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Association of diabetes mellitus with cognitive impairment and neurological disorders

Edited by
Ben Nephew, Richard Lopez, Xiaodong Sun,
Joana Nicolau and Lei Sha

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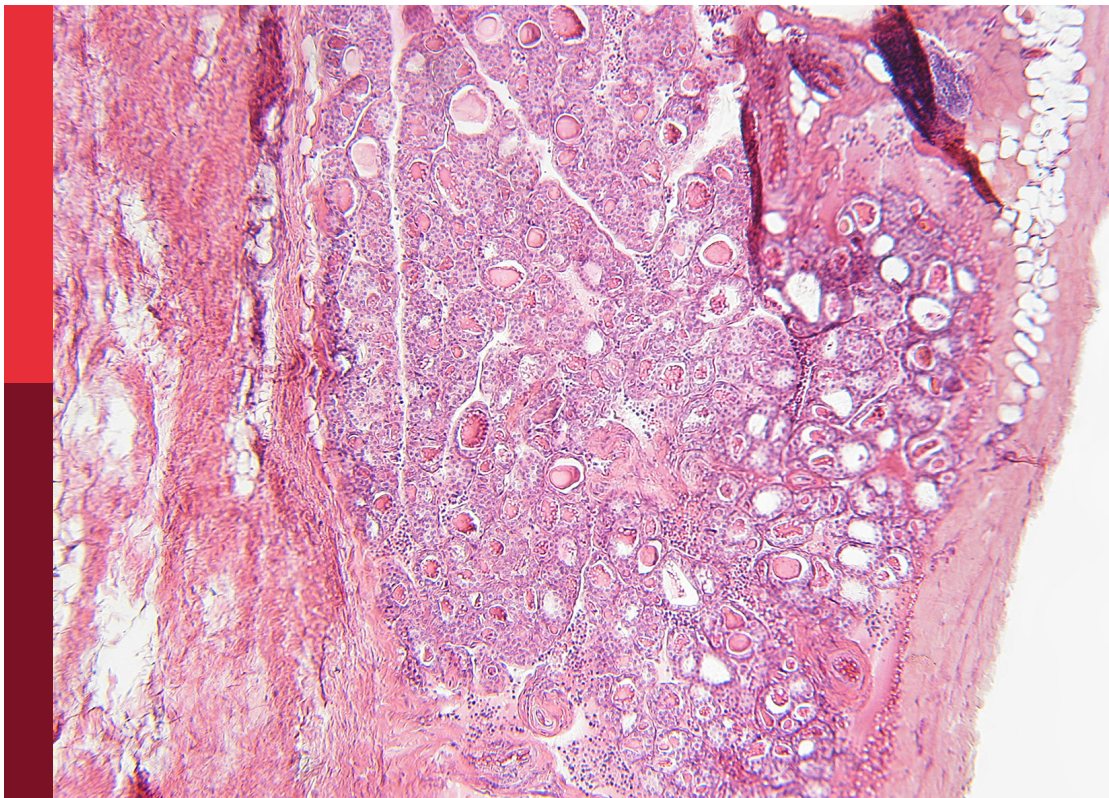

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Association of diabetes mellitus with cognitive impairment and neurological disorders

Topic editors

Ben Nephew — Worcester Polytechnic Institute, United States
Richard Lopez — Worcester Polytechnic Institute, United States
Xiaodong Sun — AFFILIATED HOSPITAL OF SHANDONG SECOND MEDICAL UNIVERSITY, China
Joana Nicolau — Son Llatzer Hospital, Spain
Lei Sha — China Medical University, China

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Hubert Vaudry,
Université de Rouen, France

*CORRESPONDENCE
Joana Nicolau
jnicolauramis@gmail.com

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Editorial: Association of diabetes mellitus with cognitive impairment and neurological disorders

Joana Nicolau* and Antelm Pujol

Vascular and Metabolic Diseases Research Group, Endocrinology Department, Son Llàtzer University Hospital, Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain

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diabetes mellitus, cognitive impairment, type 1 diabetes, electroacupuncture, phytate, mild cognitive impairment, retina

Editorial on the Research Topic
Association of diabetes mellitus with cognitive impairment and neurological disorders

The intersection of diabetes mellitus (DM) and cognitive impairment (CI) has become a critical area of research due to the rising prevalence of diabetes globally and their associated neurocognitive complications. This editorial aims to frame the research findings within this Research Topic by exploring the relationships between diabetes and cognitive decline, neurovascular asymmetry, underlying physiological mechanisms and future treatments options highlighted in the contributing articles. Each study adds valuable insights into how diabetes, both Type 1 and Type 2, affects cognitive function, posing significant public health concerns.

Tassew et al. systematically reviewed the prevalence of CI among patients with DM in Africa, finding a pooled prevalence of 43.99%. Key factors associated with CI included primary education level, poorly controlled diabetes, age over 60, and diabetes duration of more than 10 years. This review aimed to assess these associations and provide insights into the burden of cognitive dysfunction in populations with DM. Their findings highlight the need for an early diagnosis and targeted healthcare interventions to address CI in patients with DM, particularly those at higher risk due to these contributing factors mentioned before.

Moreover, having effective and low-cost screening tools is essential for early diagnosis. Lei et al. developed and validated a predictive model for cognitive dysfunction in individuals with abdominal obesity by using factors such as age, sex, education, total fat intake, red blood cell folate, depression, and physical activity. The model was built using data from 1,490 participants and assessed for predictive accuracy. Results showed strong predictive performance, with C-index values of 0.814 for the training set and 0.805 for the validation set. The nomogram effectively identified individuals at risk for cognitive dysfunction, demonstrating good clinical utility for early intervention and risk management.

In this regard, there may be additional diagnostic tools beyond cognitive tests. Some studies suggest that the retina may serve as a window to the brain. In fact, Li et al. explored the relationship between mild cognitive impairment (MCI) and retinal nerve fiber layer

(RNFL) thickness in type 2 diabetes mellitus (T2DM) patients. RNFL was assessed by using optical coherence tomography (OCT). Serum levels of IL-18, irisin, CML, and RAGE were also determined. Their results showed that T2DM patients with MCI had thinner RNFL and higher levels of IL-18, CML, and RAGE, whereas irisin levels were reduced. These markers were significantly correlated with cognitive test scores, suggesting their potential use as diagnostic indicators for MCI in T2DM patients.

Furthermore, not just retinal nerve fiber layer, but other brain changes have also been described. Samoilova et al. evaluated interhemispheric asymmetry in brain structure, metabolism, and neurovascular changes in patients with type 1 diabetes and cognitive impairment. By using MRI, spectroscopy, and perfusion imaging, their research revealed a significant asymmetry, particularly in frontal and occipital lobes. White and gray matter atrophy, along with metabolic disturbances in the hippocampus, were identified. These changes correlated with cognitive decline, particularly in attention and memory. Their findings suggest that neurovascular and metabolic alterations contribute to cognitive impairment in type 1 diabetes, highlighting the importance of early detection through neuroimaging.

Besides, an early diagnosis could present a unique opportunity to reverse brain structural and connectivity changes. In fact, Fang et al. explored cognitive reversal and brain connectivity changes in young adults with T2DM. Participants showed cognitive improvement in areas like global cognition and executive function after 18 months of a proper glycemic control. Brain connectivity, which was enhanced at baseline, normalized over time, and this reduction was linked to cognitive gains. This study suggests a potential "window period" for reversing cognitive dysfunction in early-stage diabetes. However, no clear association between glycemic control and brain connectivity was found, highlighting the complexity of the relationship between blood glucose and brain function.

Also, some emerging therapies have been proposed. Li et al. evaluated the effects of electroacupuncture (EA) on cognitive function and metabolic disorders in Alzheimer's disease (AD) model mice. EA improved cognitive abilities, reduced tau phosphorylation, and enhanced neuronal morphology. Additionally, EA regulated metabolic disorders by promoting brown adipose tissue (BAT) thermogenesis and improving peripheral glucose and lipid metabolism. EA also reduced insulin resistance and increased insulin sensitivity in the brain. These findings suggest that EA has therapeutic potential for treating AD by addressing both cognitive deficits and underlying metabolic imbalances, particularly through BAT activation and central insulin pathway regulation. In the context of human clinical trials, Pujol et al. proposed a promising clinical trial protocol which aimed to assess the effects of daily phytate supplementation on the progression of MCI, brain iron deposition, and diabetic retinopathy in T2DM patients. Over a 56-week randomized, double-blind, placebo-controlled trial, cognitive changes, brain iron accumulation using MRI, and retinal health will be evaluated. Their hypothesis is that phytate supplementation could improve cognitive function, reduce iron accumulation in the brain, and slow neurodegeneration in both the central nervous system and the retina, potentially offering a new therapeutic strategy for T2DM patients with MCI.

In conclusion, the association between diabetes mellitus and cognitive impairment represents a growing public health challenge due to the global increase in both conditions. All these studies offer valuable insights into the underlying mechanisms of this relationship, from the importance of early detection to emerging therapeutic innovations. There is an urgent need for personalized medical interventions, accessible diagnostic tools, and a comprehensive approach that addresses both metabolic disturbances and neurological symptoms to mitigate the impact of cognitive decline in patients with DM.

Author contributions

JN: Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. AP: Conceptualization, Investigation, Methodology, Writing – original draft.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Cognitive Dysfunction in Type 2 Diabetes Is Not a One-Way Process: Evidence From a Longitudinal Brain Connectivity Study

Fang Fang[1†], Yu-Jia Gong[1†], Qian Luo[2], Ren-Bin Ge[2], Mei Kang[3], Ming-Ming Ma[4], Lei Zhang[2], Di Mu[5], Da-Zhi Yin[6,7] and Yu-Fan Wang[1*]*

[1] Department of Endocrinology and Metabolism, Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China, [2] Department of Radiology, Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China, [3] Clinical Research Center, Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China, [4] Shanghai Key Laboratory of Ocular Fundus Diseases, Shanghai Engineering Center for Visual Science and Photomedicine, Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China, [5] Department of Anesthesiology, Shanghai General Hospital, Shanghai Jiao Tong University, Shanghai, China, [6] Shanghai Key Laboratory of Brain Functional Genomics (Ministry of Education), School of Psychology and Cognitive Science, East China Normal University, Shanghai, China, [7] Shanghai Changning Mental Health Center, Shanghai, China

Background: Cognitive dysfunction is an important comorbidity of diabetes characterized by brain functional hypo-connectivity. However, our recent study demonstrated an adaptive hyper-connectivity in young type 2 diabetes with cognitive decrements. This longitudinal study aimed to further explore the changes in functional connectivity and cognitive outcomes after regular glycemic control.

Methods: At 18 months after recruitment, participants underwent a second cognitive assessment and magnetic resonance imaging. Three enhanced functional connectivities previously identified at baseline were followed up. Linear mixed-effects models were performed to compare the longitudinal changes of cognition and functional connectivity in patients with type 2 diabetes and non-diabetic controls. A linear regression model was used to investigate the association between changes in functional connectivity and changes in cognitive performance.

Results: Improvements in multiple cognitive domains were observed in diabetes; however, the enhanced functional connectivity at baseline decreased significantly. Moreover, the decrease in hippocampal connectivity was correlated with an increase in the accuracy of Stroop task and the decrease in posterior cingulate cortex connectivity was correlated with an increase in Montreal Cognitive Assessment in diabetes.

Conclusion: This study suggests diabetes-related cognitive dysfunction is not a one-way process and the early-stage enhancement of brain connectivity was a potential "window period" for cognitive reversal.

Keywords: cognitive reversal, hippocampus, functional connectivity, magnetic resonance imaging, type 2 diabetes, young adult

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Ben Nephew, Worcester Polytechnic Institute, United States

Reviewed by:
Charles-Henri Malbert, Institut National de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), France
Ira Driscoll, University of Wisconsin–Milwaukee, United States

****Correspondence:***
Yu-Fan Wang
yyffwang@sina.com
Da-Zhi Yin
dzyin@psy.ecnu.edu.cn

†These authors have contributed equally to this work and share first authorship

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INTRODUCTION

Patients with type 2 diabetes are at an increased risk of cognitive dysfunction, ranging from subtle cognitive deficits to major neurocognitive disorders (1). Severe cognitive dysfunction mainly occurs in patients older than 65 years, whereas subtle cognitive deficits have been observed in diabetes of all age groups (2). Despite being within the normal cognitive range and not affecting daily life, the presence of slight cognitive deficits might be additive to the accelerated cognitive decline and might reduce the symptomatic threshold of dementia (3). Given the growing prevalence of type 2 diabetes in young individuals worldwide, efforts to elucidate the changes in brain neurophysiology in young patients have become essential and urgent.

Noninvasive multimodal magnetic resonance imaging (MRI) (e.g., structural and functional) techniques provide potential biomarkers for cognitive dysfunction (4). Previous neuroimaging studies have consistently demonstrated modest brain atrophy (5) and reduced functional connectivity among brain regions (6) in middle-aged to elderly patients with type 2 diabetes. For example, based on resting-state functional MRI (fMRI), the functional connectivity between seed regions (e.g., bilateral hippocampus (7, 8) and posterior cingulate cortex [PCC] (9, 10)) and multiple regions in the default mode network was weaker in diabetes than in controls and the decreased connectivity was correlated with cognitive decline.

