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# Presence of White Matter Lesions Associated with Diabetes-Associated Cognitive Decline in Male Rat Models of Pre-Type 2 Diabetes

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Data Interpretation D  
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**Background:** The aim of this study was to determine the association between white matter lesions (WML) and diabetes-associated cognitive decline (DACD) in rat models of type 2 diabetes (T2DM).

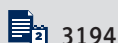
**Material/Methods:** Sixty Sprague-Dawley male rats were divided into 4 groups: control, control+metformin, T2DM, and T2DM+metformin groups. The T2DM groups were fed a diet high in fat and glucose to induce impaired glucose tolerance (IGT) and then were injected with streptozotocin to induce T2DM. The Morris water maze test was used to evaluate cognitive function. Brain diffusion tensor imaging scans were performed for WML. The expression of myelin basic protein (MBP), oligodendrocyte transcription factor 1 (OLIG1), and OLIG2 (markers of brain damage and repair) was determined using immunofluorescence. After IGT, the fractional anisotropy (FA) values of the right thalamus area were significantly lower in both T2DM groups compared with controls.

**Results:** Eight weeks after streptozotocin injection, the FA values of the thalamus were lower in the T2DM (bilateral thalamus) group and T2DM+metformin (left thalamus) group than in controls, while the FA values in the left thalamus area were lower in the T2DM+metformin group than in the control and control+metformin groups. The maze escape latency was longer and the number of rats passing through the platform was smaller in the T2DM and T2DM+metformin groups than in the control group. MBP levels were lower and OLIG1 and OLIG2 levels were higher in both T2DM groups than in controls.

**Conclusions:** WML is associated with DACD and appears before the onset of T2DM and signs of DACD and plays a role in diabetes-associated cognitive decline. Metformin reduces WMLs but does not rescue cognitive dysfunction.

**MeSH Keywords:** **Anisotropy • Diabetes Mellitus, Type 2 • Diffusion Tensor Imaging**

**Full-text PDF:** <https://www.medscimonit.com/abstract/index/idArt/918557>



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## Background

Type 2 diabetes mellitus (T2DM) is characterized by insulin deficiency caused by pancreatic  $\beta$  cell dysfunction and insulin resistance [1]. T2DM has reached epidemic proportions worldwide, with a prevalence of 8.5% in the United States in 2016–2017 [2], and 14.7% in Han Chinese, the largest Chinese ethnic group [3]. The prevalence of T2DM is even higher in the older age groups, reaching 26% in Americans >65 years old [4].

Clinical studies have indicated the association between T2DM and increased risks of brain abnormalities, including dementia [5], stroke [6], cognitive disorders [7–9], and Alzheimer disease [10,11]. A decline in working memory, information processing, attention, and executive function has been found in patients with T2DM, irrespective of age [12,13]. The magnitude of these cognitive deficits appears mild to moderate, but can significantly hamper daily functioning, adversely affecting quality of life [14]. The term ‘diabetic encephalopathy’ was introduced in 1950 when trying to describe cognitive impairment in diabetes as a complication of the disease [15], but the term ‘encephalopathy’ has not been widely accepted and some authors proposed ‘diabetes-associated cognitive decline’ (DACD) [16–19]. The exact prevalence of DACD due to T2DM is unknown because of a number of confounding factors (e.g., age, education, and apoE genotypes), but the development of DACD is considered to be closely associated with that of uncontrolled diabetes [17,20,21]. The prevalence of DACD in patients with type 1 diabetes mellitus is 35–40% [19]. Nevertheless, the factors involved in DACD are still unclear.

Recent studies suggested that white matter (WM) lesions (WML) may play a role in cognitive impairment [7,22,23]. WM plays a vital role in transferring information among gray matter regions, and the efficiency of transferring information depends on the microstructural integrity of the WM [24]. Patients with T2DM show widespread WM disruptions and a positive correlation between executive function and WM integrity in the left anterior limb of the internal capsule and the left external capsule [25,26]. Disruption of the WM network is also related to the slowing of information processing in patients with T2DM [27,28]. Nevertheless, when WML appears in the brain in relation to diabetes development is unclear. Whether WML contributes to cognitive disorders during the progression of diabetes also remains unknown.

The thalamus is the most important sensory conduction relay station in the human body. Sensory conduction pathways from the whole body (except for olfaction) replace neurons in the thalamus and then project into the cerebral cortex. In addition to receiving various sensory inputs, the thalamus has extensive and complex connective fibers linking different brain regions. Multiple studies have shown that the thalamus affects

cognitive functions, including language, memory, attention, visual space, and executive function [29,30]. Therefore, this study selected the bilateral thalamus areas as the regions of interest (ROI) for the study of DACD.

The integrity of WM can be evaluated using imaging and histological examinations. Diffusion tensor imaging (DTI) is a sensitive neuroimaging method for the study of early WML [31]. Fractional anisotropy (FA) is a sensitive indicator of WM health [32,33]. Myelin basic protein (MBP) is a major component of the myelin sheath and is a specific indicator of changes in demyelination. Oligodendrocyte transcription factor 1 (OLIG1) and oligodendrocyte transcription factor 2 (OLIG2) are expressed in myelinating oligodendrocytes (OL) and OL progenitor cells. When the central nervous system (CNS) is injured, overexpression of OLIG1 and OLIG2 can be observed [34]. Therefore, WML can be estimated by assessing the expression of MBP, OLIG1, and OLIG2, in addition to the imaging of brain microstructural alterations.

