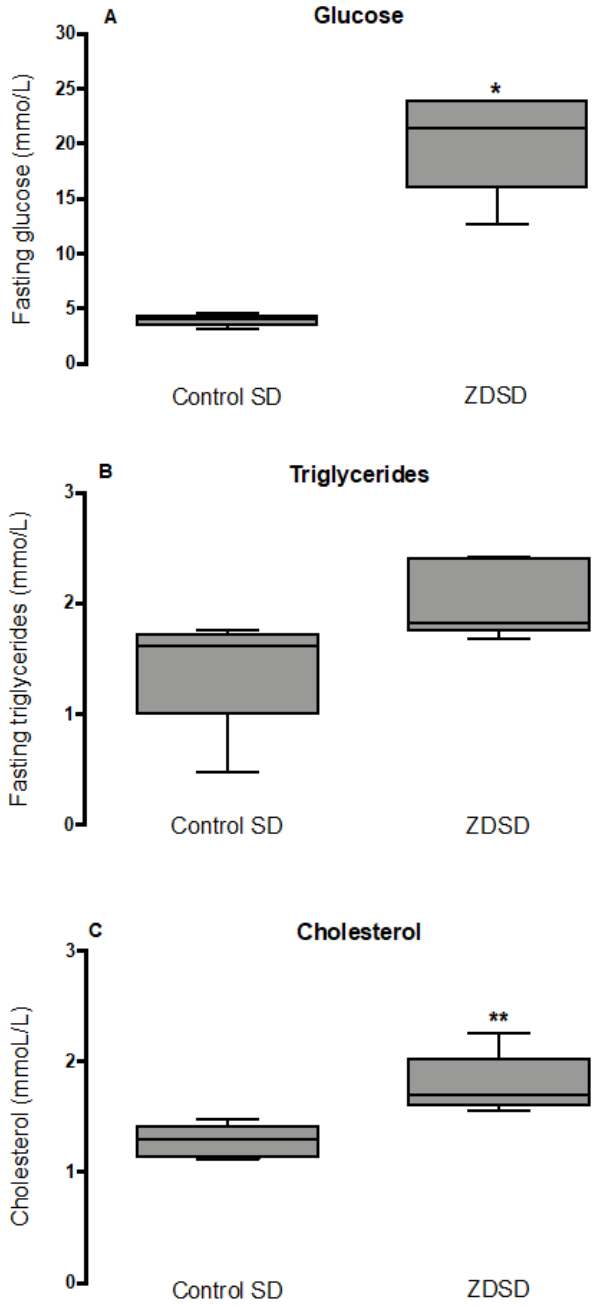

Fig. 2.- Fasting glucose **(A)**, triglycerides **(B)** and cholesterol **(C)** (mean and SD) in Zucker Diabetic Sprague Dawley rats and normal Sprague Dawley rats (Control SD) at 20 weeks of age. * indicates glucose concentration in ZDSD significantly different from Control SD (P = 0.0321); ** indicates cholesterol concentration in ZDSD significantly different from Control SD (P = 0.0072).

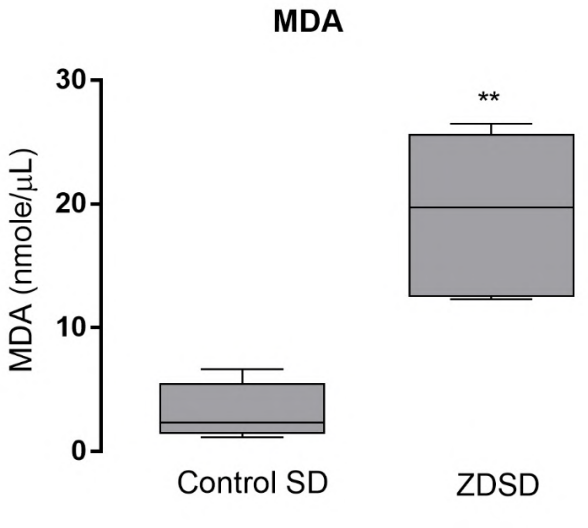

Fig. 3.- Malondialdehyde (MDA) concentration in normal Sprague Dawley rats (Control SD) and Zucker Diabetic Sprague Dawley (ZDSD) rats at 20 weeks of age. ** indicates MDA concentration in ZDSD significantly different from Control SD (p=0.001).