In contrast, in our recent study, young adults with type 2 diabetes (age: <40 years) at an early stage of disease progression (no detectable microvascular complications) had an enhanced resting-state functional connectivity compared with non-diabetes controls, which is related to cognitive decrements and occurs before brain morphometric change (11). These findings suggest a compensation of brain function to counteract the insidious cognitive decline during the early stage of type 2 diabetes. Moreover, this increase in intrinsic functional connectivity has been found in young type 1 diabetic children (12) and in type 1 diabetic adults without microvascular complications (13). Overall, the emerging neuroimaging studies suggest nonlinear changes in brain connectivity at different stages of diabetes progression. However, the evolution of brain hyperconnectivity in the early stages of diabetes and its effects on neuropsychological performance under glycemic control have not been demonstrated.

In the current longitudinal study, the three enhanced functional connectivities previously identified at baseline (i.e., connectivity of the left hippocampus with the left inferior frontal gyrus [IFG] and left inferior parietal lobule [IPL] and connectivity of the posterior cingulate cortex [PCC] with the left IPL) were further investigated both in young adults with type 2 diabetes and controls. In addition, voxel-based morphometric (VBM) analysis was performed to investigate the change in brain volume. We hypothesized that the enhancement of functional connectivity was temporary, which would disappear and contribute to the change of cognitive function.

MATERIALS AND METHODS

Participants and Recruitment

This was an extension of our previous cross-sectional study (11). The recruited patients with type 2 diabetes (age: < 40 years) and non-diabetic controls were followed up. Patients had no peripheral microvascular complications such as diabetic retinopathy, diabetic nephropathy, and diabetic peripheral neuropathy at recruitment. Participants with a history of diabetic ketosis or ketoacidosis, hypoglycemia within 48 hours, clinical evidence of cardiovascular or cerebrovascular diseases, a history of alcohol consumption, thyroid dysfunction, anemia, and any MRI contraindications were excluded. After recruitment, patients were provided diabetes education and individualized treatment. All licensed antidiabetic medications were permitted. No target hemoglobin A1c was predetermined. The follow-up visits targeted at 18 months after recruitment. Of the 67 participants (35 patients with type 2 diabetes and 32 controls) recruited at baseline, 50 (74.6%) participants (26 patients with type 2 diabetes and 24 controls) consented to participate in the extensional study. Participants were given separate informed consents for this longitudinal study. The study protocol was reviewed and approved by Shanghai General Hospital Ethics Committee, approval number 2019SQ082.

Neuropsychological and Clinical Assessments

Cognitive function was assessed at both time-points using the same methodology (11) and by the same physician. Briefly, the cognitive battery included tests for global cognitive function (Montreal Cognitive Assessment [MoCA]), executive function (the accuracy and reaction time of Stroop Color Word Test - part C), memory function (Rey Auditory Verbal Learning Test [RAVLT]), and language function (Verbal Fluency Test [VFT] and Boston Naming Test [BNT]).

Age, sex, education level, and follow-up interval (i.e., the time from baseline to follow-up MRI scan) were recorded for statistical correction. The levels of hemoglobin A1c (HbA1c), fasting plasma glucose, and fasting serum C-peptide were assessed to determine the glycemic control at the two time points. Homeostasis model assessment (HOMA) (14) was used to assess β-cell function (HOMA-%β) and insulin resistance (HOMA-IR). Antidiabetic drugs used by patients with type 2 diabetes at recruitment and follow-up visits were recorded. The laboratory data were collected a day before the MRI scan.

Microvascular complications were assessed in patients with diabetes at the two time points. A dilated fundus examination using a 90-diopter pan-fundus lens was performed by an ophthalmologist. Diabetic retinopathy was defined as the presence of any of the following lesions: retinal microaneurysms, hemorrhages, hard exudates, soft exudates, neovascularization, or evidence (or history) of laser photocoagulation. The urinary albumin-to-creatinine ratio (UACR) was measured in first-void clean-catch urine samples collected on two consecutive days. The average UACR for two consecutive days was used for the analysis.

MRI Data Acquisition and Preprocessing

The baseline and follow-up MRI were performed using a 3-Tesla MRI scanner (Ingenia, Philips Healthcare, Best, NL). fMRI data were acquired using a gradient-echo-planar imaging sequence with the same parameters and instructions (e.g., eyes closed): repetition time (TR), 2000 ms; echo time (TE), 30 ms; field of view, 224 × 224 mm^2; flip angle, 90°; slices, 33; slice thickness, 3.5 mm; slice spacing, 0.7 mm; matrix, 64 × 62; volumes, 240; and acquisition time, 8 min. High-resolution, T1-weighted images were obtained using a magnetization-prepared rapid gradient-echo sequence (TR = 7.0 ms, TE = 3.2 ms, flip angle = 7°, inversion time = 1100 ms, and voxel size = 1 × 1 × 1 mm^3).

The fMRI data were preprocessed using Data Processing Assistant for Resting-State fMRI software (15) and the following parameters, as in our previous study (11): 1) slice timing; 2) spatial realignment to correct for head motion; 3) spatial normalization of images to the Montreal Neurological Institute (MNI) space using a unified segmentation algorithm (16) and resampling to 3-mm isotropic voxels; 4) spatial smoothing with a 6-mm full-width-half-maximum (FWHM) Gaussian kernel; and 5) linear detrending, bandpass filtering (0.01–0.08 Hz), and regressing out several covariates such as six head-motion parameters and white matter (WM) and cerebrospinal fluid (CSF) signals.

Clinical and Neuropsychological Analyses

Subjects with both baseline and follow-up data were enrolled for the statistical analysis. The between-group differences at each time point were analyzed using a two-tailed two-sample t-test, Mann–Whitney U test, or chi-square test. Neuropsychological scores were normalized by logarithmic transformation. Thereafter, linear mixed-effects models were performed to compare the longitudinal changes of neuropsychological scores in patients with diabetes and non-diabetic controls between the two time-points, with adjustment for sex, age at baseline, education level and follow-up interval.

Resting-State Functional Connectivity Analysis

Functional connectivity analysis using the Resting-State fMRI Data Analysis Toolkit (REST, http://restfmri.net) was performed using bilateral hippocampus and PCC as the seed region of interest (ROI) in our previous study (11). The three enhanced functional connectivities which were founded in previous between-group analysis were further investigated. The value of each functional connectivity for each subject at both time points was extracted. We first performed between-group analysis at each time point using a two-tailed two-sample t-test. We then performed linear mixed-effects models to compare the longitudinal changes of brain functional connectivity in patients with diabetes and controls between the two time-points, adjusted for sex, age at baseline, education level and follow-up interval.

Finally, to determine the associations between the changes in the three functional connectivities and the changes in neuropsychological performance, we performed linear regression analyses adjusting sex, age at baseline, education level, and follow-up interval in each group. The associations between changes in the aforementioned connectivity and changes in hyperglycemia-related variables (i.e., HbA1c level, fasting plasma glucose level, fasting serum C-peptide level, HOMA-%β, and HOMA-IR) were also determined in patients with diabetes.

VBM Analysis

We performed VBM analysis on T1-weighted images using the VBM8 toolbox in the SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm/). Cerebral tissues from each participant were segmented into gray matter (GM), WM, and CSF. Images of each participant's GM, WM, and CSF were nonlinearly registered using the DARTEL method and transformed into the MNI standard space. The warped GM images were modulated by Jacobian determinants to include the information of volume in these modulated images. Finally, the resultant maps were smoothed with a 6-mm FWHM Gaussian kernel. In addition, the GM, WM, CSF, and total brain volumes for each participant were obtained simultaneously.

At the voxel level, between-group differences in the GM volume were determined using random-effects two-sample t-tests with age, sex, educational level, and BMI as nuisance covariates. The threshold was set at a corrected P value of < 0.05, with multiple comparisons corrected using the AlphaSim program (http://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf) determined by 1000 Monte Carlo simulations (i.e., single voxel $P < 0.001$, combining a minimum cluster size). In addition, we performed between-group analysis for the mean volumes of GM, WM, and CSF, as well as total brain volume.

RESULTS

Demographic and Clinical Data

The mean follow-up intervals for patients and controls were 18.0 ± 5.5 and 18.0 ± 4.9 months respectively ($P = 0.891$). The characteristics of subjects who withdrew from the study are presented in **Supplemental Table 1**.

Compared with the controls, the patients with type 2 diabetes had higher body mass index ($P = 0.017$), HbA1c level ($P < 0.001$), fasting blood glucose ($P = 0.011$), HOMA-%β ($P = 0.003$), and lower HDL cholesterol level ($P = 0.002$) at the follow-up visit as expected. However, there were no differences in the level of HOMA-%β ($P = 0.965$), triglyceride ($P = 0.083$), and uric acid ($P = 0.129$) at the follow-up visit, and these variables were statistically different between groups at baseline. Notably, the number of patients who were taking antidiabetic medicines increased from 42.3% to 92.3% from baseline to follow-up. A large proportion of patients were using metformin (73.1%) and/or glucagon-like peptide 1 receptor agonists (42.3%) at the follow-up visit. The demographic and clinical data of the participants are shown in **Table 1**.

TABLE 1 | Demographic and Clinical data at baseline and at the follow-up visit.

	Baseline			Follow-up		
	Controls N = 24	**Diabetes N = 26**	***P* values**	**Controls N = 24**	**Diabetes N = 26**	***P* values**
Age, year	34.1 ± 4.4	33.0 ± 5.5	0.444	35.4 ± 4.2	34.6 ± 5.6	0.574
Male	13 (54.2)	20 (76.9)	0.090	13 (54.2)	20 (76.9)	0.090
Education, year	14.2 ± 4.6	13.2 ± 2.7	0.324	14.2 ± 4.6	13.2 ± 2.7	0.324
Body Mass Index, kg/m^2	23.8 ± 3.0	26.7 ± 3.7	0.004*	23.9 ± 3.0	26.1 ± 3.5	0.017*
History of smoking	10 (41.7)	10 (38.5)	0.817	10 (41.7)	11 (42.3)	0.963
Presence of hypertension	5 (20.8)	8 (30.8)	0.424	5 (20.8)	8 (30.8)	0.424
Statin treatment	0 (0)	5 (19.2)	0.051	0 (0)	7 (26.9)	0.010*
Diabetes treatment	–	11 (42.3)	–	–	24 (92.3)	–
Metformin	–	9 (34.6)	–	–	19 (73.1)	–
Sulfonylureas	–	3 (11.5)	–	–	0 (0)	–
Glinides	–	2 (7.7)	–	–	1 (3.8)	–
Thiazolidinediones	–	0 (0)	–	–	1 (3.8)	–
Acarbose	–	2 (7.7)	–	–	8 (30.8)	–
DPP-4i	–	2 (7.7)	–	–	7 (26.9)	–
SGLT-2i	–	(0)	–	–	2 (7.7)	–
GLP-1A	–	(0)	–	–	11 (42.3)	–
Insulin	–	3 (11.5)	–	–	4 (15.4)	–
HbA1c, %	5.5 ± 0.3	10.0 ± 2.2	< 0.001*	5.2 ± 0.2	6.5 ± 1.2	< 0.001*
Fasting plasma glucose, mmol/L	4.74 ± 0.55	8.45 ± 3.55	< 0.001*	5.16 ± 0.25	7.14 ± 3.07	0.011*
Fasting serum C-peptide, pmol/L	507.0 ± 200.7	578.3 ± 306.2	0.372	449. 0 ± 190.2	673.1 ± 326.8	0.015*
HOMA-%β	116.6 ± 41.6	53.4 ± 30.7	< 0.001*	85.8 ± 21.1	85.1 ± 54.0	0.965
HOMA-IR	1.09 ± 0.43	1.58 ± 0.98	0.046*	0.94 ± 0.39	1.62 ± 0.77	0.003*
Total Cholesterol, mmol/L	4.92 ± 1.14	4.99 ± 0.94	0.805	4.75 ± 0.97	4.80 ± 0.75	0.837
Triglyceride, mmol/L	1.31 ± 0.79	2.74 ± 1.50	< 0.001*	1.56 ± 1.39	2.60 ± 2.13	0.083
HDL cholesterol, mmol/L	1.32 ± 0.34	0.90 ± 0.23	< 0.001*	1.25 ± 0.30	0.99 ± 0.21	0.002*
LDL cholesterol, mmol/L	3.14 ± 0.89	3.08 ± 0.77	0.809	2.59 ± 0.76	2.57 ± 0.78	0.944
Uric acid, μmol/L	296.0 ± 80.2	372.7 ± 134.0	0.023*	334.8 ± 92.0	391.7 ± 130.4	0.129
Urine ACR, mg/g	9.38 (4.5, 21.7)	14.0 (2.1, 29.9)	0.058	9.08 (5.9, 28.0)	6.25 (2.5, 307)	0.419
Diabetic retinopathy	–	0 (0)	–	–	3 (1.2)	–
Diabetes duration, year	–	1.5 (0, 10)	–	–	2.8 (1.1, 11.3)	–

*Data are represented as mean ± SD, n (%), or median (range). *P <0.05. DPP-4i, Dipeptidyl peptidase 4 inhibitors; SGLT-2i, Sodium glucose cotransporter 2 inhibitors; GLP-1A, Glucagon-like peptide 1 receptor agonists; HOMA-%β, homeostasis model assessment of β-cell function; HOMA-IR, homeostasis model assessment of insulin resistance; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and ACR, albumin-to-creatinine ratio.*

Neuropsychological Tests

The between-group differences in the neuropsychological tests at baseline are consistent with our former study, which revealed longer Stroop Reaction Time in patients with type 2 diabetes than in controls (P = 0.033 in the current sample), indicating poorer executive function in the patients group. At the follow-up visit, interestingly, patients with type 2 diabetes had a higher MoCA than the controls (P = 0.039), which indicated better global cognitive function in the patients group. No significant differences were noted in any other neuropsychological performances between the two groups at the follow-up visit (**Figure 1**).