This study aimed to explore when WML occurs during T2DM development and whether WML is associated with DACD. A T2DM rat model was established and the cognitive function of the rats was tested at different time points. Metformin is the first-line treatment for T2DM. It is effective and safe, and it is listed in the World Health Organization’s List of Essential Medicines. It lowers blood glucose levels, but without affecting body weight [35–37]. Preliminary data revealed that WML develops prior to overt T2DM.

## Material and Methods

### Animals

All experimental procedures were approved by the Ethics Committee for the Use of Experimental Animals of the Second Affiliated Hospital of Zhengzhou University. All experiments were carried out according to the regulations from the National Institutes of Health and the Chinese government. All animals were cared for humanely and all means were taken to avoid or minimize suffering.

Sixty specific-pathogen-free grade 14-week old Sprague-Dawley (SD) male rats (Jinan Pengyue Experimental Animal Breeding Co., Jinan, China), weighing  $88.9 \pm 3.9$  g, were kept at the Experimental Center of Henan Academy of Traditional Chinese Medicine (license number SYXK(Yu)2012-009). The rats were kept 5/cage. The floor litter was replaced every 3 days. The cage was disinfected once a week. The temperature of the animal room was controlled at 23–26°C, relative humidity at 60%, and the light/dark cycle was 12/12 h. They were randomly divided into 4 groups after 7 days of adaptive feeding: normal control

(control group,  $n=15$ ), normal control+metformin group (C+MET group,  $n=15$ ), T2DM (T2DM group,  $n=15$ ), and T2DM+metformin group (T2DM+MET group,  $n=15$ ). The normal control group and C+MET group were fed standard diet, whereas the T2DM and T2DM+MET groups were fed a high-fat and high-sugar diet (79% normal diet, 10% sucrose, 5% lard, and 1% cholesterol; Jiangsu Collaborative Pharmaceutical Bio-engineering Co., Nanjing, China) for 8 weeks. The chow was from Jiangsu Synergy Pharmaceutical Bio-engineering Co. (Industrial and commercial registration number: 320000000044691). The rats were provided free access to water.

Streptozotocin (STZ; 30 mg/kg, intraperitoneal) was administered after overnight fasting [38,39]. Weight and fasting blood glucose (FBG) were measured at the beginning, after 8 weeks of study diet, and 3 days after STZ injection. FBG was measured using blood from the tail vein and a blood glucometer (OneTouch, UltraEasy; Lifescan, Inc., Milpitas, CA, USA). The rats with FBG  $>16.65$  mmol/L were considered diabetic [40]. After the T2DM model was established successfully, the rats in the C+MET group and T2DM+MET groups received metformin (Glucophage, 200 mg/kg/day) by gavage. The control and T2DM groups received the same volume of normal saline by gavage every day for 8 weeks.

### Cognitive behavioral testing

The procedural, visuospatial, and recognition memories of each rat were tested in a Morris water maze at the beginning of the study, after 8 weeks of study feeding, and 8 weeks after STZ injection. Each Morris water maze test lasted for 6 days [41]. The first 5 days were for the positioning cruise experiment and the sixth day was for the spatial probe test. The testing was performed after feeding and used a black circular pool (200 cm in diameter and 20 cm in height) filled with water at 25°C. Black ink was used as an opacifier. The position of the hidden platform (radius of 10 cm) remained fixed for all testing during the entire study period. In the positioning cruise experiment, each rat was placed at an identical starting position in every quadrant. The rats were left to swim until they located the platform, climbed onto it, and stayed on it for at least 2 s, or when 60 s elapsed. The latency to reach the platform was recorded with each test (escape latency), and the same test was repeated for each rat after 15 min. In the spatial probe test, the platform was removed and then a fixed entry point in the pool was chosen. The swimming trajectory within 1.0 min and the number of times the rat crossed the original location of the platform were recorded.

### Magnetic resonance imaging

After every Morris water maze test, the rats were anesthetized using 10% chloral hydrate (300 mg/100 g), which is rapidly

absorbed by the digestive tract and rapidly metabolized by the liver without any residual effects. All rats were first submitted to behavioral studies in the water maze, and then received anesthesia for magnetic resonance imaging (MRI). Therefore, chloral hydrate anesthesia did not affect behavior. They underwent brain coronal MRI scanning, including T1-weighted imaging (T1WI), T2-weighted imaging (T2WI), fluid-attenuated inversion recovery (FLAIR), and DTI, using a 3.0 T MRI scanner (MR750; GE Healthcare, Waukesha, WI, USA) and a special coil for rats (Shanghai Chenguang Medical Technologies Co., Shanghai, China). The rat was fixed in supine position.

The scan parameters were as follows. T1WI: TR/TE=1813 ms/24 ms; slice thickness=1.8 mm; interslice gap=0.2; number of slices=14; matrix size=192×192; field of view=5.0 cm; and scanning time=122 s. T2WI: TR/TE=3647 ms/85 ms; scanning time=110 s; the other parameters were the same as those of T1WI. DTI: TR/TE=2500 ms/92.2 ms; slice thickness=3.0 mm; no interslice gap; number of slices=7; matrix size=128×128; and field of view=110×110 mm<sup>2</sup>.

### DTI image preprocessing

DTI image preprocessing and analysis were implemented using an offline Advantage Windows workstation, version 4.5 (GE Healthcare, Waukesha, WI, USA), with the FuncTool software package. The threshold was adjusted and the ROI in the bilateral thalamus area of rats was chosen to measure FA. Every position was repeated 3 times and averaged.