Ki67 and DCX Immunoreactivity

Ki 67-ir cells were observed in the subgranular zone (SGZ) of the hippocampal dentate gyrus of the Control SD (Fig. 5A, C) and ZDSD rats (Figs. 5B, D). The number of Ki-67-ir cells in the SGZ of the ZDSD (9.6 ± 0.96) rats was significantly reduced by approximately 55% compared to the Control SD (20.7 ± 1.38) rats (P<0.002, Fig. 7A).

Similarly, DCX-ir cells were observed in the SGZ and granule cell layer (GCL) of the hippocampal dentate gyrus in the Control SD (Figs. 6A, C) and ZDSD (Figs. 6B, D) rats. The number of DCX-ir cells in the SGZ and GCL of the ZDSD (58.6 ± 3.97) rats was significantly reduced by approximately 65% compared to the Control SD (165.9 ± 11.13) rats (P<0.001, Fig. 7B). Additionally, DCX-ir neuroblasts in the SGZ of the hippocampal DG of Control SD rats had well developed vertical dendrites that extended into the molecular layer (Fig. 6C). Conversely, DCX-ir neuroblasts in the hippocampal DG of ZDSD rats had poorly developed dendrites (Fig. 6D).

Table 1. Pearson correlation of MDA with Cholesterol, Triglycerides, Glucose as well as DCX and KI-67 immunopositive cells. MDA is the proxy for oxidative stress. Diabetic parameters are cholesterol, triglycerides, and glucose. DCX and Ki-67 are makers of cells neuronal replication. MDA = Malondialdehyde; DCX =Doublecortin.

		Cholesterol	Triglycerides	Glucose	DCX	KI-67
MDA	Correlation	0.859	0,631	0.884	-0.868	-0.866
	P value	0,001	0,049	0,001	0,001	0,001