The linear mixed-effects model demonstrated that the longitudinal trajectories of log-transformed MoCA and RAVLT differed significantly between the two groups. Compared with controls, patients with diabetes had a 1.5% greater incremental rate of MoCA score (β = 0.015, 95% CI: 0.00013 – 0.030) and a 5% greater incremental rate of RAVLT (β = 0.050, 95% CI: 0.0002 – 0.100) on a logarithmic scale from baseline to follow-up visit. Furthermore, significant increase of log-transformed Stroop Accuracy ((β = -0.009, 95% CI: -0.016 – -0.002) and decrease of Stroop Reaction Time ((β = 0.030, 95% CI: 0.010 – 0.049)) were observed among patients with diabetes, which indicated that patients had better executive function at follow-up compared to that at baseline.

Functional Connectivity Measures

Baseline between-group analyses revealed that the three functional connectivities were significantly higher in patients with type 2 diabetes than in the controls (P < 0.001 for the connectivity of the left hippocampus with the left IFG and the left IPL; P = 0.001 for the connectivity of the PCC with the left IPL), which were also consistent with our former study. At the follow-up visit, however, the between-group differences disappeared (**Figure 2**).

Moreover, the linear mixed-effects model demonstrated the longitudinal changes of the connectivity of the left hippocampus with the left IFG and the left IPL differed significantly between diabetic group and controls. Compared with controls, patients with diabetes had a 20.7% greater decremental rate in the connectivity of the left hippocampus with the left IFG (β = -0.207, 95% CI: -0.334 – -0.080) and a 16.0% greater decremental rate in the connectivity of the left hippocampus with the left IPL (β = -0.160, 95% CI: -0.271 – -0.048) from baseline to follow-up visit (**Table 2**).

Considering that the antidiabetic drugs might have a direct effect on brain functional connectivity (7), we further divided the

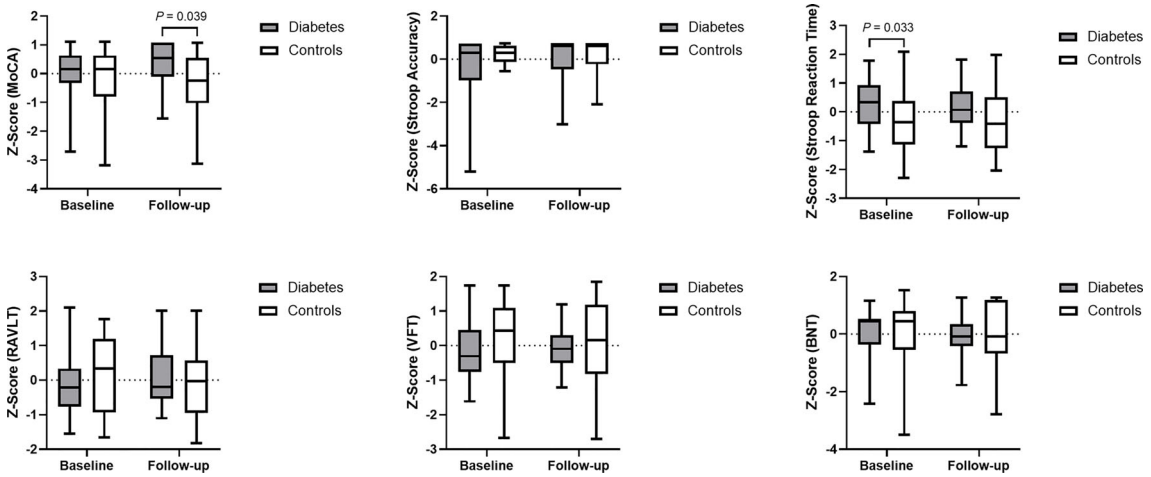

FIGURE 1 | Data distribution and between-group comparison of cognitive performance. Z-score was used for standardization of data. Higher cognitive scores and lower Stroop Reaction Time indicate better cognitive function. MoCA, Montreal Cognitive Assessment; Stroop Accuracy, the accuracy of Stroop Color Word Test - part C; Stroop Reaction Time, the reaction time of Stroop Color Word Test - part C; RAVLT, Rey Auditory Verbal Learning Test; VFT, Verbal Fluency Test; and BNT, Boston Naming Test.

patients into two subgroups according to whether they took antidiabetic drugs at baseline (Medicated subgroup, $N = 11$; non-Medicated subgroup, $N = 15$) to obtain a comprehensive understanding of the brain neurophysiology in young patients with type 2 diabetes. Between-subgroup analyze demonstrated that the functional connectivity between PCC and left IPL was lower in medicated subgroup compared with that in non-medicated subgroup at baseline. No between-subgroup differences were observed in cognitive performance at baseline, the changes of cognitive performance and the changes of functional connectivity from baseline to the follow-up visit (**Supplemental Table 2**). The decreased intrinsic functional connectivity in medicated subgroup at baseline provided some clues to the further research on the effect of antidiabetic drugs on brain function.

Brain Volume Measures

No significant between-group differences were observed in brain morphometric analyses (both voxel-based and global brain volume analyses) at both time-points. (**Supplemental Table 3**).

Regression Analysis

In patients with type 2 diabetes, the change in Stroop Accuracy was negatively correlated with the change in functional connectivity between the left hippocampus and the left IPL after adjusting for age at baseline, sex, education level, and

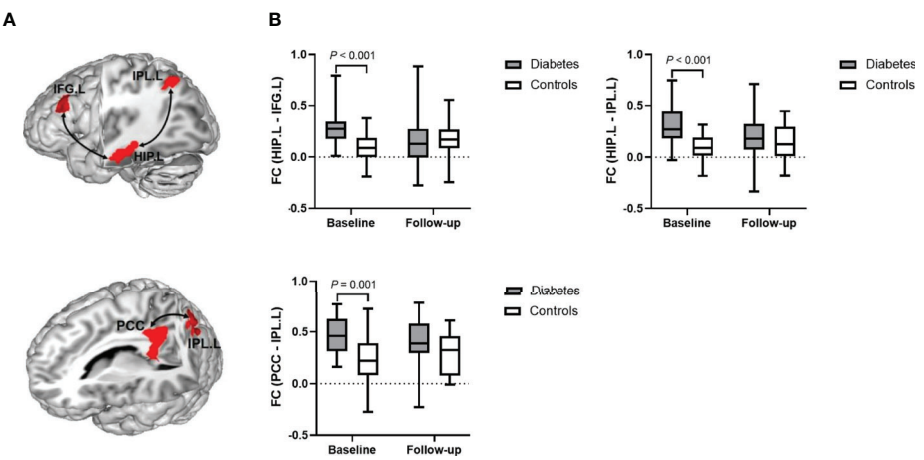

FIGURE 2 | **(A)** A representative model of the enhanced functional connectivity at baseline. **(B)** Data distribution and between-group comparisons of functional connectivity. HIP.L, left hippocampus; IFG.L, left inferior frontal gyrus; IPL.L, left inferior parietal lobule; PCC, posterior cingulate cortex; and FC, functional connectivity.

TABLE 2 | Linear mixed-effects models for the changes of cognition and functional connectivity in patients with diabetes and controls from baseline to follow-up visit.

	β	95% CI	*P* values
Cognition (Log-transformed)			
MoCA			
Group	-0.022	(-0.034, -0.009)	0.001*
Visit	-0.012	(-0.022, -0.002)	0.026*
Group * Visit	0.015	(0.0001, 0.030)	0.048*
Stroop Accuracy			
Group	0.002	(-0.003, 0.007)	0.385
Visit	-0.009	(-0.016, -0.002)	0.011*
Group * Visit †	0.011	(-0.003, 0.024)	0.111
Stroop Reaction Time			
Group	-0.037	(-0.082, 0.007)	0.100
Visit	0.030	(0.010, 0.049)	0.004*
Group * Visit †	-0.009	(-0.048, 0.030)	0.636
RAVLT			
Group	-0.045	(-0.095, 0.005)	0.078
Visit	-0.053	(-0.087, -0.018)	0.004*
Group * Visit	0.050	(0.0002,0.100)	0.049*
VFT			
Group	0.005	(-0.040, 0.050)	0.818
Visit	-0.007	(-0.024, 0.011)	0.452
Group * Visit †	0.017	(-0.017, 0.052)	0.324
BNT			
Group	-0.0007	(-0.025, 0.024)	0.956
Visit	-0.008	(-0.017, 0.0009)	0.078
Group * Visit †	0.005	(-0.013, 0.023)	0.537
Functional Connectivity			
HIP.L-IFG.L			
Group	0.026	(-0.101, 0.152)	0.684
Visit	0.146	(0.058, 0.234)	0.002*
Group * Visit	-0.207	(-0.334, -0.080)	0.002*
HIP.L-IPL.L			
Group	-0.041	(-0.160, 0.079)	0.495
Visit	0.100	(0.023, 0.178)	0.012*
Group * Visit	-0.160	(-0.271, -0.048)	0.006*
PCC-IPL.L			
Group	-0.129	(-0.259, 0.002)	0.053
Visit	-0.050	(-0.041, 0.141)	0.276
Group * Visit †	-0.122	(-0.253, 0.010)	0.069

*Model adjusted for sex, age at baseline, education level and follow-up interval. †Interaction was not adopted in the final model since there was no statistical difference of the interactive effect. *P <0.05.*

β coefficient for "Group" represents the difference of the variable (i.e., MoCA, Stroop Accuracy, Stroop Reaction Time, RAVLT, VFT, BNT, FC [HIP.L-IFG.L], FC [HIP.L-IPL.L], or FC [PCC-IPL.L]) levels between patients with diabetes and non-diabetic controls at the follow-up visit.

β coefficient for "Visit" represents the longitudinal change of the variable (i.e., MoCA, Stroop Accuracy, Stroop Reaction Time, RAVLT, VFT, BNT, FC [HIP.L-IFG.L], FC [HIP.L-IPL.L], or FC [PCC-IPL.L] levels) levels from baseline to follow-up visit among patients with diabetes.

*β coefficient for "Group * Visit" represents the difference in longitudinal change of the variable (i.e., MoCA, Stroop Accuracy, Stroop Reaction Time, RAVLT, VFT, BNT, FC [HIP.L-IFG.L], FC [HIP.L-IPL.L], or FC [PCC-IPL.L] levels) levels from baseline to follow-up visit between patients with diabetes and non-diabetic controls.*

MoCA, Montreal Cognitive Assessment; Stroop Accuracy, the accuracy of Stroop Color Word Test – part C; Stroop Reaction Time, the reaction time of Stroop Color Word Test – part C; RAVLT, Rey auditory verbal learning test; VFT, Verbal fluency test; BNT, Boston naming test; FC (HIP.L-IFG.L), the functional connectivity of the left hippocampus with the left inferior frontal gyrus; FC (HIP.L-IPL.L), the functional connectivity of the left hippocampus with the left inferior parietal lobule; FC (PCC-IPL.L), the functional connectivity of the posterior cingulated cortex with the left inferior parietal lobule.

follow-up interval (β = -0.441, $P = 0.045$). This indicated that the greater the decline in the hippocampal connectivity, the greater the improvement of executive function. Moreover, the change in MoCA was negatively correlated with the change in functional connectivity between the PCC and left IPL after adjusting for the aforementioned potential confounders (β = −0.486, $P = 0.037$), indicating that the greater the decline in PCC connectivity, the greater the improvement of global cognition. In contrast, no association was observed between the changes in functional connectivity and the changes in cognitive performance in the controls (**Figure 3**).

The associations between the changes in the three functional connectivities and the changes in the HbA1c level as well as other hyperglycemia-related variables (fasting plasma glucose level, fasting serum C-peptide level, HOMA-%β, and HOMA-IR) were also investigated in patients with type 2 diabetes. However, no significant associations were found after adjusting for potential confounders (**Supplemental Table 4**).

DISCUSSION

Diabetes-associated cognitive dysfunction progresses extremely insidiously and is almost irreversible once the pathology is present in the brain (17). However, the current longitudinal study demonstrated an improvement in cognition in young type 2 diabetic patients with early stage disease after a mean follow-up of 18.0 months. Increased cognition abated baseline cognitive decrements of the diabetic patients, indicating a cognitive reversal.