### Biochemistry

After the third MRI scan, the rats were fixed in supine position. After regular disinfection, the abdominal cavity was cut open along the ventral midline and the abdominal aorta was exposed. About 4 ml of blood was sampled to measure arterial FBG (AFBG) and fasting insulin (FINS) using an automatic biochemistry analyzer (Blood Glucose Kit and Insulin Kit: Express Technology Co., Automatic Biochemistry Analyzer: HA-8160; Arkray, Inc., Kyoto, Japan). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the last evaluation of FBG and FINS ( $HOMA-IR=FBG \times FINS / 22.5$ ).

### Tissue harvesting and immunofluorescence

After drawing blood from the abdominal aorta, the rats were sacrificed with an overdose of pentobarbital intraperitoneally and cervical dislocation. The brain tissues were harvested, the thalamus was fixed in 4% polyformaldehyde, and the remaining brain was frozen in liquid nitrogen. The whole process was completed within 30 s. The brain tissues were then embedded in paraffin. Sections (10- $\mu$ m-thick) were cut for morphological assessment and immunofluorescence staining.

**Table 1.** Biochemical and cognitive data of each group.

|                                   |   | Control group<br>(n=15) | Control+MET<br>group (n=15) | T2DM<br>group (n=15)     | T2DM+MET<br>group (n=15) | Test<br>value (F/ $\chi^2$ ) | P     |
|-----------------------------------|---|-------------------------|-----------------------------|--------------------------|--------------------------|------------------------------|-------|
| Before group<br>feeding           | Weight (g)  | 88.60±4.70              | 88.80±2.51                  | 88.93±3.99               | 89.27±4.51               | 0.073                        | 0.974 |
|                                   | DFBG (mmol/L)                                     | 5.13±0.45               | 5.21±0.40                   | 5.31±0.33                | 5.37±0.40                | 1.098                        | 0.358 |
|                                   | Escape latency<br>(s)                             | 38.35<br>(18.75, 60.02) | 37.59<br>(22.14, 59.81)     | 39.12<br>(14.56, 60.02)  | 35.82<br>(14.15, 60.00)  | 0.755 <sup>#</sup>           | 0.860 |
|                                   | Number of rats<br>passing through<br>the platform | 2.00±1.69               | 2.13±1.55                   | 2.00±0.76                | 2.25±1.58                | 0.034                        | 0.991 |
|                                   | FA (left)   | 0.208±0.023             | 0.210±0.022                 | 0.207±0.010              | 0.207±0.015              | 0.109                        | 0.955 |
|                                   | FA (right)  | 0.209±0.017             | 0.210±0.012                 | 0.200±0.009              | 0.200±0.009              | 1.776                        | 0.162 |
| 8 weeks<br>after group<br>feeding | Weight (g)  | 475.47±16.70            | 475.33±11.82                | 497.87±17.16*            | 494.27±16.80*            | 8.711                        | 0.001 |
|                                   | DFBG (mmol/L)                                     | 5.25±0.37               | 5.34±0.37                   | 7.31±0.96*               | 6.98±0.76*               | 39.370                       | 0.001 |
|                                   | Escape latency<br>(s)                             | 8.12<br>(4.46, 18.87)   | 9.79<br>(5.03, 15.98)       | 8.09<br>(4.45, 20.48)    | 7.69<br>(4.07, 25.36)    | 2.209 <sup>#</sup>           | 0.530 |
|                                   | Number of rats<br>passing through<br>the platform | 3.00±1.73               | 2.80±1.37                   | 2.67±1.32                | 2.50±2.61                | 0.049                        | 0.985 |
|                                   | FA (left)   | 0.227±0.010             | 0.226±0.013                 | 0.216±0.019              | 0.221±0.015              | 1.776                        | 0.162 |
|                                   | FA (right)  | 0.241±0.019             | 0.245±0.019                 | 0.210±0.012*             | 0.212±0.013*             | 19.947                       | 0.001 |
| 8 weeks<br>after using<br>STZ     | Weight (g)  | 486.73±17.61            | 485.73±11.59                | 478.53±12.74             | 490.27±13.85             | 1.821                        | 0.154 |
|                                   | AFBG (mmol/L)                                     | 5.61±0.34               | 5.46±0.62                   | 18.98±1.24*              | 14.25±3.31**,**          | 206.789                      | 0.001 |
|                                   | Escape latency<br>(s)                             | 8.91<br>(5.03, 26.67)   | 8.56<br>(5.67, 28.47)       | 40.19<br>(21.05, 60.02)* | 28.47<br>(18.84, 60.02)* | 15.576 <sup>#</sup>          | 0.001 |
|                                   | Number of rats<br>passing through<br>the platform | 3.53±0.99               | 3.80±1.08                   | 1.93±1.34*               | 2.27±1.39*               | 8.692                        | 0.001 |
|                                   | FA (left)   | 0.263±0.026             | 0.266±0.019                 | 0.224±0.020*             | 0.238±0.013*,            | 15.615                       | 0.001 |
|                                   | FA (right)  | 0.267±0.030             | 0.267±0.023                 | 0.235±0.011*             | 0.266±0.019**            | 7.631                        | 0.001 |
|                                   | HOMA-IR   | 2.09±0.18               | 2.12±0.25                   | 4.77±0.45*               | 4.33±0.21*               | 184.873                      | 0.001 |

AFBG – arterial blood fasting blood glucose; DFBG – distal fasting blood glucose; FA – fractional anisotropy; HOMA-IR – homeostasis model assessment of insulin resistance. \* Statistically significant difference compared with the control group; \*\* statistically significant difference compared with the T2DM group; <sup>#</sup> Chi-square test value.