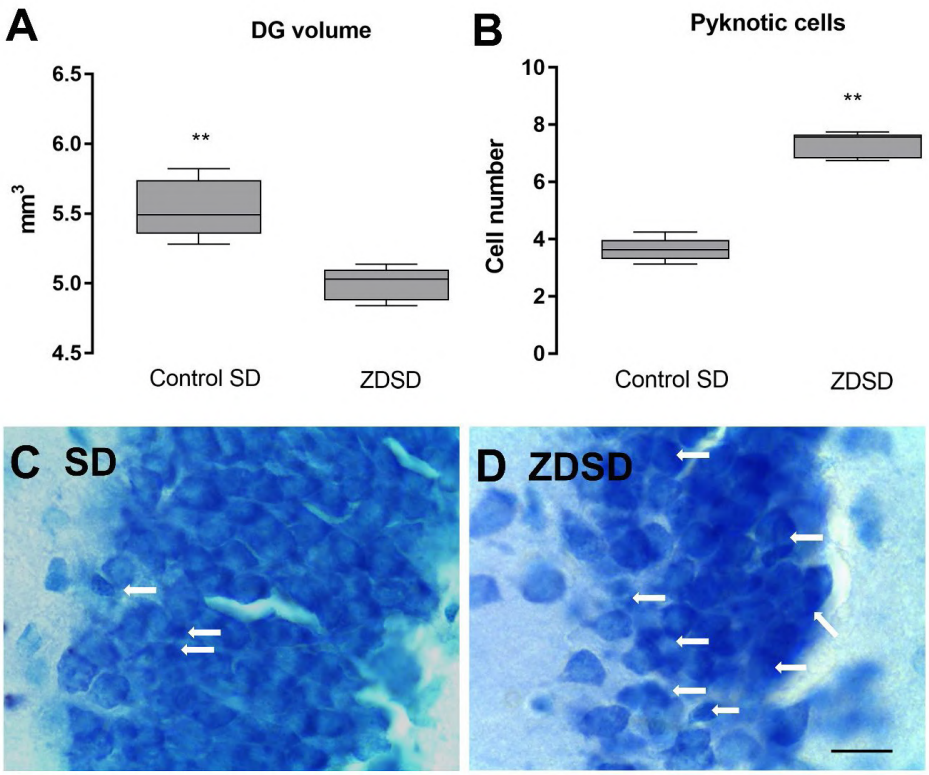

Fig. 4.- Dentate gyrus volume and pyknotic cells. **A,** DG volume (mean ±SD) in both the SD control rats and ZDSD rats. ** indicates that hippocampal DG volume was significantly reduced in the brains of ZDSD rats compared to SD control rats (P<0.0001). **B,** pyknotic cell count (mean±SD) in the granule cell layer in both the SD control rats and ZDSD; ** indicates that the pyknotic cell were significantly more in the brains of ZDSD rats compared to SD control rats (P< 0.0001). **C** and **D**, representative photomicrographs of pyknotic cells (arrows) in the SD and ZDSD rats, respectively. DG, dentate gyrus. Scale bar = 20 µm.

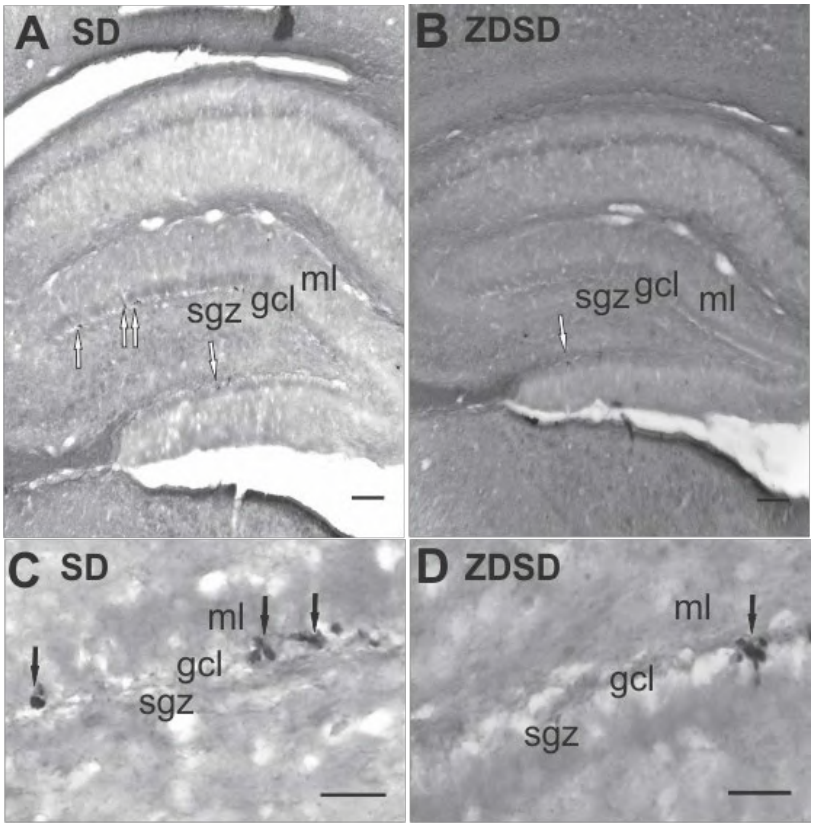

Fig. 5.- Photomicrographs showing Ki-67-ir cells in the sub granular zone of the hippocampal dentate gyrus in Control SD (A, C) and ZDSD (B, D) rats. White arrows in A&B and black arrows in C&D show Ki-67-ir cells in the dentate gyrus. ml, molecular layer; gcl, granular cell layer; sgz, subgranular zone. Scale bar in **A**, **B** = 200 µm and in **C**, **D** = 10 µm.