Meanwhile, the enhanced functional connectivity at baseline was significantly decreased in the patients with type 2 diabetes, resulting in a level similar to that of the controls at the follow-up visit. Intriguingly, the decline in the connectivity of the left hippocampus with the left IPL was related to the increase in executive function, whereas the decrease in the connectivity of the PCC with the left IPL was related to the increase in MoCA. IPL is a major hub (critically important for information integration) of the frontoparietal control system (18) which is involved in processing a diverse range of higher cognitive functions (19). It suggests that the baseline functional hyperconnectivity could be normalized in response to improved cognition. Therefore, the early brain hyperconnectivity might serve as a biomarker for an important preclinical stage, or so-called "window period" for cognitive reversal. During the "window period," pathological changes in the brain are not accumulated. However, brain function is disturbed by risk factors such as hyperglycemia, which can be detected by brain connectivity.

Practice effects of repeated cognitive testing, which are often observed in cognitively healthy adults (20), might play a role in cognitive performance both in diabetic group and non-diabetic control. However, compared with controls, patients with diabetes had greater incremental rate of both MoCA score and RAVLT from baseline to follow-up visit. Therefore, the observed

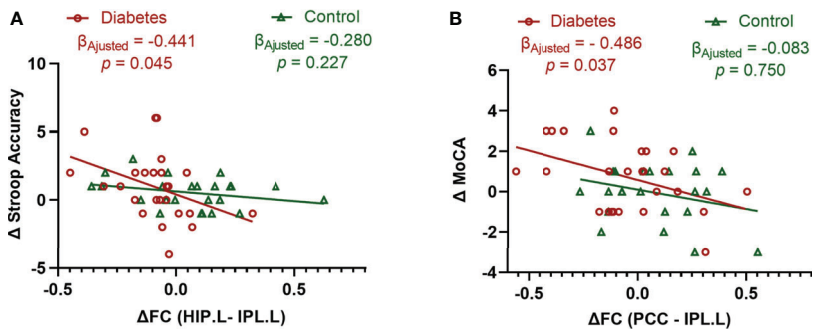

FIGURE 3 | Associations between the changes in cognitive performance and the changes in functional connectivity. **(A)** Significant association between the changes in Stroop Accuracy and the changes in the functional connectivity between the left hippocampus and left inferior parietal lobule in type 2 diabetic patients after adjusting for age, sex, education level, and follow-up interval. **(B)** Significant association between the changes in MoCA and the changes in the functional connectivity between the posterior cingulate cortex and left inferior parietal lobule in type 2 diabetic patients after adjusting for age, sex, education level, and follow-up interval. Δ is the value obtained after subtracting the baseline value from the follow-up value. Data exceeding 2.5 Standard Deviation were removed from the analyses (Analyses with original data are in **Supplemental Figure 1**). Stroop Accuracy, the accuracy of Stroop Color Word Test - part C; MoCA, Montreal Cognitive Assessment; HIP.L, left hippocampus; IPL.L, left inferior parietal lobule; PCC, posterior cingulate cortex; and FC, functional connectivity.

cognitive improvement in patients with type 2 diabetes was not exclusively attributed to practice effects.

Interestingly, patients with type 2 diabetes had higher MoCA than controls at the follow-up visit, indicating better global cognitive function in diabetic group. A possible explanation might be the neuroprotective effect of some antidiabetic agents (21) such as glucagon-like peptide-1 receptor agonist (22) and metformin (23), which were widely used in this study cohort at the follow-up visit. Recently, the REWIND trial (24), which also used MoCA as one of the two primary endpoints, revealed that glucagon-like peptide-1 receptor agonist might reduce cognitive impairment in patients with type 2 diabetes. Therefore, randomize controlled study is needed to investigate the relationship between the antidiabetic drugs, brain functional connectivity and cognitive outcome.

The mean HbA1c level moderated from 10% to 6.5% during the study period in patients with type 2 diabetes. However, no association was observed between the changes in the HbA1c level and the changes in brain functional connectivity. Moreover, no association between the changes in any other hyperglycemia-related variables (i.e., fasting plasma glucose level, fasting serum C-peptide level, HOMA-%β, and HOMA-IR) and the changes in brain connectivity was found. This result indicated that hyperglycemia-related variables in peripheral blood might not be sufficient to specifically reflect the brain activity. For example, the brain does not depend on insulin to use glucose, and the insulin action in the central nervous system is substantially different from that in peripheral tissues (25). Furthermore, some antidiabetic drugs might increase insulin sensitivity or improve glucose uptake in the brain (1). Therefore, in addition to lowering blood glucose, antidiabetic drugs might have a direct effect on brain functional connectivity. It further complicated the intricate relationship between hyperglycemia and brain functional connectivity. It is possible that no direct or specific link exists between peripheral hyperglycemia-related variables and brain function. Instead, fMRI signals are thought to be a valuable tool to unmask brain activity and are well related to cognitive changes. Therefore, in addition to concerns regarding early metabolic control in patients with type 2 diabetes, researchers should pay more attention to the changes in central nervous system (e.g., brain neuroimaging marker) in the future.

Regarding brain morphometry, no between-group differences (at both voxel and global levels) were observed at both the time points, supporting our previous hypothesis that functional changes in the brain occurred before structural changes (11). However, the stage at which substantial changes in brain structure occur during type 2 diabetes progression and effects of structural atrophy on cognitive dysfunction are unclear.

This study has several limitations. First, the current study was an observational study. Hence, whether cognitive reversal is related to a certain intervention is unclear. A well-designed randomized controlled study is required to elucidate whether the cognitive benefits are attributed to the "window period," to certain interventions, or both. Second, it is still unclear whether the preclinical stage would be present in the middle-aged to elderly population. Finally, the conclusions from this pilot study should be used with caution because relatively small number of subjects were included. A study with longer follow-up and larger sample size is required to verify the current results.

In conclusion, the current study demonstrates the recovery of cognitive function coupled with the normalization of brain functional hyperconnectivity. These findings suggest that diabetes-related cognitive dysfunction is not a one-way process. There might be a potential "window period" for cognitive reversal in the early stage of type 2 diabetes, and the enhancement of brain functional connectivity may serve as the marker.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Shanghai General Hospital Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FF researched data and wrote the manuscript. Y-JG performed cognitive testing. QL and R-BG performed MRI scanning. MK contributed to the statistical analysis of clinical data. M-MM detected the diabetic retinopathy. DM collected the clinical data. D-ZY and LZ oversaw MRI analyses. Y-FW and D-ZY designed the study, reviewed and edited the manuscript. Y-FW and D-ZY are the guarantors of this work. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2022.874538/full#supplementary-material

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EDITED BY
Neil James MacLusky,
University of Guelph, Canada

REVIEWED BY
Vadim Klimontov,
Institute of Cytology and Genetics (RAS), Russia
Lingling Xu,
Peking Union Medical College Hospital (CAMS), China

*CORRESPONDENCE
Stephen Olaide Aremu
arethomps@gmail.com

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Interhemispheric asymmetry of the brain in patients with type 1 diabetes mellitus and cognitive impairment

Yulia Gennadevna Samoilova[1], Mariia Vladimirovna Matveeva[1], Olga Sergeevna Tonkih[2], Dmitry Anatolievich Kudlay[3], Oxana Alekseevna Oleynik[1], Stephen Olaide Aremu[1]*, Oksana Yurievna Kilina[4], Alexander Federovich Kanev[5] and Olga Mihailovna Gerget[6]

[1]Department of Children Diseases, Siberian State Medical University, Tomsk, Russia, [2]Department of Tomographic Research Methods, Siberian State Medical University, Tomsk, Russia, [3]Department of Pharmacology, Ivan Mikhailovich Sechenov First Moscow State Medical University, Moscow, Russia, [4]Department of Internal Medicine, Katanov Khakass State University, Abakan, Republic of Khakassia, Russia, [5]Department of Internal Medicine with a Course of Therapy of Pediatrics Faculty, Siberian State Medical University, Tomsk, Russia, [6]Department of Information Technology of the Engineering School of Information Technology and Robotics, Tomsk Polytechnic University, Tomsk, Russia

With an ageing of population and a splurging epidemic of diabetes mellitus (DM), the prevalence of complications associated with pathology of the central nervous system are expected to increase, which in the future may have serious consequences for public health. It is known that one of the main manifestations of brain damage in type 1 diabetes is cognitive impairment, which is possibly associated with the peculiarities of vascularization and interhemispheric asymmetry, which requires in-depth analysis using modern neuroimaging methods. The aim of the study is to assess the symmetry of structural, metabolic and neurovascularization changes in the brain in patients with type 1 diabetes and cognitive impairment. The study included 120 patients with type 1 diabetes aged 18 to 45 years suffering from cognitive impairment, and 30 people without cognitive decline and the control group (n=30) healthy people without diabetes. Neuropsychological testing included the Montreal Cognitive Dysfunction Assessment Scale (MoCA test). For neuroimaging methods, standard magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), contrast and non-contrast-enhanced perfusion were used. Statistical processing was carried out using the SPSS Statistic 2020 software. In patients with type 1 diabetes with cognitive impairment, as manifested by impaired memory and/or attention, perfusion imaging revealed the presence of brain asymmetry zones. Standard MRI allowed to demonstrate changes in the white, gray matter and hippocampus in the right hemisphere. The results obtained were refined taking into account the topical localization, so during the perfusion study, regions with asymmetric blood flow were identified - namely, the white matter of the frontal lobe and the gray matter in the occipital lobe. Spectroscopy of the brain revealed that it was in

these areas of the brain that the most significant metabolic disorders were noted – in the form of significantly altered ratio of N-acetylaspartate (NAA)/ choline (Cho) on the left, along with the asymmetry in phosphocreatine level (Cr 2) on the right. In conclusion, early preclinical predictive diagnostics with the use of modern neuroimaging methods allows for timely detection of impaired vascularization and brain metabolism in this group of patients, However, decreased perfusion in the region within the region of frontal lobe white matter and temporal lobe grey matter, and hippocampal cell metabolism by spectra should be highlighted among the parameters Cr right and NAA/ Cho left.

KEYWORDS

type 1 diabetes mellitus, interhemispheric asymmetry, brain, cognitive impairment, neuroimaging

Introduction

Diabetes mellitus (DM) is a metabolic disease associated with the development of acute and chronic complications (1, 2). Among the complications of diabetes, relatively less attention is paid to cognitive impairments, which are verified in some patients with type 1 and 2 diabetes. (3). Interestingly, in the early 20th century, researchers and doctors recognized that diabetic patients often complain of poor memory and lack of attention. In 1922, Miles et al. have shown, examining memory and attention, that diabetic patients performed poorly on cognitive tasks (4). The term diabetic encephalopathy was introduced in 1950 to describe complications of diabetes associated with the central nervous system (5). Other terms, such as functional disorders of the brain and central neuropathy, have also been used in the literature to describe the cognitive dysfunctions associated with diabetes (6). Modern methods make it possible to non-invasively study the morphology and functioning of the brain in diabetic patients, determining the possibilities of predictive diagnostics.

Currently, there is a sufficient number of descriptive studies of the brain in type 1 diabetes, which describe the phenomenon of focal atrophy of the white and/or gray matter, more often the frontal, temporal and occipital lobes (7, 8). Another neuroimaging method is Magnetic resonance spectroscopy (MRS) - an advanced biochemical analysis technique that detects changes in metabolic neurochemical levels of metabolites in various areas of the brain *in vivo*. In type 1 diabetes, changes in the levels of NAA, Cho and the NAA/Cr (creatine) ratio are most often recorded (9–11). Considering the peculiarity of the development of microangiopathies in patients with type 1 diabetes, the assessment of cerebral perfusion also plays an important diagnostic role, allowing for the demonstration of changes in cerebral blood flow and other characteristics of the cortical and subcortical formations of the brain (12). Therefore, the use of these methods in a complex way allows us to clarify the features of morphofunctional associations in patients with cognitive impairments. At the same time, there are data in the literature that indicate a possible connection between the asymmetry of the cerebral hemispheres and various pathological conditions, which are not caused by genetic prerequisites (13, 14).

Thus, due to the lack of systematized data on the symmetry of brain structures, we formulated the aim of the study: to assess the symmetry of structural, metabolic and neurovascularization changes in the brain in patients with type 1 diabetes mellitus and cognitive impairments.

Methods

The study protocol was approved by the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education Siberian State Medical University of the Ministry of Health of Russia (conclusion No. 5265 of 05/02/2017). Inclusion criteria: patients with type 1 diabetes mellitus aged 18-35 years who have signed an informed consent. Exclusion criteria: other types of diabetes mellitus (type 2 or gestational diabetes mellitus), organic brain disease, psychiatric disorders, contraindications to MRI, glomerular filtration rate less than 60 ml/min, severe visual and hearing loss. The sample required for this study was calculated using IBM SPSS Sample Power software. The minimum sample size was 100 people. To increase the power of the sample size, taking into account the possibility of losing 10% of the data during the study, the sample level was 120 people. This number of participants will ensure the representativeness of the obtained sample and will allow extrapolating the obtained data to the population. A

computerized randomization was used to create a list from the diabetes registry for patient recruitment.