All examinations were performed on the bilateral thalamus. Three sections were made in each side of the thalamus. Paraffin-embedded sections were routinely dewaxed. The sections were boiled in an EDTA solution and heated for 18 min for antigen retrieval. For myelin and oligodendrocyte detection, monoclonal mouse anti-OLIG1 (ab68105; Abcam, Cambridge, UK; RRID: AB\_1142042; host: rabbit; antigen: synthetic peptide: SAR PDA KEE QQQ QLR-C, corresponding to amino acids 93-107 of Human Olig1), monoclonal mouse anti-OLIG2 (ab42453; Abcam; RRID: AB\_1603899; host: rabbit; antigen: a region within synthetic

peptide: SLP GSG LPS VGS IRP PHG LLK SPS AAA AAP LGG GGG GSG ASG GFQ HWG GM, corresponding to amino acids 242-291 of Olig2), and monoclonal mouse anti-MBP (Santa Cruz Biotechnology, Santa Cruz, CA, USA; RRID: AB\_297862; host: mouse; antigen: purified human MBP) were incubated overnight at 4°C. Then, secondary antibodies (MBP: FITC-goat anti-mouse IgG, ZSGB-Bio, Inc., Beijing, China, ZF-0312, OLIG1 and OLIG2: TRITC-goat anti-rabbit IgG, ZSGB-Bio, Inc., Beijing, China, ZF-0316) were incubated for 1 h at room temperature. The fluorescence signals were observed under a fluorescence

**Table 2.** Results of the Morris water maze test in rats.

|                             |   | Control group<br>(n=15) | T2DM group<br>(n=15)     | T2DM+MET<br>group (n=15) | (F/ $\chi^2$ )      | P     |
|-----------------------------|---|-------------------------|--------------------------|--------------------------|---------------------|-------|
| Before group feeding        | Escape latency (s) <sup>#</sup>             | 58.71<br>(22.14, 60.02) | 39.12<br>(14.55, 60.02)  | 35.82<br>(14.15, 60.00)  | 3.484 <sup>#</sup>  | 0.175 |
|                             | Number of rats passing through the platform | 2.00<br>(0.25, 3.75)    | 2.00<br>(1.00, 2.00)     | 2.50<br>(0.5, 3.75)      | 0.344 <sup>#</sup>  | 0.842 |
| 8 weeks after group feeding | Escape latency (s) <sup>#</sup>             | 8.12<br>(4.46, 18.87)   | 8.09<br>(4.45, 20.48)    | 7.69<br>(4.07, 25.36)    | 0.032 <sup>#</sup>  | 0.984 |
|                             | Number of rats passing through the platform | 3.00±1.87               | 2.67±1.53                | 2.50±3.54                | 0.060               | 0.942 |
| 8 weeks after using STZ     | Escape latency (s) <sup>#</sup>             | 8.91<br>(5.03, 26.67)   | 40.19<br>(21.05, 60.02)* | 28.47<br>(18.84, 60.02)* | 10.737 <sup>#</sup> | 0.005 |
|                             | Number of rats passing through the platform | 3.53±0.99               | 1.93±1.34*               | 2.27±1.39*               | 6.843               | 0.003 |

\* Statistically significant difference compared with the control group; \*\* statistically significant difference compared with the T2DM group; # not normally distributed and presented as median (P25, P75).

microscope (BX53, Olympus, Tokyo, Japan). Optical density values of MBP, OLIG1, and OLIG2 were analyzed using Image-Pro Plus software (Media Cybernetics, Inc., Rockville, MD, USA). Each slide was analyzed by 3 different pathologists, and the mean value of the 3 measurements was used for analysis. The bilateral thalamic regions were examined in each rat.

### Statistical analysis

The data were analyzed using SPSS 20.0 (IBM Corp., Armonk, NY, USA). The Shapiro-Wilk test was used to assess the continuous data for normal distribution. Normally distributed data are expressed as means±standard error and analyzed using analysis of variance (ANOVA). Non-normally distributed data are expressed as median (P25, P75) and were analyzed using the Kruskal-Wallis test. Pearson and Spearman correlation analyses were used to analyze the correlations. A *P* value <0.05 was considered significant.

## Results

### Rat models of T2DM

Table 1 presents the characteristics of the rats, showing that there were no differences with regard to body weight and FBG levels before modeling. After 8 weeks of high-fat and high-sugar diet, the rats had impaired fasting glycemia (IFG), but did not yet have T2DM. T2DM appeared in all rats of the T2DM and T2DM+MET groups 8 weeks after STZ injection. The AFBG levels were significantly higher in the T2DM and T2DM+MET groups than in the control and C + MET groups ( $F=206.789$ ,  $P=0.001$ ). The HOMA-IR values were higher in the T2DM and

T2DM+MET groups than in the control and C+MET groups ( $F=184.873$ ,  $P=0.001$ ). The weights of rats in each group were comparable ( $F=1.821$ ,  $P=0.154$ ) (Table 1).