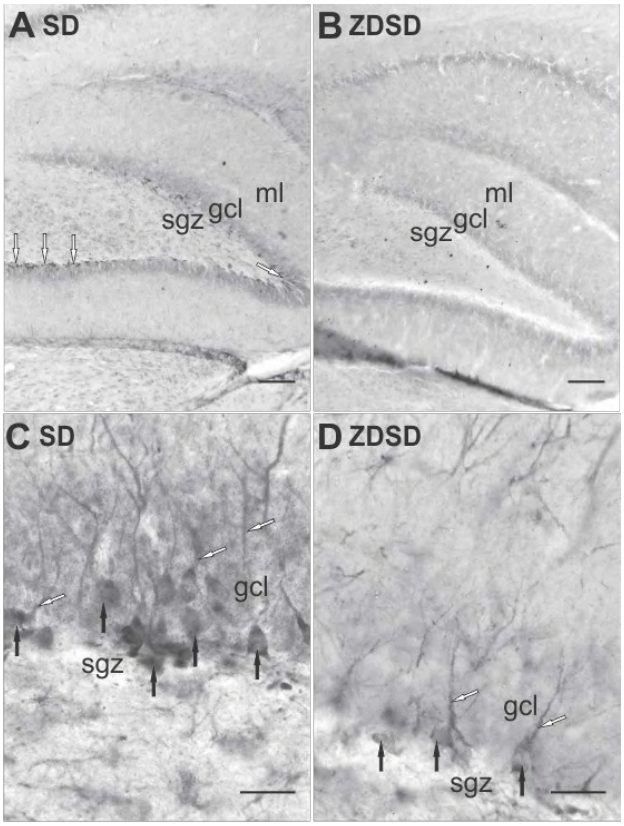

Fig. 6.- Photomicrographs showing DCX-ir cells in the sub granular zone of the hippocampal dentate gyrus in Control SD (A, C) and ZDSD (B, D) rats. White arrows in **A**, **B** and black arrows in **C**, **D** show DCX-ir cells in the dentate gyrus. ml, molecular layer; gcl, granular cell layer; sgz, subgranular zone. Scale bar in A, B = 200 µm and in C, D = 10 µm.

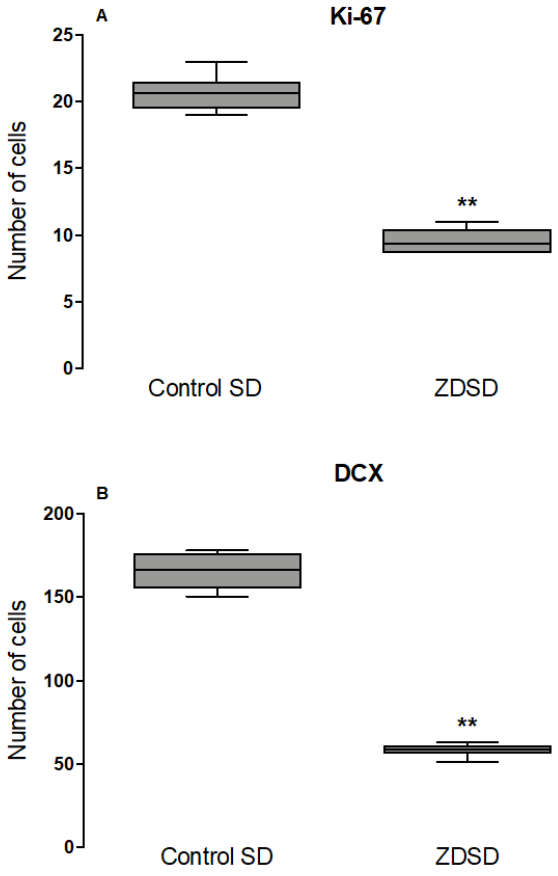

Fig. 7.- Ki-67 **(A)** and DCX **(B)** immunoreactive cells in the hippocampal dentate gyrus of Zucker Diabetic Sprague Dawley rats and normal Sprague Dawley rats (Control SD) at 20 weeks of age. ** indicates Ki 67 and DCX in hippocampal dentate gyrus of ZDSD is significantly lower than that of Control SD rats ($P<0.001$).

Correlation of oxidative stress with diabetes parameters and makers of cell neuronal replication

Significant positive correlations were observed between serum MDA levels and cholesterol (P=0.001), triglycerides (P=0.040), glucose (P=0.001) (Table 1). On the contrary, strong negative correlations were also observed between serum MDA levels and DCX (P=0.001), Ki 67 (P=0.001) (Table 1).