The study included 120 patients with type 1 diabetes with cognitive impairment. The control group (n = 30) was comparable in age (26 [23:39] years) and disease duration (13 [2:24] years). The control group consisted 30 healthy volunteers selected randomly. Screening for cognitive disorders was performed using the Montreal Cognitive Assessment Scale (MoCA test). The degree of cognitive impairment was established in strict accordance with generally accepted criteria, according to the classification of Academician of the Russian Academy of Medical Sciences (15), distinguishing between severe, moderate and mild cognitive impairments.

Standard MRI examination of the brain was performed in axial, sagittal and coronal projections using T2 (TR - time of repetition) 4932 ms, TE (Echotime) 90 ms, T1 (TR 280 MS, te 6.1 MS) modes, using the programs with free water signal suppression (Fluid Attenuated Inversion Recovery, FLAIR, TR 8000 ms, TE 105 ms, TI - time in version 2200 ms) on a Signa Creator "E" magnetic resonance scanner, GE Healthcare, 1.5 T, China.

Dynamic contrast MRI was performed, using Gadovist contrast agent administered as a 5 ml intravenous bolus with acquisition of images weighted by the inhomogeneity of the magnetic field (dynamic susceptibility contrast MR), as well as the technique of arterial spin labeling (ASL), which does not require the administration of contrast agent and allows one to quantify cerebral blood flow.

To process the MRI results, the FreeSurfer program was used, which is designed to analyze and visualize the structural and functional parameters of neuroimaging from cross-sectional or longitudinal studies, which was developed by the Computational Neuroimaging Laboratory at the Center for Biomedical Imaging (http://surfer.nmr.mgh.harvard.edu/). Proton spectroscopy of the brain was performed in a multivoxel mode; in the hippocampus region, the main spectra of NAA, Cho, Cr, and Cr2, as well as their ratios, were recorded.

Statistical analysis and data processing were performed using SPSS Statistica software version 25.0 on Windows 7/XP Pro operating systems. Methods of descriptive statistics were: mean value and standard deviation - for normally distributed data; quartiles - for non-normally distributed data. Qualitative binary signs were presented in the form of relative frequency (%) and its 95% confidence interval. Testing of statistical hypotheses of normally distributed quantitative parameters was performed using the following parametric criteria: Student's t - test for paired comparison (when assessing independent samples), Student's t - test for dependent data (dependent), analysis of variance for multiple comparisons. Correlation analysis was assessed using Pearson's criterion. Nonparametric criteria were used to test hypotheses for non-normally distributed quantitative parameters: Mann-Whitney (independent samples) and Wilcoxon (dependent samples), Kruskal-Wallace criteria for paired comparison. For correlation analysis - nonparametric Spearman criterion. To assess reliability of differences in qualitative characteristics, we used conjugation tables with the calculation of χ^2 (chi-square). The null hypothesis was rejected at the level of statistical significance $p < 0.05$.

Results

According to the study, in patients with type 1 diabetes, cognitive impairment was presented as mild in 50.8% (n = 61) of the patients, moderate in 40% (n = 48) and severe in 9.2% (n = 11). Neuropsychological testing data showed a decrease in the overall score of the MoCA test and lower scores on the tasks for attention (serial subtraction) and memory ($p < 0.001$).

The characteristics of the patients are presented in Table 1, the groups were comparable, with the exception of the level of fasting glycemia.

Next, we assessed the presence of microvascular complications in patients in both groups (Table 2). Differences in the incidence of retinopathy and nephropathy between groups were found.

Anamnesis analysis of patients with type 1 diabetes revealed the following concomitant thyroid diseases: autoimmune thyroiditis, nodular goiter in compensation stage - 6 (5%) cases in patients with cognitive impairment and 2 (6,6%) without, allergic reactions to food or medications - 12 (10%) and in 3 (10%) patients, gastrointestinal diseases (gallstone disease) and kidney diseases (chronic pyelonephritis) - 2 cases (1.6%) and in 1 (3,3%).

Patients with cognitive impairment were more likely to be on multiple insulin injections - 90 (75%), when in the group without cognitive impairment most received insulin on a pump regimen -24 (80%).

Factor analysis revealed that patients with type 1 diabetes and cognitive impairment were more often on artificial feeding 30% (n=36), regularly consumed coffee 85%(n=102) and alcohol 58,3% (n=70), smoked a third of all patients 40% (n=48), and half had higher education 50% (n=60) and in more than half of the cases had a full family 78,3% (n=94). Whereas patients with type 1 diabetes and without cognitive impairment were naturally feeding 73,3% (n=22), frequently consumed coffee 93,3% (n=28), and less than half smoked 40% (n=12), alcohol was consumed by 20% of the subjects (n=6), almost all of the patients had a college education 86,6% (n=26) and most had full families 83,3% (n=25).

Interhemispheric asymmetry according to standard brain MRI

Initially, the method of segmentation was used to assess the volume of brain structures, as a result of which the total volumes

TABLE 1 Characteristics of patients with type 1 DM (Me [Q1; Q3]).

Parameters	Patients with type 1 DM and cognitive impairment, n = 120	Patients with type 1 DM without cognitive impairment, n = 30	Patients without DM, n = 30	*P*
Age, years	27[18:45]	26 [23:39]	27 [23:39]	0,2
Disease duration, years	11 [1:32]	13 [2:24]	–	0,2
Fasting plasma glucose, mmol/l	9.1 [6.4:16.4]	7,9 [5.5:18.3]	4,9 [3.8:5.3]	0,05
HbA1c,%	7.6 [6:12.4]	6.9 [4.5:10.3]	4.9 [3.8:5.8]	0,2
Body weight index, kg/m^2	22.6 [17.4:30.6]	21.8 [16:30.4]	21.7 [18:29.3]	0,2

$p \leq 0,05$ – significant differences.

of white, gray matter, including the hemisphere and hippocampus, were obtained (Table 3).

In patients with type 1 diabetes and cognitive impairments, changes were noted in the volumes of white matter of both hemispheres, gray matter and the hippocampus on the right, compared to the patients from the control group, which indicate signs of atrophy in aforementioned areas.

Interhemispheric asymmetry according to contrast and non-contrast cerebral perfusion

We defined the null hypothesis as the absence of significant differences between the perfusion indices of the right and left cerebral hemispheres, while the alternative hypothesis is the presence of significant differences between the samples. To accept or reject this paradigm, the Mann-Whitney test was applied. Table 3 shows the values of the Mann-Whitney U-statistics for which the alternative hypothesis is accepted, that is, statistically significant differences between the samples are proved.

Thus, during contrast perfusion in the area of the white matter of the frontal lobe, asymmetry was observed in the parameter of the average time of blood passage. When assessing non-contrast perfusion, differences were shown in the main parameter of cerebral blood flow in the occipital lobe of the gray matter in patients with type 1 diabetes and cognitive impairment. Apparently, this is an example of how the processes of neuroplasticity are implemented, aimed at compensating for cognitive impairments (Figure 1).

Cerebral perfusion was lower in the group with moderate cognitive impairment than in patients with mild ($p \leq 0,009$), whereas no significant differences were recorded in the temporal lobe region (Table 4).

Interhemispheric asymmetry as assessed using proton spectroscopy of the brain

Based on the initial data of proton spectroscopy, a correlation matrix was built to check the presence of variables that have a high degree of connection with each other during proton spectroscopy of the brain (Table 5).

The figure shows that the relationship between the variables exists, but it is not pronounced enough to exclude any variable, since the maximum coefficient does not exceed 0.8. Therefore, based on the correlation matrix, no feature can be rejected as uninformative. The Kruskal-Wallis test was used to check the informativeness of the features (Table 6).

Based on the data in the table, we can conclude that for each variable the hypothesis of significance does not change at the significance level of 0.5, because the p-value does not exceed this figure. Figure 2 shows a diagram of the distribution of the importance of features

Figure 2 demonstrates that the least informative trait is Cr on the left, with the most informative being NAA/Cho on the left and Cr2 on the right. Thus, the asymmetry of the hippocampal region on the right is associated with a change in metabolism depicted as the change in the NAA/Cho ratio.

TABLE 2 Characteristics of patient groups according to the presence of microvascular complications of type 1 DM.

Complications	Patients with type 1 DM and cognitive impairment, n = 120	Patients with type 1 DM without cognitive impairment, n = 30	*P*
Angioretinopathy,%	75%	63,3%	0,045
Polyneuropathy,%	62,4%	70%	0,359
Nephropathy, %	45,8	20%	0,025

$p \leq 0,05$ – significant differences.

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TABLE 3 Brain segmentation in patients with type 1 DM.

Anatomic region	Patients with type 1 DM, n = 150		Patients without DM, n = 30	*P*
	With cognitive impairment, n = 120	Without cognitive impairment, n = 30		
Grey matter, mm^3	478009 [457669,1-511273,8]	497704 [442993,1-586559,6]	626496 [593716; 649388]	0,106
Grey matter, left hemisphere, mm^3	225046 [212392,9-232197,2]	252441 [224292,9-271860,4]	295734 [278564; 301837]	0,0004
Gray matter, right hemisphere, mm^3	235085 [219200,2-254713,2]	253587 [223200,3-270387,4]	298546 [278058;320759]	0,015
White matter, mm^3	455968 [421138,4- 473940,5]	503517 [440036,6- 509720,7]	659724 [6391765;689266]	0,005
White matter, left hemisphere, mm^3	232213 [217884,9-239910,2]	241831 [217516,8-264238,8]	359624 [338616;378166]	0,639
White matter, right hemisphere, mm^3	235509 [211609,8-237965,5]	270207 [228287,6-310513,8]	365926 [329764;387165]	0,046
Left hippocampus, mm^3	73 [72,1-73,4]	73 [72,9-74,8]	77,8 [76,8;79,2]	0,141
Right hippocampus, mm^3	72 [71,1-73,2]	73 [72,4-75,0]	78,3 [76,3;79]	0,005

$p \leq 0,05$ – significant differences.

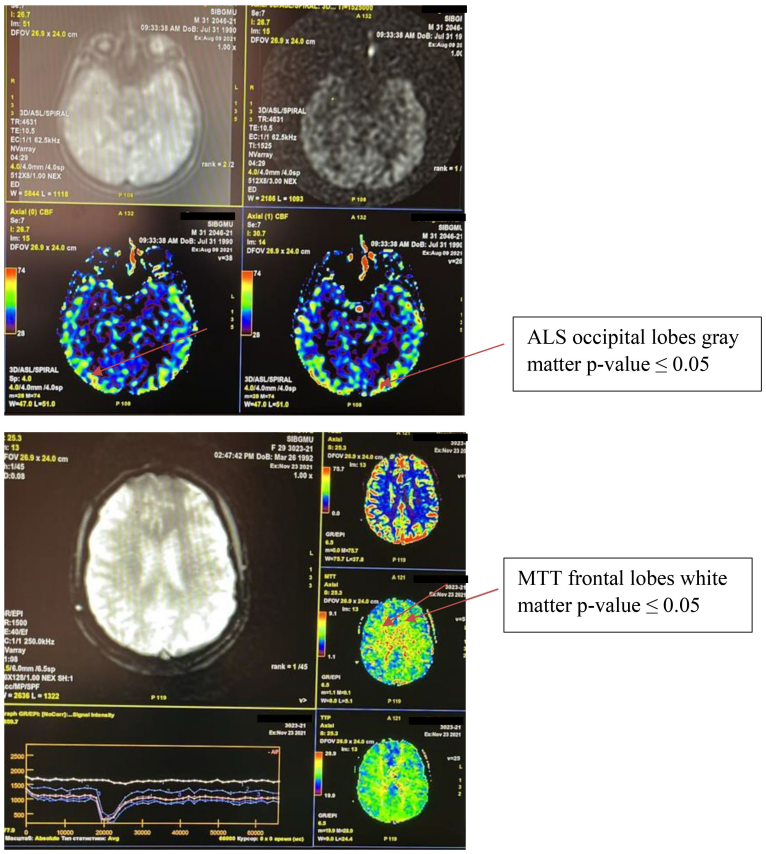

FIGURE 1
An image depicting an example of asymmetry perfusion in patent with diabetes type 1.

TABLE 4 Asymmetry of neurovascularization according to contrast and non-contrast perfusion in patients with type 1 diabetes mellitus and cognitive impairment.

Localization and indices of perfusion	U-statistics	P value
Frontal lobe, white matter, MTT/sec	201,5000	0,032032
Temporal lobe, grey matter, ASL/CBF	201,5000	0,032032

U-Mann-Whitney test, p-value ≤ 0.05; MTT, mean transit time; AS, arterial spin labeling - method of spin labeling of arterial blood; CBF, cerebral blood flow.

An analysis was performed during the study, which showed the following clinical features in patients who had interhemispheric asymmetry by all MRI methods of investigation: duration of disease more than 5 years, HbA1c more than 7.5% (and time in the target range less than 65%) and those who were on multiple insulin injections, by neuropsychological tests they usually had 2 domains of cognitive functions affected-attention and another of those presented (memory, visual-construction skills, speech fluency). These data confirm the importance of distinguishing risk groups of patients for the development of cognitive impairment and morpho-functional changes of the brain in diabetes mellitus especially at a young age.