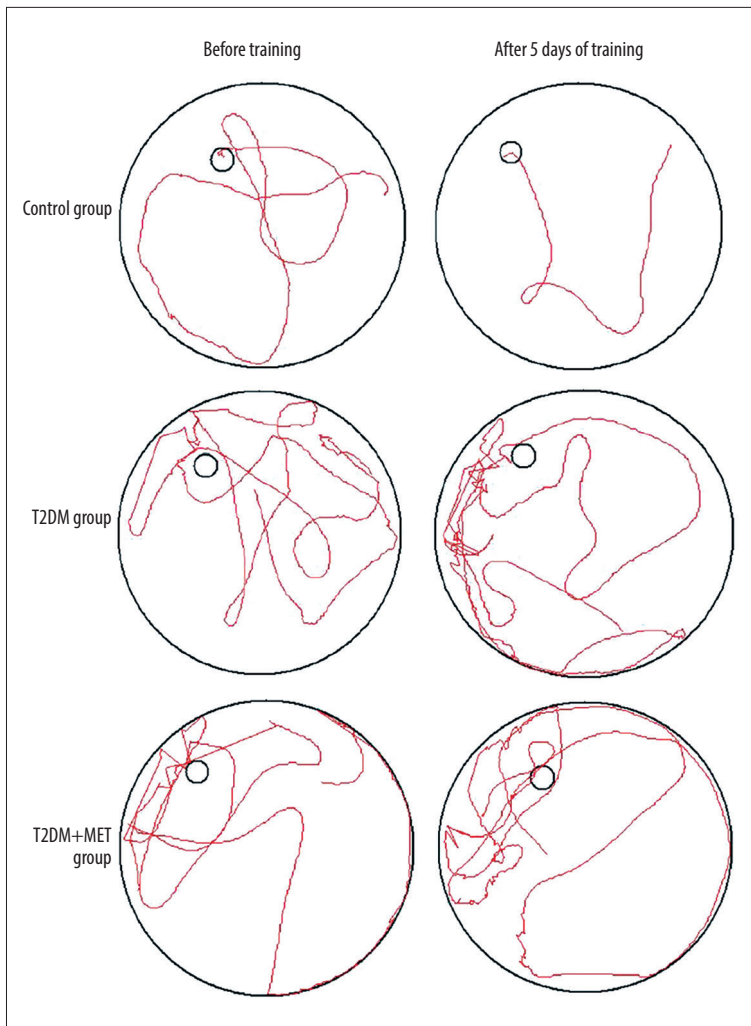
### Cognitive function was impaired in rats with T2DM

There were no differences in the escape latency and the number of rats passing through the platform before STZ injection (Table 2). Eight weeks after STZ injection, the escape latency was longer in the T2DM and T2DM+MET groups than in the control and C+MET groups ( $\chi^2=15.576$ ,  $P=0.001$ ) and they crossed the platform fewer times ( $F=8.692$ ,  $P=0.001$ ) (Table 2). The positioning cruise experiments also showed that the swimming trajectory of the rats with T2DM was more chaotic, while that of the T2DM+MET group was less chaotic (Figure 1), indicating that the cognitive function of T2DM rats was impaired.

### WML developed before T2DM and was correlated with cognitive dysfunction in rats with T2DM

After 8 weeks of study feeding (i.e., in the IFG state, before T2DM), the FA values of the right thalamus area were already significantly lower in the T2DM and T2DM+MET groups than in the control and C + MET groups ( $F=19.947$ ,  $P=0.001$ ) (Table 3). Eight weeks after STZ injection, the FA values of the left thalamus area of rats were lower in the T2DM and T2DM+MET groups than in the control and C+MET groups ( $F=15.615$ ,  $P=0.001$ , Figure 2), and only the FA values of the right thalamus area in T2DM group were lower than in the control and C+MET groups ( $F=7.631$ ,  $P=0.001$ , Figure 2). Meanwhile, the FA values of the right thalamus area in the T2DM+MET group were significantly higher than in the T2DM group (Figure 2).





**Figure 1.** Representative swimming trajectories of rats before (left panel) and after training (right panel). Eight weeks after STZ injection, the swimming trajectory of the rats in the T2DM and T2DM+MET groups were obviously more chaotic than in the Control and Control+MET groups and did not improve after 5 days of training.