DISCUSSION

Type 2 diabetes and its associated cognitive decline is a growing concern worldwide. The cognitive decline in diabetes is understood to be a consequence of impaired neuronal activity and increased apoptosis in the central nervous system (Ramos-Rodriguez et al., 2014). Our study focused on the hippocampal dentate gyrus, a region of the brain crucial for cognitive functions such as memory and learning. We intended to establish whether neurogenic activity in the hippocampal dentate gyrus would be impaired in a rodent model of diabetes that has an intact leptin signaling pathway, the ZDSD rat. Previous studies have reported reduced neurogenic activity in both chemical induced (El-Adli et al., 2023) and genetic rat models of diabetes (Bonds et al., 2020). In the present study, we confirmed successful modeling of type 2 diabetes by monitoring body mass, fasting blood glucose, OGTTs and lipid profile. Immunolabeling of neurons with the anti-Ki-67 and DCX antibodies revealed fewer proliferating and differentiating neuroblasts, respectively in the ZDSD rat dentate gyrus. Additionally, the DCX immunoreactive neuroblasts in subgranular of the ZDSD rat dentate gyrus had fewer dendritic extensions to the molecular layer compared to that of the SD controls.

High levels of cholesterol were observed in the ZDSD group, suggesting a potential role of hypercholesterolemia in neuronal damage. The brain normally synthesizes its own cholesterol as the blood brain barrier prevents systemic cholesterol from entering the central nervous system (Pfrieger and Ungerer, 2011). However, hyperlipidemia can elicit production of pro- inflammatory cytokines such as TNF-α and IL-1β, which may compromise the structural integrity of the blood brain barrier (Yang et al., 2017). This may allow systemic cholesterol and toxic substances to enter the brain with consequential neuronal damage.

Cholesterol is an important component of synapses and their optimal transmission of action potentials, and as such, its homeostasis is highly regulated to maintain conductive abilities of neurons (Pfrieger, 2003). Rodent studies show that hypercholesterolemia is linked to neurodegenerative diseases through increased oxidative stress and alterations at the synaptic connections (Ettcheto et al., 2015).

Elevated triglyceride levels were detected in the present study. Hypertriglyceridemia is reported to be the most common lipid derangement in diabetes (Hirano, 2018). Triglycerides cross the blood brain barrier (BBB) and cause insulin resistance (Banks et al., 2018), and hyperglycemia impair the transport of insulin across the BBB (Rhea and Banks, 2019). This suggests that glucose use by neurons in diabetes may be compromised through resistance to the available insulin coupled with the restricted access to insulin. Neurogenic activity in the hippocampal dentate gyrus is crucial for cognitive function. There is reduced neurogenesis in diabetic rodents has been extensively reviewed in (Bachor and Suburo, 2012). In the present study of the dentate gyrus, we observed diminished neuronal production as evidenced by DCX and Ki67 immunoreactive cells. This is similar to previous findings (Johnson et al., 2022).

The interaction of the BBB, neurons and insulin may be a plausible explanation as to the decrease neurogenesis and fewer dendritic extensions observed in the present study of the ZDSD rat.

Malondialdehyde (MDA) is derived from lipid peroxidation, consequential to oxidative stress (Khoubnasabjafari et al., 2015). Oxidative stress occurs when the free radical production is substantially greater than free radical scavenging processes (Bajaj and Khan, 2012).

Hyperglycemia increases oxidative stress as previously described (Johnson et al., 2023; Sharma et al., 2010). In the present study, the concentration of MDA in ZDSD rats was significantly

higher than that of SD control rats. This means that ZDSD rats had more oxidative stress. This is similar to previous studies that reported significantly higher MDA levels (Atalay and Laaksonen, 2002; Johnson et al., 2022). Free radicals generated from oxidative stress can affect DNA and may cause structural changes to important proteins which may impair normal cell function (Sharifi-Rad et al., 2020).