Discussion

As a result of the study, it was revealed that type 1 diabetes mellitus is characterized by mild to moderate cognitive impairment with a predominance of impaired attention and memory. It should be noted that memory impairment in this type of diabetes has not been previously recorded, which may require the use of specific tests to understand the phenomenology of this result. These data contradict the results of many authors, who believe that with type 1 diabetes, the neurodynamic component of cognitive functions is mainly affected, namely the pace and the ability to concentrate (16), although there are studies that focus on memory impairment primarily associated with diabetes (17, 18).

For example, a study conducted by Weinger etal. (19) using magnetic resonance imaging as the diagnostic tool and with a focus on the cerebral white mater revealed that the participants who had type 1 diabetes had comparatively lower scores to the controls on one measure of executive function (Sorting Test), short-term memory, delayed recall, vocabulary, and psychomotor efficiency.

With regard to the issues of asymmetry of the cerebral hemispheres, many studies are devoted to the issues of

TABLE 5 Correlation matrix for checking the normality of parameters of proton spectroscopy of the brain.

	NAA left	NAA right	Cho left	Cho right	Cr left	Cr right	Cr2 left	Cr2 right	NAA/ Cr left	NAA/Cr right	NAA/ Cho left	NAA/Cho right	Cho/Cr left	Cho/Cr right
NAA left	1	0,5	0,01	-0,9	0,2	0,3	0,3	0,4	-0,1	0,02	-0,5	-0,2	-0,1	-0,4
NAA right	0,5	1	0,22	0,2	0,3	0,3	0,6	0,4	0,1	0,2	-0,3	-0,4	-0,0	-0,4
Cho left	0,01	0,22	1	0,4	0,2	0,4	0,1	0,1	0,7	0,7	0,4	0,2	0,6	-0,2
Cho right	-0,9	0,2	0,4	1	-0	-0	0,1	-0,1	0.1	0,1	0	0,1	0,45	0,4
Cr left	0,2	0,3	0,2	-0	1	0,3	0,1	0,2	0,3	0,1	-0	0	-0,3	-2,3
Cr right	0,3	0,3	0,4	-0	0,3	1	0,1	0,4	0,6	0,6	0,1	0,2	0,1	-0,5
Cr2 left	0,3	0,6	0,1	0,1	0,1	0,1	1	0,5	-0,1	0	-0,4	-0,4	0	-0,3
Cr2 right	0,4	0,4	0,1	-0,1	0,2	0,4	0,5	1	0,16	0,15	-0,1	-0,1	0	-0,3
NAA/Cr left	-0,1	0,1	0,7	0,1	0,3	0,6	-0,1	0,16	1	0,8	0,65	0,4	0,4	-0,2
NAA/Cr right	0,02	0,2	0,7	0,3	0,1	0,6	0	0,15	0,8	1	0,35	0,47	0,6	-0,3
NAA/Cho left	-0,5	-0,3	0,4	0	-0	0,1	-0,4	-0,1	0,65	0,35	1	0,5	0,2	0,3
NAA/Cho right	-0,2	-0,4	0,2	0,1	0	0,2	-0,4	-0,1	0,4	0,47	0,5	1	0,2	0,4
Cho/Cr left	-0,1	-0,0	0,6	0,45	-0,3	0,1	0	0	0,4	0,6	0,2	0,2	1	0,1
Cho/Cr right	-0,4	-0,4	-0,2	0,4	-2,3	-0,5	-0,3	-0,3	-0,2	-0,3	0,3	0,4	0,1	1

NAA, N-acetylaspartate; Cho, choline; Cr, creatine; Cr2, phosphocreatine.

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TABLE 6 Values of the Kruskal-Wallis rank test for parameters of proton spectroscopy of the brain of patients with type 1 diabetes mellitus.

Parameter	Statistics	p-value
NAA left	22,62228	0,0001
NAA right	26,53589	0,0003
Cho left	28,52084	0,0000
Cho right	14,18308	0,0027
Cr left	5,721290	0,1260
Cr right	33,81858	0,0000
Cr2 left	29,96852	0,0002
Cr2 right	34,03594	0,0001
NAA/Cr left	33,19992	0,0000
Naa/Cr right	31,45471	0,0001
NAA/Cho left	34,10368	0,0004
Naa/Cho right	33,72973	0,0001
Cho/Cr left	23,58656	0,0000
Cho/Cr right	18,70439	0,0003

NAA, N-acetylaspartate; Cho, choline; Cr, creatine; Cr2, phosphocreatine.

sensorimotor integration (20, 21). On the other hand, the different functioning of the cerebral hemispheres is an important phenomenon in injury, employing the rehabilitation potential and neuroplasticity of the central nervous system (22). The only large meta-analysis in neurology on hemispheric asymmetry was presented in 2019, consisting of 159 publications on voxel-based morphometry (registration of 4469 patients and 4307 controls), showing that asymmetry does exist in neurodegenerative diseases. Regions with asymmetric brain decline were located in areas primarily affected by neurodegeneration. Thus, with moderate cognitive impairment, the region of the right hippocampus is most vulnerable (23).

The data obtained in this study deserve attention from the perspective of preventive medicine, since early preclinical diagnosis of cognitive impairment and related dysfunctions, including microangiopathies, can reduce health care costs and improve the quality of life of patients with type 1 diabetes. Information on non-invasive techniques seems promising: non-contrast perfusion, in which the area of interest is the gray matter of the occipital lobes; and proton spectroscopy of the hippocampus, the informative signs for which are the NAA/Cho ratio on the left and the Cr2 content on the right.

The study isn't without limitations. For instance, the associations used in this type of study does not allow for the assessments of the direction of causality, however the long duration diabetes in the participants makes causation in the reverse direction less probable. Furthermore, the study has relatively low power given the limited sample size; hence, an expansion of the patient sample is required in further studies. It's

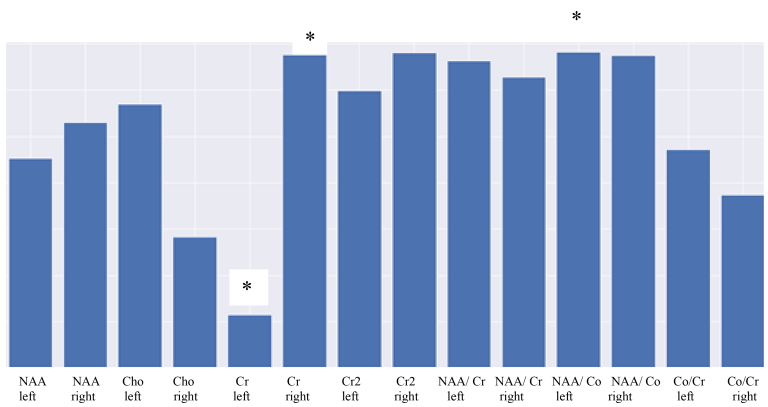

FIGURE 2
Diagram showing the significance of MRS parameters highlighting the importance of features. * - statistical significance ($p \leq 0,05$).

also important that longitudinal studies are planned to confirm the findings of the study and to determine whether they can predict cognitive impairment and associated neuroimaging changes as well as related dysfunctions, including microangiopathies, in type 1 diabetes mellitus. The strengths of the study include the study sample source, the holistic nature of the cognitive assessment that included clinical and neuropsychological assessments and the neuroimaging methodology that comprise of updated, advanced, automated volume measures that gave precise and accurate measures and assessments (24)

Conclusion

In patients with type 1 diabetes with a disease duration of more than 10 years, the neurodynamic type of cognitive impairment can turn into cortical-subcortical one, taking into account the topical localization of the revealed changes. Asymmetry of the hemispheres is characteristic of patients with type 1 diabetes and cognitive impairment. Early preclinical predictive diagnostics with the use of modern neuroimaging methods allows for timely detection of impaired vascularization and brain metabolism in this group of patients.

Thus, asymmetry according to various methods may be key in the predictive diagnosis of cognitive impairment in type 1 diabetes mellitus. Asymmetry in the volume of gray, white matter of the brain and hippocampus, decreased perfusion in the region within the region of frontal lobe white matter and temporal lobe grey matter, and hippocampal cell metabolism by spectra should be highlighted among the parameters Cr right and NAA/Cho left.

However, further work is needed to validate the findings and provide a better understanding of the functional role of interhemispheric asymmetry, for example, in the context of cognitive reserve and compensation.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by The study protocol was approved by the Ethics Committee of the Federal State Budgetary Educational Institution of Higher Education Siberian State Medical University of the Ministry of Health of Russia (conclusion No. 5265 of 05/02/2017). The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Ben Nephew,
Worcester Polytechnic Institute, United States

REVIEWED BY
Essam Mohamed Elmatbouly Saber,
Benha University, Egypt
Pengpeng Ye,
Chinese Center For Disease Control and Prevention, China

*CORRESPONDENCE
Shihong Chen
chenshihong26@163.com
Xianghua Zhuang
sd73095760653@163.com

†These authors have contributed equally to this work

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Correlation of mild cognitive impairment with the thickness of retinal nerve fiber layer and serum indicators in type 2 diabetic patients

Renshi Li†, Fengjie Zheng†, Peichen Xu, Li Lv, Yapeng Mu, Xianghua Zhuang* and Shihong Chen*

Department of Endocrinology and Metabolism, Multidisciplinary Innovation Center for Nephrology of the Second Hospital of Shandong University, The Second Hospital of Shandong University, Ji'nan, Shandong, China

Background: Cognitive Impairment arising from type 2 diabetes mellitus (T2DM) has garnered significant attention in recent times. However, there are few studies on the identification and diagnosis of markers of cognitive impairment. Notably, alterations in the Retinal Nerve Fiber Layer's (RNFL) thickness can potentially serve as an indicative measure of central nervous system changes. Further investigations have indicated that the decline in cognitive function within T2DM patients is intricately linked to persistent systemic inflammation and the accumulation of advanced glycosylation end products. Comprehensive studies are warranted to unveil these complex associations.

Objective: This study aims to explore the potential of utilizing the RNFL thickness and serological concentrations of IL-18, irisin, CML, and RAGE as diagnostic indicators for Mild Cognitive Impairment (MCI) among individuals with T2DM.

Methods: The thickness of RNFL were determined in all patients and controls using optical coherence tomography (OCT). The serum levels of IL-18, irisin, CML and RAGE were detected by ELISA kit. In addition, Cognitive assessment was performed by the Mini-Mental State Examination (MMSE) and the Montreal Cognitive assessment (MoCA).

Results: The average RNFL thickness in the right eye were decreased in T2DM and T2DM combined with MCI (T2DM-MCI) patients and were positively correlated with MoCA and MMSE scores. The serum levels of IL-18, CML and RAGE in T2DM and T2DM-MCI increased significantly ($p<0.05$) and were negative correlated with MoCA and MMSE scores. The level of irisin in T2DM and T2DM-MCI decreased significantly ($p<0.05$) and were positively correlated with MoCA and MMSE scores. The area under the ROC curve of T2DM-MCI predicted by the average RNFL thickness in the right eye, CML and RAGE were 0.853, 0.874 and 0.815. The diagnostic efficacy of the combination of average RNFL thickness in the right eye, CML, and RAGE for the diagnosis of T2DM-MCI was 0.969.

Conclusion: The average RNFL thickness in the right eye, CML and RAGE have possible diagnostic value in T2DM-MCI patients.

KEYWORDS

type 2 diabetes mellitus, the thickness of retinal nerve fiber layer, interleukin-18, irisin, carboxymethyl lysine, receptors for AGEs, mild cognitive impairment

Introduction

Diabetes Mellitus (DM) and cognitive impairment are prevalent conditions among older individuals. DM, a metabolic disorder with multiple underlying causes, is characterized by chronic hyperglycemia and stands as one of the fastest-growing diseases. Its global prevalence is expected to affect around 693 million adults by 2045, making it a significant health concern (1). In 2019, statistics indicated that approximately 1 in 11 individuals had DM, with a global total of 463 million cases, out of which about 90% were classified as Type 2 diabetes mellitus (T2DM) (2). DM's complications are diverse, encompassing cardiovascular, ocular, renal, and neuropathic issues, as well as disorders of the immune system.

Cognitive impairment resulting from T2DM has garnered significant attention in recent times. This impairment can be categorized into two main classifications: dementia and mild cognitive impairment (MCI). Research has unequivocally demonstrated a connection between T2DM and cognitive decline. Individuals afflicted with T2DM are inherently predisposed to a heightened risk of developing both MCI and full-fledged dementia when compared to those without diabetes (3). In the advanced stages of dementia, the observable symptoms in patients are conspicuous. However, treatment options remain limited. Consequently, this situation places a substantial burden not only on the affected families but also on society as a whole. Therefore, the imperative of identifying early markers for dementia diagnosis and implementing timely interventions has become more pressing than ever before.

Contemporary research underscores the intricate mechanisms underlying cognitive impairment in DM, encompassing disrupted lipid metabolism, inflammatory responses, mitochondrial dysfunction, and oxidative stress. These cumulative factors culminate in a diminished frequency of neuronal activity, the apoptotic demise of nerve cells, anomalous central nervous system integration, distorted information processing, degenerative necrosis, and the demyelination of nerve cells and fibers (4). Of note, inflammation emerges as a pivotal player in the pathogenesis of cognitive impairment in DM. Consequently, our endeavors have been directed towards the identification of pertinent serological indicators for the diagnosis of T2DM in conjunction with MCI, with a specific focus on the inflammatory dimension.