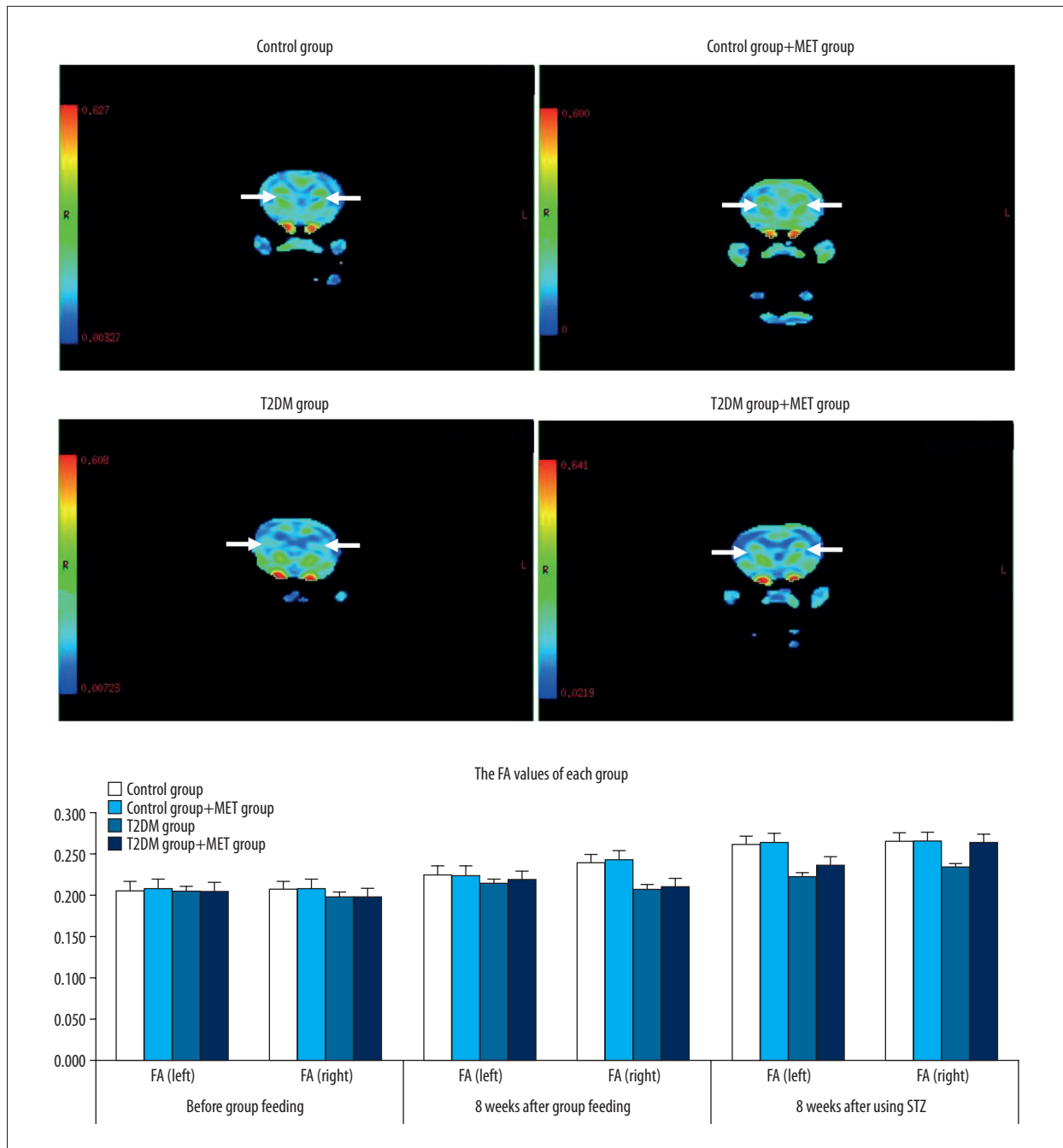
**Table 3.** Fractional anisotropy of the bilateral thalamus in rats.

|                             |            | Control group<br>(n=15) | T2DM group<br>(n=15) | T2DM+MET<br>group (n=15) | (F/ $\chi^2$ ) | P      |
|-----------------------------|------------|-------------------------|----------------------|--------------------------|----------------|--------|
| Before group feeding        | FA (left)  | 0.208±0.023             | 0.207±0.010          | 0.207±0.015              | 0.023          | 0.977  |
|                             | FA (right) | 0.208±0.017             | 0.201±0.009          | 0.202±0.009              | 1.620          | 0.210  |
| 8 weeks after group feeding | FA (left)  | 0.227±0.010             | 0.216±0.019          | 0.221±0.015              | 2.022          | 0.145  |
|                             | FA (right) | 0.241±0.019             | 0.210±0.012*         | 0.212±0.013*             | 19.591         | <0.001 |
| 8 weeks after using STZ     | FA (left)  | 0.263±0.032             | 0.224±0.020*         | 0.238±0.014***           | 16.057         | <0.001 |
|                             | FA (right) | 0.267±0.035             | 0.235±0.010*         | 0.266±0.020**            | 14.287         | <0.001 |

FA – fractional anisotropy. \* Statistically significant difference compared with the control group; \*\* statistically significant difference compared with the T2DM group.

The escape latency was correlated with the left FA value (Spearman,  $r=0.902$ ,  $P=0.001$ ), and the right FA value (Spearman,  $r=0.580$ ,  $P=0.001$ ). The number of rats crossing the platform was negatively correlated with the left FA value

(Pearson,  $r=0.814$ ,  $P=0.001$ ) and the right FA value (Pearson,  $r=0.681$ ,  $P=0.001$ ).