The elevated oxidative stress as shown by high MDA levels in the ZDSD rats is most likely a consequent the hypertriglyceridemia, hypercholesterolemia with concurrent hyperglycaemia detected in the present study. This is confirmed by a strong positive correlation of MDA with fasting glucose triglyceride, and cholesterol levels observed in this study. Additionally, the present study found a strong negative correlation between MDA and neurogenesis (DCX and Ki-67). Therefore, it is plausible to deduce that oxidative stress can be among the causes of impaired neurogenesis in diabetes (Johnson et al., 2022).

CONCLUSION

Our findings show that the ZDSD exhibits impaired neurogenic activity similar to what is observed in other rodent models of diabetes and in humans. Decreased neuronal production coupled with fewer dendritic extensions in the molecular layer of the dentate gyrus are characteristic of features exhibited in the ZDSD. These results complement the explanations given in scientific literature about the role of the neurons of the hippocampal dentate gyrus in the cognitive decline observed in type 2 diabetes. Studies are required to determine whether reversing hyperglycemia to the normoglycemic state would reverse the impaired adult hippocampal neurogenesis and restore normal neuronal architecture.

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REFERENCES

ARTOLA A, KAMAL A, RAMAKERS GM, GARDONI F, DI LUCA M, BIESSELS GJ, CATTABENI F, GISPEN WH (2002) Synaptic plasticity in the diabetic brain: advanced aging? *Prog Brain Res*, 138: 305-314.

ATALAY M, LAAKSONEN DE (2002) Diabetes, oxidative stress and physical exercise. *J Sports Sci Med*, 1(1): 1-14.

BACHOR TP, SUBURO AM (2012) Neural stem cells in the diabetic brain. *Stem Cells Int*, 2012: 820790.

BAJAJ S, KHAN A (2012) Antioxidants and diabetes. *Indian J Endocrinol Metab*, 16(Suppl 2): S267-271.

BANKS WA, FARR SA, SALAMEH TS, NIEHOFF ML, RHEA EM, MORLEY JE, HANSON AJ, HANSEN KM, CRAFT S (2018) Triglycerides cross the blood-brain barrier and induce central leptin and insulin receptor resistance. *Int J Obes (Lond)*, 42(3): 391-397.

BEAUQUIS J, HOMO-DELARCHE F, GIROIX MH, EHSES J, COULAUD J, ROIG P, PORTHA B, DE NICOLA AF, SARAVIA F (2010) Hippocampal neurovascular and hypothalamic-pituitary-adrenal axis alterations in spontaneously type 2 diabetic GK rats. *Exp Neurol*, 222(1): 125-134.

BONDS JA, SHETTI A, STEPHEN TKL, BONINI MG, MINSHALL RD, LAZAROV O (2020) Deficits in hippocampal neurogenesis in obesity-dependent and -independent type-2 diabetes mellitus mouse models. *Sci Rep*, 10(1): 16368.

BROWN JP, COUILLARD-DESPRES S, COOPER-KUHN CM, WINKLER J, AIGNER L, KUHN HG (2003) Transient expression of doublecortin during adult neurogenesis. *J Comp Neurol*, 467(1): 1-10.

COOPER-KUHN CM, GEORG KUHN H (2002) Is it all DNA repair? *Dev Brain Res*, 134(1-2): 13-21.

DORSEMANS AC, COURET D, HOARAU A, MEILHAC O, LEFEBVRE D'HELLENCOURT C, DIOTEL N (2017) Diabetes, adult neurogenesis and brain remodeling: new insights from rodent and zebrafish models. *Neurogenesis (Austin)*, 4(1): e1281862.

EL-ADLI D, GAWISH SA, ABDELFATTAH AAM, SOLIMAN MF (2023) The effect of experimentally-induced diabetes on rat hippocampus and the potential neuroprotective effect of Cerebrolysin combined with insulin. A histological and immunohistochemical study. *Egypt J Basic Appl Sci*, 10(1): 255-273.

ETTCHETO M, PETROV D, PEDROS I, DE LEMOS L, PALLAS M, ALEGRET M, LAGUNA JC, FOLCH J, CAMINS A (2015) Hypercholesterolemia and neurodegeneration. Comparison of hippocampal phenotypes in LDLr knockout and APPswe/PS1dE9 mice. *Exp Gerontol*, 65: 69-78.