Currently, MCI can be diagnosed by magnetic resonance imaging, positron emission tomography, cerebrospinal fluid (CSF) tests and neuropsychological assessment. But these tests have the disadvantages of being costly, invasive, insensitive and specific. However, to our knowledge, there are no reports of observed changes in RNFL thickness in T2DM-MCI patients. Exploring potential markers for MCI in T2DM is critical to better comprehend its pathogenesis and monitor disease progression. Considering that the potential relation between RNFL degeneration and cognitive deterioration in T2DM remains undiscovered, it would be valuable and fascinating to investigate whether RNFL thickness could become a potential indicator for MCI in T2DM. The aim of this study was to first determine thickness of RNFL in T2DM and T2DM-MCI patients, and to further clarify the correlation between RNFL thickness, concentrations of serum IL18, irisin, CML, RAGEs and cognitive function in T2DM-MCI patients.

Materials and methods

Patients

A total of 108 patients diagnosed with T2DM, who were hospitalized in the Endocrinology Department of the Second Hospital of Shandong University between June 2021 and December 2021, were selected for this study. Among them, there were 60 T2DM patients without MCI (34 males and 26 females), and 48 T2DM-MCI patients (34 males and 26 females). Additionally, 23 individuals were recruited as a normal control group (8 males and 15 females). The study was approved by the Ethics Committee of the Second Hospital of Shandong University. Every participant was required to provide a written consent for this study to allow investigators to measure their clinical status. This study has been engaged in the clinical trial registration at http://www.chictr.org.cn/showproj.aspx?proj=186818. And the clinical trial registration number is ChiCTR2300070232.

Inclusion and exclusion criteria

Inclusion criteria were: 1. Control group: Fasting blood glucose <6.1mmol/l and blood glucose <7.8mmol/l after 2h of Oral Glucose Tolerance Test (OGTT). A score of ≥26 on the Montreal Cognitive assessment (MoCA) and ≥27 on the Mini-Mental State

Examination (MMSE). Other acute and chronic diseases were excluded. 2.T2DM group: According to the 2019 WHO diagnostic criteria for diabetes: (1) DM is typically characterized by the symptoms of "three excesses and one deficit (excessive drinking, excessive urination, excessive eating and weight loss)", random blood glucose level ≥11.1mmol/l. (2) Fasting blood glucose level ≥ 7 mmol/l. (3) OGTT: blood glucose ≥11.1mmol/l after 2h of OGTT. (4) Glycated hemoglobin (HbA1c) ≥6.5%. Patients are diagnosed with diabetes when any of these criteria are met within 2 consecutive days, while excluding type 1 diabetes, gestational diabetes and specific types of diabetes. And the MoCA score ≥ 26 and MMSE score ≥ 27. 3.T2DM-MCI group: Meets the 2019 WHO criteria for diagnosis of DM. And MoCA score is of 18-26 and MMSE score is of 21-27. 4. In all three groups above: best corrected visual acuity ≥ 0.2. Diopter requirements: Spherical lenses -3.00S∼+3.00S, cylindrical lenses -3.00C∼+3.00C, refractive error ≤ 2D in both eyes. IOP <21mmHg in both eyes on all three measurements.

Subjects with any of the following conditions were excluded: 1.Diseases affecting the optic nerve such as glaucoma, retinal nerve fiber layer disorders, high myopia and a history of eye surgery within six months. 2. Other neurodegenerative diseases that may cause changes of the retinal nerve fiber layer, such as multiple sclerosis. 3. Refractive interstitial clouding is evident. 4. Heart failure, malignant hypertension, acute myocardial infarction, stroke, history of renal failure, severe cardiac arrhythmias. 5.Presence of other neurological disorders that cause cognitive impairment, such as brain tumors, Parkinson's, encephalitis, epilepsy, traumatic brain injury, etc. 6.Presence of other conditions that can cause cognitive impairment, such as hypothyroidism, severe anemia, folic acid and vitamin B12 deficiency, syphilis, AIDS, alcohol and drug abuse, etc. 7.Are using medications that affect cognition, such as sedatives, anti-anxiety medications, hypnotic medications, etc. 8.Those who are unable to cooperate with the inspection.

Research methodology

Data collection

(1) General information: Inquire about and record the name, gender, age, hospitalization number, duration of T2DM, education level, previous medical history, family history of the individuals. (2) Basic measurements: height, weight, Body Mass Index (BMI), systolic blood pressure, diastolic blood pressure. (3) Biochemical parameters: fasting blood glucose, HbA1c, triglycerides, total cholesterol, high density liptein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), estimated glomerular filtration rate (eGFR), liver function, uric acid.

Cognitive function

Cognitive function assessments were conducted by the same physician for all participants, utilizing both the MMSE and the MoCA. The cognitive evaluations for the enrolled patients were carried out on the same day as the blood sample collection.

OCT image acquisition

All participants underwent bilateral OCT examinations conducted by experienced ophthalmologists from the Second Hospital of Shandong University, and the doctors were unaware of the grouping of participants. The peripapillary region surrounding the optic disk was segmented into four parts, being, superior, inferior, temporal and nasal sectors. The global RNFL thickness represents the average thickness of these four sectors.

Testing of serological indicators

Morning fasting blood samples were taken from all participants following a fasting period of 8-10 hours. These samples were then placed in specialized inert separator gel procoagulation tubes. Subsequently, they underwent centrifugation at a speed of 3000 revolutions per minute for a duration of 10 minutes. The resulting supernatant, meticulously isolated, was then stored within a temperature-controlled environment at -80°C until the time of analysis.

Data analysis

The required sample size for this study was calculated using the PASS 15 software. The specific parameter settings are as follows: The One-Way Analysis of Variance model was selected, and the significance level α was set to 0.05, and the power of the test (1-β) was set to 0.90. The Number of Groups was set to 3, and Hypothesized Means were set as 110 80 70. The Standard Deviation of Subjects was set to 15. After calculation, the study requires a sample size of 15 individuals. Considering a dropout rate of 20%, each group should include a minimum of 18 patients. All experimental data were statistically analyzed using SPSS 25.0. Means ± standard deviations (x±s) were used for measures meeting normal distribution, and one-way ANOVA was used for data comparison between the three groups. Independent samples *t*-test and paired *t*-test were used for data comparison between the two groups. Non-normally distributed measures were expressed using median and interquartile spacing, and data were compared between the three groups using non-parametric tests and between the two groups using the Mann-Whitney rank sum test. Frequency, percentages or composition ratios were used to describe the count data, and the chi-square test was used for comparison between groups. Correlation analysis was performed using Pearson correlation analysis for normally distributed measures and Spearman for non-normally distributed measures. Binary logistic regression was applied to analyze the independent risk factors and protective factors for MCI in patients with T2DM. Analysis of the diagnostic efficacy of specificity indicators for MCI in patients with T2DM using ROC curves. A *p*-value of less than 0.05 was considered statistically significant.

Results

The study encompassed a cohort of 23 normal controls, 60 T2DM patients, and 48 T2DM-MCI patients. However, for various reasons such as suboptimal image quality, imaging artefacts, or the

presence of macular oedema in OCT imaging, a total of 5 normal controls, 19 T2DM patients, and 12 T2DM-MCI patients were excluded from the subsequent analysis. Therefore, the final dataset subjected to analysis consisted of 18 normal controls, 41 T2DM patients, and 36 T2DM-MCI patients.

Clinical information for each group

As depicted in Table 1, notable statistically significant differences were observed among the three groups with respect to gender, years of education, diastolic blood pressure, blood glucose, HbA1c, and BMI ($p<0.05$). Conversely, when comparing the T2DM group with the T2DM-MCI group, no statistically significant disparities were evident in terms of gender, years of education, diastolic blood pressure, blood glucose, HbA1c, and BMI ($p<0.05$).

RNFL thickness, serum marker concentrations in male and female

As presented in Table 2 and Table 3, there was no difference in RNFL thickness, serological indicator concentrations between male and female ($p>0.05$).

RNFL thickness of the study subjects

As illustrated in Table 4 and Figure 1, the average RNFL thickness of four quadrants and RNFL thickness in each quadrant of the right eye exhibited notable disparities among the three participant groups ($p<0.05$). When compared to the control group, differences in RNFL thickness within each quadrant and the average RNFL thickness in T2DM patients were statistically insignificant for the left eye (all $p>0.05$). However, these measurements were notably reduced in the temporal quadrant and the average RNFL thickness in the right eye (all $p<0.05$). Furthermore, the T2DM-MCI group displayed reduced average RNFL values and RNFL thickness in each quadrant of the right eye (all $p<0.05$) in comparison to controls. When comparing the T2DM group with the T2DM-MCI group, the latter exhibited significant thinning in the lower left eye, each quadrant of the right eye, and the average RNFL thickness (all $p<0.05$).

Serum index concentration of each group

Table 5 presents the concentrations of serological indicators within each group. Statistically significant differences were observed in the concentrations of IL-18, irisin, CML, and RAGE among the

TABLE 1 Basic information of the study subjects.

	Control (n=18)	T2DM (n=41)	T2DM-MCI (n=36)	*p*
Gender (Male)	7(38.9%)	27(65.9%)[a]	23(63.9%)[a]	**0.016**
Age (year)	53.78±12.82	56.93±9.22	59.78±7.68	0.088
Diabetes history (year)	–	9.90±7.41	10.04±6.86	0.932
years of education (year)	14.72±5.69	9.34±3.64[a]	8.78±2.26[a]	**<0.001**
Systolic pressure (mmHg)	129.22±3.62	134.46±15.89	131.86±15.37	0.408
Diastolic blood pressure (mmHg)	77.33±3.91	85.78±10.94[a]	83.36±8.82[a]	**0.007**
BMI (kg/m^2)	23.41±0.63	26.10±3.54[a]	26.25±3.45[a]	**0.005**
Blood glucose (mmol/l)	5.48±0.34	9.56±2.84[a]	8.92±2.78[a]	**<0.001**
HbA1c(;%)	5.46±0.27	8.60±1.51[a]	8.75±2.13[a]	**<0.001**
ALT(U/L)	22.00(13.50-27.00)	18.00(12.00-24.50)	18.00(12.00-26.00)	0.755
AST(U/L)	17.00(15.50-22.50)	18.00(15.00-23.00)	18.50(13.00-22.25)	0.619
Triglycerides (mmol/l)	1.13±0.58	1.70±1.64	1.56±0.97	0.277
Total cholesterol (mmol/l)	4.51±0.89	4.79±1.67	4.20±1.38	0.202
HDL-L (mmol/l)	1.31±0.31	1.23±0.55	1.17±0.38	0.575
LDL-C (mmol/l)	2.43±0.76	2.84±0.90	2.52±1.02	0.186
Urine (mmol/l)	5.61(4.83-5.98)	5.60(4.90-6.60)	5.80(4.90-6.90)	0.526
Creatinine (μmol/l)	70.78±7.45	72.52±16.68	75.41±23.64	0.661
Uric acid (μmol/l)	271.97±60.25	302.47±74.87	291.85±82.07	0.385
eGFR (ml/min/1.73m^2)	91.90±13.64	95.39±19.00	89.77±21.40	0.447

[a]versus control, $p<0.05$.
[b]versus T2DM, $p<0.05$. Significant P values (<0.05) are indicated in bold.

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TABLE 2 RNFL thickness in males and females.

	Male	Female	*p*
RNFL thickness in left eye(μm)			
Superior	133.33±18.68	141.58±48.01	0.244
Inferior	124.88±18.22	129.55±22.12	0.264
Nasal	65.93±14.37	71.39±14.94	**0.077**
Temporal	78.12±19.11	81.79±33.75	0.501
Global	101.04±11.25	106.24±23.44	0.151
RNFL thickness in right eye(μm)			
Superior	127.65±19.22	129.79±15.99	0.572
Inferior	124.28±23.03	132.24±21.00	0.091
Nasal	69.77±13.57	74.32±21.22	0.206
Temporal	81.55±21.57	88.66±29.96	0.213
Global	101.02±13.29	106.45±14.25	0.061

Significant P values (<0.05) are indicated in bold.

groups encompassing T2DM, T2DM-MCI, and the control subjects. When compared to the normal control group, IL-18, CML, and RAGE exhibited significant increments in both the T2DM and T2DM-MCI groups, while the levels of irisin showed a noteworthy reduction. Furthermore, in comparison to the T2DM group, the concentrations of IL-18, CML, and RAGE in the T2DM-MCI group experienced a substantial increase, concomitant with a marked decrease in irisin levels.

Correlation analysis of Moca, MMSE and other indicators

Correlation analysis of clinical features, RNFL thickness, IL-18, irisin, CML, RAGE with MoCA and MMSE was performed in Table 6. Average RNFL thickness, age, years of education, HbA1c, total cholesterol, LDL-C, irisin, CML, IL-18, and RAGE were significantly associated with MoCA and MMSE.