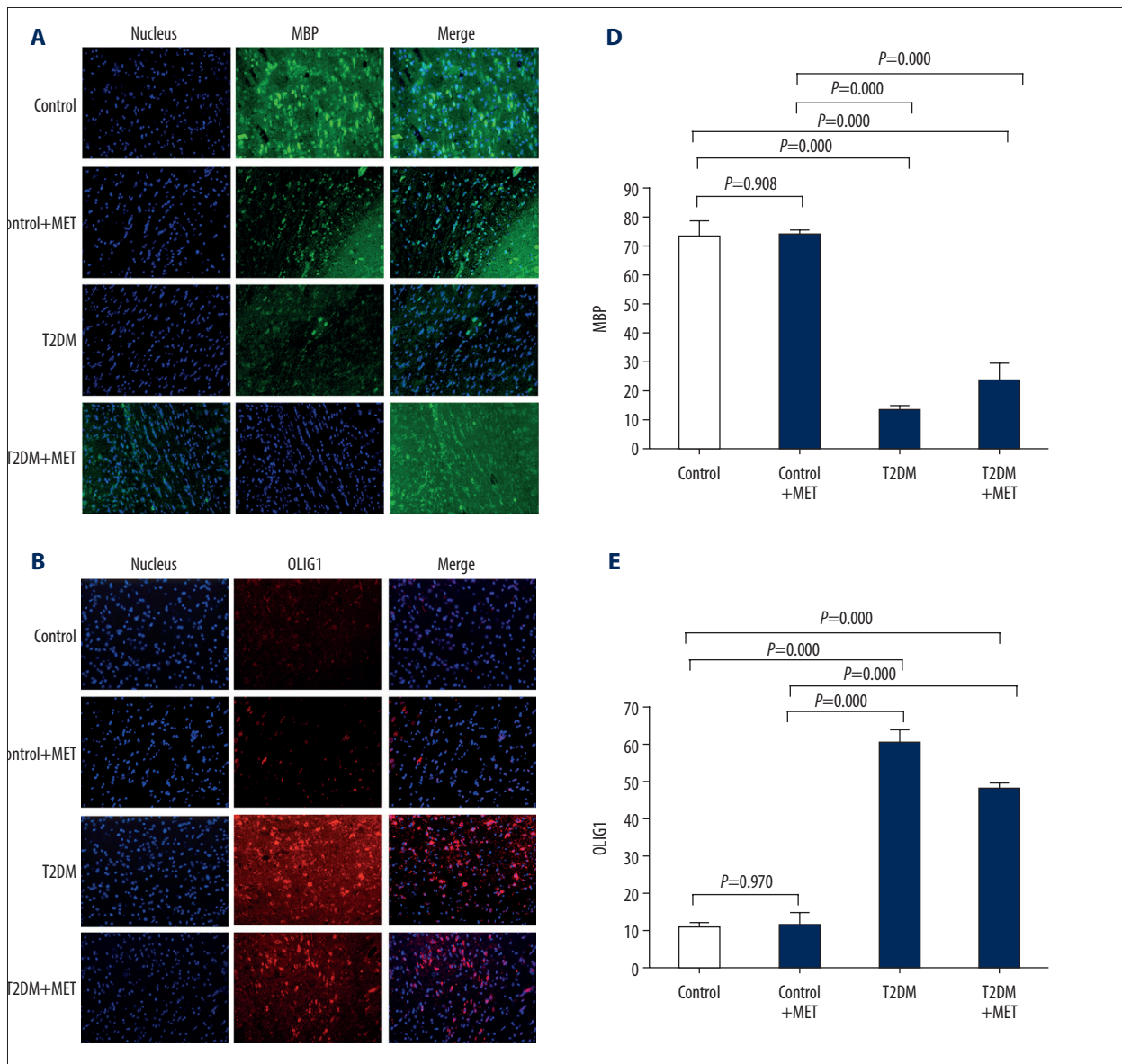
**Figure 2.** Representative DTI images 8 weeks after STZ injection. The FA values of the bilateral thalamus area (white arrow) were detected. Obvious white matter damage could be seen in the bilateral thalamus area in rats in the T2DM group. The color bar on the left side represents FA values. From bottom to top, FA value increases gradually from blue to red. Control group: the white arrows indicate the bilateral thalamus. The left FA value was 0.285 and the right FA value was 0.322. Control+MET group: the white arrows indicate the bilateral thalamus. The left FA value was xxx and the right FA value was xxx. T2DM group: the white arrows indicate the bilateral thalamus. The left FA value was 0.229 and the right FA value was 0.219. T2DM+MET group: the white arrows indicate the bilateral thalamus. The left FA value was 0.265 and the right FA value was 0.271.

**WML in rats with T2DM might be due to the demyelination of oligodendrocytes in the thalamus**

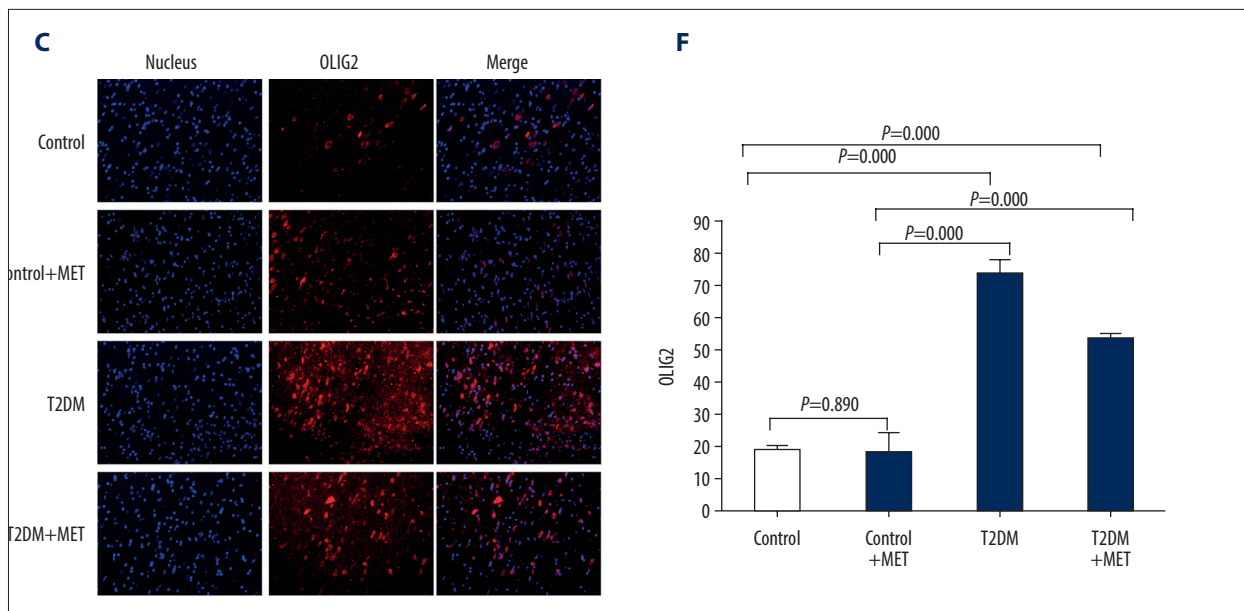
The expression of MBP was lower in the T2DM and T2DM+MET groups than in the control and C+MET groups ( $F=68.707$ ,  $P=0.001$ ). The expression of OLIG1 and OLIG2 were higher in the T2DM and T2DM+MET groups than in the control and C+MET groups (OLIG1:  $F=103.462$ ,  $P=0.001$ ; OLIG2:  $F=59.183$ ,  $P=0.001$ ) (Figure 3). These data suggest that WML in rats with T2DM might develop, at least partially, due to impaired differentiation and myelination of oligodendrocytes.