FORBES JM, COOPER ME (2013) Mechanisms of diabetic complications. *Physiol Rev*, 93(1): 137-188.

HIRANO T (2018) Pathophysiology of diabetic dyslipidemia. *J Atheroscler Thromb*, 25(9): 771-782.

HO N, SOMMERS MS, LUCKI I (2013) Effects of diabetes on hippocampal neurogenesis: links to cognition and depression. *Neurosci Biobehav Rev*, 37(8): 1346-1362.

HWANG IK, YI SS, KIM YN, KIM IY, LEE IS, YOON YS, SEONG JK (2008) Reduced hippocampal cell differentiation in the subgranular zone of the dentate gyrus in a rat model of type II diabetes. *Neurochem Res*, 33(3): 394-400.

JOHNSON AJ, NDOU R, MBAJIORGU EF (2022) Combination antiretroviral therapy (cART) in diabetes exacerbates diabetogenic effects on hippocampal microstructure, neurogenesis and cytokine perturbation in male Sprague Dawley rats. *Diagnostics (Basel)*, 12(4).

JOHNSON JA, NDOU R, MBAJIORGU EF (2023) Interactions of alcohol and combination antiretroviral (cART) drug in diabetic male Sprague Dawley rats: Hippocampal perturbations and toxicosis. *Toxicol Rep*, 10: 155-170.

KHOUBNASABJAFARI M, ANSARIN K, JOUYBAN A (2015) Reliability of malondialdehyde as a biomarker of oxidative stress in psychological disorders. *Bioimpacts*, 5(3): 123-127.

KIM B, FELDMAN EL (2012) Insulin resistance in the nervous system. *Trends Endocrinol Metab,* 23(3): 133-141.

LANG BT, YAN Y, DEMPSEY RJ, VEMUGANTI R (2009) Impaired neurogenesis in adult type-2 diabetic rats. *Brain Res,* 1258: 25-33.

LI J, LIU B, CAI M, LIN X, LOU S (2019) Glucose metabolic alterations in hippocampus of diabetes mellitus rats and the regulation of aerobic exercise. *Behav Brain Res,* 364: 447-456.

MONEREO-SÁNCHEZ J, JANSEN JFA, KÖHLER S, VAN BOXTEL MPJ, BACKES WH, STEHOUWER CDA, KROON AA, KOOMAN JP, SCHALKWIJK CG, LINDEN DEJ, SCHRAM MT (2023) The association of prediabetes and type 2 diabetes with hippocampal subfields volume: The Maastricht study. *NeuroImage: Clinical,* 39: 103455.

PETERSON RG, JACKSON CV, ZIMMERMAN K, DE WINTER W, HUEBERT N, HANSEN MK (2015) Characterization of the ZDSD rat: a translational model for the study of metabolic syndrome and type 2 diabetes. *J Diabetes Res,* 2015: 487816.

PFRIEGER FW (2003) Cholesterol homeostasis and function in neurons of the central nervous system. *Cell Mol Life Sci,* 60(6): 1158-1171.

PFRIEGER FW, UNGERER N (2011) Cholesterol metabolism in neurons and astrocytes. *Prog Lipid Res,* 50(4): 357-371.

RAMOS-RODRIGUEZ JJ, MOLINA-GIL S, ORTIZ-BARAJAS O, JIMENEZ-PALOMARES M, PERDOMO G, COZAR-CASTELLANO I, LECHUGA-SANCHO AM, GARCIA-ALLOZA M (2014) Central proliferation and neurogenesis is impaired in type 2 diabetes and prediabetes animal models. *PLoS One,* 9(2): e89229.

RHEA EM, BANKS WA (2019) Role of the blood-brain barrier in central nervous system insulin resistance. *Front Neurosci,* 13: 521.

SCHNEIDER CA, RASBAND WS, ELICEIRI KW (2012) NIH Image to ImageJ: 25 years of image analysis. *Nat Methods,* 9(7): 671-675.