**Discussion**

In this study, a rat model of T2DM was established to observe the development of WML during the development of T2DM, and the relationship between WML and DACD was investigated. After 8 weeks of high-fat and high-sugar diet, WML appeared, even though the FBG did not meet the diagnostic criteria for T2DM, and WML was further aggravated after overt T2DM. The cognitive function of rats with T2DM was lower than in the control rats. FA values in some parts of the brain were lower in rats with T2DM compared with controls. Metformin in rats with T2DM alleviated in part the impact of diabetes on those parameters.







**Figure 3.** Immunofluorescence images and quantification of MBP, OLIG1, and OLIG2 expression in each group. (A) The immunofluorescence of nuclei (blue) and MBP (green). (B) The MBP levels. (C) The immunofluorescence of nuclei (blue) and OLIG1 (red). (D) The OLIG1 levels in each group. (E) The immunofluorescence of nuclei (blue) and OLIG2 (red) in each group. (F) The OLIG2 levels.

It was reported that cognitive dysfunction appears in pre-diabetes [42,43]. WML was already detectable before the onset of T2DM in the present study, which might be due to the mildly elevated FBG. Impaired FBG levels are associated with increased WM hyperintensity burden in older individuals with or without T2DM [26]. Interestingly, higher FBG was associated with greater WM hyperintensity burden in the right hemisphere, but not the left, and particularly in the frontal and temporal lobes [44]. Higher “normal” blood glucose levels are also notably associated with lower WM volumes in the right hemisphere [45]. The animal experiments showed that the FA values of the right thalamus region were lower in the T2DM and T2DM+MET groups than in the control group after 8 weeks of study feeding. These findings indicated that mildly elevated FBG had already damaged the WM. The FA values of the right thalamus in the T2DM and T2DM+MET groups were lower in the pre-diabetes stage, but the cognitive function in each group had no difference, indicating that significant WML appeared earlier than the cognitive dysfunction, since no obvious cognitive dysfunction was found before the onset of T2DM.

The hypothesis that T2DM can cause cognitive dysfunction has been confirmed by several studies [11–13], but the mechanisms are still not clear. Some studies suggested that DACD is associated with WML [13,27,46]. The bilateral thalamic regions of rats mainly include some sensory projection fibers and nerve nucleus, which have a variety of neural connections with cortical regions [47]. WM microstructural impairments disrupt the sensory projection fiber networks and underlie various

cognitive dysfunctions. Damage to the thalamus has been reported to be associated with neuropsychological dysfunction and ischemia-related dementia [48]. After successful T2DM modeling, WML was further aggravated and cognitive function declined rapidly, suggesting that WML was indeed related to DACD. The correlation analysis also supported this conclusion that WML was indeed a risk factor for DACD.

Previous studies suggested that WML are mainly due to axonal loss and nerve fiber demyelination [49]. In the present study, the immunofluorescence assay for MBP, OLIG1, and OLIG2 was conducted to try to understand the pathological changes in WML. When the CNS was injured, the expression of MBP decreased and the proliferation and differentiation of OLs increased to repair the damage. The results showed that the expression of MBP was significantly lower in the T2DM and T2DM+MET groups than in the control group, while the expression levels of OLIG1 and OLIG2 were significantly higher in the T2DM and T2DM+MET groups than in the control group, indicating that the OLs were injured and demyelinated, but that OLs proliferated and differentiated to repair the injury. Nevertheless, previous studies revealed that diabetic neuropathy could be electrophysiologically axonal, demyelinating, or both [50–52]. The possibility that T2DM also causes WML via axonal loss could not be excluded, but was not examined in the present study. The molecular mechanisms underlying demyelinating WML caused by T2DM or hyperglycemia warrants further exploration. Axonal WML during T2DM would be an interesting topic to investigate. MET treatment alleviated the

WML, but not cognitive function, in rats with T2DM. Whether metformin contributes to or protects from cognitive decline in patients with T2DM is controversial [53].

## Conclusions

WML appears in the IFG phase and positive correlations were observed between WML and DACD in rats with T2DM. Metformin can reduce WML, but fails to obviously rescue cognitive dysfunction. Future studies are needed to use MRI to

monitor WML in patients and for analysis in T2DM patients. Moreover, the molecular mechanisms underlying WML occurrence during diabetes need further investigation. Future research will examine the effect of different commonly used anti-diabetic drugs on the development and progression of WML. The exact mechanisms of WML development under high blood glucose conditions also need to be determined.

## Conflict of interest

None.

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