SHARIFI-RAD M, ANIL KUMAR NV, ZUCCA P, VARONI EM, DINI L, PANZARINI E, RAJKOVIC J, TSOUH FOKOU PV, AZZINI E, PELUSO I, PRAKASH MISHRA A, NIGAM M, EL RAYESS Y, BEYROUTHY ME, POLITO L, IRITI M, MARTINS N, MARTORELL M, DOCEA AO, SETZER WN, CALINA D, CHO WC, SHARIFI-RAD J (2020) Lifestyle, oxidative stress, and antioxidants: back and forth in the Pathophysiology of chronic diseases. *Front Physiol,* 11: 694.

SHARMA R, BURAS E, TERASHIMA T, SERRANO F, MASSAAD CA, HU L, BITNER B, INOUE T, CHAN L, PAUTLER RG (2010) Hyperglycemia induces oxidative stress and impairs axonal transport rates in mice. *PLoS One,* 5(10): e13463.

STRANAHAN AM, ARUMUGAM TV, CUTLER RG, LEE K, EGAN JM, MATTSON MP (2008) Diabetes impairs hippocampal function through glucocorticoid-mediated effects on new and mature neurons. *Nature Neuroscience,* 11(3): 309-317.

SUN H, SAEEDI P, KARURANGA S, PINKEPANK M, OGURTSOVA K, DUNCAN BB, STEIN C, BASIT A, CHAN JCN, MBANYA JC, PAVKOV ME, RAMACHANDARAN A, WILD SH, JAMES S, HERMAN WH, ZHANG P, BOMMER C, KUO S, BOYKO EJ, MAGLIANO DJ (2022) IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract,* 183: 109119.

WANG AN, CARLOS J, FRASER GM, MCGUIRE JJ (2022) Zucker Diabetic-Sprague Dawley (ZDSD) rat: Type 2 diabetes translational research model. *Exp Physiol,* 107(4): 265-282.

WANG B, CHANDRASEKERA PC, PIPPIN JJ (2014) Leptin- and leptin receptor-deficient rodent models: relevance for human type 2 diabetes. *Curr Diabetes Rev,* 10(2): 131-145.

WANG SH, SUN ZL, GUO YJ, YUAN Y, YANG BQ (2009) Diabetes impairs hippocampal function via advanced glycation end product mediated new neuron generation in animals with diabetes-related depression. *Toxicol Sci,* 111(1): 72-79.

WILLIAMSON R, MCNEILLY A, SUTHERLAND C (2012) Insulin resistance in the brain: an old-age or new-age problem? *Biochem Pharmacol,* 84(6): 737-745.

WRIGHTEN SA, PIROLI GG, GRILLO CA, REAGAN LP (2009) A look inside the diabetic brain: Contributors to diabetes-induced brain aging. *Biochim Biophys Acta,* 1792(5): 444-453.

YANG W, SHI H, ZHANG J, SHEN Z, ZHOU G, HU M (2017) Effects of the duration of hyperlipidemia on cerebral lipids, vessels and neurons in rats. *Lipids Health Dis,* 16(1): 26.

YI SS, HWANG IK, YOO KY, PARK OK, YU J, YAN B, KIM IY, KIM YN, PAI T, SONG W, LEE IS, WON MH, SEONG JK, YOON YS (2009) Effects of treadmill exercise on cell proliferation and differentiation in the subgranular zone of the dentate gyrus in a rat model of type II diabetes. *Neurochem Res,* 34(6): 1039-1046.

YONAMINE CY, MICHALANI MLE, MOREIRA RJ, MACHADO UF (2023) Glucose transport and utilization in the hippocampus: from neurophysiology to diabetes-related development of dementia. *Int J Mol Sci,* 24(22): 16480.

ZHANG S, ZHANG Y, WEN Z, YANG Y, BU T, BU X, NI Q (2023) Cognitive dysfunction in diabetes: abnormal glucose metabolic regulation in the brain. *Front Endocrinol,* 14: 1192602.

ZHANG WJ, TAN YF, YUE JT, VRANIC M, WOJTOWICZ JM (2008) Impairment of hippocampal neurogenesis in streptozotocin-treated diabetic rats. *Acta Neurol Scand,* 117(3): 205-210.