Logistic regression analysis of MCI in T2DM patients

We employed two distinct models in our study to investigate the associations between RNFL thickness, IL-18, CML, RAGE, and irisin levels, and their relationship to the presence of MCI in patients with T2DM (refer to Table 7). Given the known correlations between cognitive function and variables such as age, years of education, HbA1c, total cholesterol, and LDL-C, we initially addressed logistic regression analysis in model 1. This allowed us to correct for the influence of age and years of education, thus enabling a focused exploration of the links between MCI and the average RNFL, IL-18, irisin, CML, and RAGE in T2DM patients. Our findings revealed significant associations. Specifically, we observed that the average RNFL thickness in the right eye was inversely related to the presence of T2DM with MCI (OR=0.699, CI: 0.532-0.920, p=0.011). Moreover, CML (OR=1.011, 95% CI: 1.001-1.021, p=0.030) and RAGE (OR=1.007, 95% CI: 1.001-1.013, p=0.017) levels in the right eye were positively associated with T2DM patients having MCI. Specifically, higher average RNFL thickness in the right eye appeared to be a protective factor, while elevated levels of CML and RAGE emerged were as risk factors for MCI in this context. To account for potential confounding effects stemming from other variables, we conducted further adjustments in model 2, incorporating HbA1c, total cholesterol, and LDL-C. Our results from this analysis continued to highlight significant relationships. Notably, the average RNFL thickness in the right eye remained a protective factor against the presence of T2DM with MCI (OR=0.695, 95% CI: 0.521-0.928, p=0.013). Conversely, CML (OR=1.012, 95% CI: 1.001-1.022, p=0.037) and RAGE (OR=1.007, 95% CI: 1.002-1.012, p=0.007) levels remained as identified risk factors associated with MCI in T2DM patients, even after accounting for the effects of HbA1c, total cholesterol, and LDL-C. Upon examination, the goodness-of-fit for both models were satisfactory.

TABLE 3 Serological indicator concentrations in males and females.

	Male	Female	*p*
Irisin(ng/ml)	34.97(9.34-87.34)	27.85(9.79-66.56)	0.682
CML(pg/ml)	175.32(98.99-324.76)	204.02(83.52-285.28)	0.805
IL-18(pg/ml)	46.74(16.60-171.19)	60.07(16.66-183.89)	0.571
RAGE(pg/ml)	436.30(239.00-703.31)	439.07(266.46-616.01)	0.878

Right eye RNFL thickness, CML, RAGE predict MCI in T2DM patients

After adjustment for confounding factors, it was found that average thickness of RNFL, CML and rage in the right eye were still related to cognitive function. In Figure 2, ROC curve was used to analyze the diagnostic efficacy of these three indicators for MCI in T2DM. The area under the ROC curve of average RNFL thickness in the right eye for MCI in T2DM was 0.853, the sensitivity was 91.7%, and the specificity was 61.0%. The area under the ROC curve of CML was 0.874, the sensitivity was 82.9%, and the specificity was 90.2%. The area under the ROC curve of RAGE was 0.815, the sensitivity was 68.6%, and the specificity was 82.9%. In order to further explore the significance of the combined diagnosis of OCT and serological indexes, firstly, the average RNFL thickness and CML of the right eye were used as diagnostic indexes to observe whether the combined diagnosis of the two could improve the diagnostic efficacy of MCI in patients with T2DM. The diagnostic efficacy of the combined diagnosis of the two was 0.948 (sensitivity 88.6%, specificity 95.1%), which was significantly higher than that of RNFL thickness and CML of the right eye. The diagnostic efficacy was significantly higher than that of RNFL thickness and CML in the right eye. Next, the diagnostic efficacy of the combined diagnosis

TABLE 4 RNFL thickness in different groups.

	Control	T2DM	T2DM -MCI	*p*
RNFL thickness in left eye (μm)				
Superior	139.78±11.26	137.17±14.98	134.44±52.06	0.855
Inferior	131.61±25.94	131.17±15.07	119.28±19.59[b]	**0.015**
Nasal	71.33±16.13	68.07±13.26	66.56±15.83	0.538
Temporal	75.06±15.30	80.05±12.60	81.33±38.58	0.699
Global	104.50±9.14	104.37±8.98	101.00±25.70	0.652
RNFL thickness in right eye (μm)				
Superior	133.33±14.08	134.68±13.40	119.06±20.35[a,b]	**<0.001**
Inferior	141.83±18.62	133.83±15.56	113.03±23.29[a,b]	**<0.001**
Nasal	81.06±25.79	72.95±12.09	65.31±14.32[a,b]	**0.004**
Temporal	111.59±43.43	82.90±14.45[a]	73.33±11.15[a,b]	**<0.001**
Global	115.56±15.94	106.34±7.65[a]	93.42±11.74[a,b]	**<0.001**

[a]versus control, p<0.05.
[b]versus T2DM, p<0.05. Significant P values (<0.05) are indicated in bold.

of average RNFL thickness and RAGE in the right eye was observed, and it was found that the diagnostic efficacy of the combined diagnosis of the two (0.939) was higher than that of RNFL thickness and RAGE in the right eye. Finally, the diagnostic efficacy of the combined diagnosis of RNFL thickness, CML, and RAGE in the right eye was found to be significantly higher (0.969). (Table 8, Figure 3).

Discussion

Under physiological conditions, the curves depicting RNFL thickness exhibit a distinctive "bimodal" configuration. This intriguing pattern manifests with the superior and inferior RNFL thickness surpassing that of the nasal and temporal regions. Notably, the superior and inferior nerve fibers exhibit the highest density, rendering the RNFL in these two quadrants particularly vulnerable to initial damage. In our current investigation, the RNFL thickness profiles of the three patient groups conspicuously displayed this intriguing "bimodal" attribute. Furthermore, a comprehensive retrospective and meta-analytic inquiry unveiled compelling findings. It discerned a significant reduction in the average RNFL thickness among patients afflicted with Alzheimer's Disease (AD) when compared to their cognitively intact counterparts. In AD patients, all quadrants exhibited a pronounced thinning of the RNFL. Intriguingly, a discernible trend towards a thinner RNFL was also observable among patients with MCI. However, it is noteworthy that the extent of this thinning in MCI patients did not reach statistical significance (5). In a community-centered investigation encompassing a substantial cohort of individuals in good health, those devoid of substantial cognitive impairment but exhibiting thinner RNFL measurements at the study's onset were observed to face an elevated risk of experiencing diminished performance on subsequent cognitive assessments (6). The investigation encompassed measurements of peripapillary RNFL thickness using OCT in both AD and MCI patients. The findings revealed a noteworthy pattern of thinning in the RNFL, with particular prominence in the upper quadrant, observed in both AD and MCI patients. Moreover, AD patients exhibited thinning not only in the upper quadrant but also in the lower quadrant when compared to the control group (7). In the initial stages of AD, a discernible pattern emerges involving a selective reduction in the RNFL, which is predominantly localized in the upper quadrant. However, as AD advances, the degenerative process extends beyond the upper quadrant, and also affects the lower quadrant of the

TABLE 5 Serum index concentrations of the three groups.

	Control	T2DM	T2DM-MCI	*p*
IL-18(pg/ml)	13.86(11.30-15.58)	32.00(19.83-157.62)[a]	136.36(80.95-407.87)[a,b]	**<0.001**
Irisin(ng/ml)	116.22(77.00-159.01)	32.81(9.07-81.67)[a]	15.69(4.71-31.61)[a,b]	**<0.001**
CML(pg/ml)	16.02(5.08-35.30)	180.09(132.51-237.05)[a]	322.43(277.33-583.87)[a,b]	**<0.001**
RAGE(pg/ml)	193.79(109.40-263.86)	433.02(284.18-566.38)[a]	703.29(475.96-1117.90)[a,b]	**<0.001**

[a]versus control, p<0.05.
[b]versus T2DM, p<0.05. Significant P values (<0.05) are indicated in bold.

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TABLE 6 Correlation between Moca, MMSE and other variables.

	MoCA		MMSE	
	r	p	r	p
inferior quadrant in left eye (μm)	-0.157	0.129	-0.374	**<0.001**
superior quadrant in left eye (μm)	0.163	0.115	0.113	0.275
nasal quadrant in left eye (μm)	-0.074	0.475	-0.132	0.203
temporal quadrant in left eye (μm)	-0.251	**0.014**	-0.417	**<0.001**
average RNFL thickness in left eye (μm)	-0.146	0.158	-0.340	**0.001**
inferior quadrant in right eye (μm)	0.447	**<0.001**	0.346	**0.001**
superior quadrant in right eye (μm)	0.399	**<0.001**	0.284	**0.005**
nasal quadrant in right eye (μm)	0.209	**0.042**	0.145	0.161
temporal quadrant in right eye (μm)	0.365	**<0.001**	0.292	**0.004**
average RNFL thickness in right eye (μm)	0.517	**<0.001**	0.396	**<0.001**
Age (year)	-0.283	**0.005**	-0.331	**0.001**
Diabetes history (year)	0.011	0.912	-0.047	0.684
years of education (year)	0.385	**<0.001**	0.396	**<0.001**
Systolic pressure (mmHg)	0.026	0.805	-0.111	0.285
Diastolic blood pressure (mmHg)	0.056	0.590	-0.029	0.781
BMI (kg/m^2)	-0.037	0.723	-0.046	0.661
Blood glucose (mmol/l)	-0.031	0.769	-0.025	0.812
HbA1c(;%)	-0.272	**0.009**	-0.283	**0.006**
Triglycerides (mmol/l)	0.131	0.206	0.157	0.128
Total cholesterol (mmol/l)	0.305	**0.003**	0.217	**0.035**
HDL-L (mmol/l)	0.030	0.776	0.010	0.924
LDL-L (mmol/l)	0.218	**0.034**	0.262	**0.010**
eGFR ($ml/min/1.73m^2$)	0.025	0.816	-0.030	0.779
Irisin (pg/ml)	0.724	**<0.001**	0.456	**<0.001**
CML(pg/ml)	-0.773	**<0.001**	-0.475	**<0.001**
IL-18(pg/ml)	-0.775	**<0.001**	-0.544	**<0.001**
RAGE(pg/ml)	-0.735	**<0.001**	-0.352	**<0.001**

Significant P values (<0.05) are indicated in bold.

RNFL. From an anatomical standpoint, the axons originating from the upper quadrant of the retina project through the parietal segment of the optic radiations, eventually connecting to the cuneate gyrus within the primary visual cortex. Conversely, axons originating from the lower quadrant of the retina project to the lingual gyrus. Notably, the density of senile plaques and neurofibrillary tangles in the cuneate gyrus is greater than that in the lingual gyrus, and this difference may be the reason for the major RNFL lacunae in the middle and upper quadrant of AD (8). Post-mortem examinations of individuals with AD, juxtaposed with control subjects, have unequivocally revealed optic nerve degeneration and a notable decline in retinal ganglion cells (RGC) (9). This observed phenomenon may be attributable to the accumulation of amyloid deposits within the retina, a consequence of cognitive impairment, which subsequently leads to the depletion of RGCs. Collectively, the findings from these aforementioned studies underscore the correlation between the thinning of the RNFL and the progression of Alzheimer's Disease, reaffirming the potential utility of RNFL thickness as an indicative marker for AD development.

In the early stages of neurodegeneration in DM, a cascade of events unfolds, primarily affecting the function and integrity of retinal neurons. This multifaceted process encompasses several key phenomena, including the dysfunction and degeneration of retinal neurons, apoptosis of retinal nerve cells induced by hyperglycemia, heightened neurofilament phosphorylation, increased reactivity of glial cells due to metabolic stress, activation of microglia, and perturbations in the regulation of glutamate levels. Notably, these

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TABLE 7 Logistic regression analysis of the associations of NPDR with IL-17A, IL-22, and irisin in T2DM patients.

Characteristics	Model 1		Model 2	
	OR (95%CI)	*p*	OR (95%CI)	*p*
Age (year)	0.959(0.854-1.077)	0.481	0.943(0.826-1.076)	0.380
years of education (year)	0.960(0.728-1.266)	0.772	1.018(0.752-1.378)	0.907
HbA1c (%)	–	–	1.468(0.736-2.929)	0.276
LDL-C (mmol/L)	–	–	0.678(0.212-2.161)	0.511
Average RNFL thickness in left eye (μm)	1.161(0.967-1.394)	0.110	1.154(0.938-1.418)	0.175
Average RNFL thickness in right eye (μm)	0.699(0.532-0.920)	**0.011**	0.695(0.521-0.928)	**0.013**
IL-18 (pg/ml)	1.002(0.993-1.012)	0.598	1.002(0.992-1.013)	0.675
CML (pg/ml)	1.011(1.001-1.021)	**0.030**	1.012(1.001-1.022)	**0.037**
RAGE (pg/ml)	1.007(1.001-1.013)	**0.017**	1.007(1.002-1.012)	**0.007**
Irisin (pg/ml)	1.019(0.952-1.092)	0.585	1.022(0.951-1.100)	0.550

Significant P values (<0.05) are indicated in bold.

initial alterations predominantly target the inner retina and manifest as a reduction in the thickness of the RNFL and the loss of ganglion cell bodies (10). Srinivasan et al. reported a notable reduction in the overall thickness of the retina, along with discernible thinning of the RNFL specifically within the pars plana and periocular regions among patients diagnosed with T2DM (11). Among patients afflicted with T2DM who do not exhibit concurrent diabetic retinopathy, evidence of retinal neurodegeneration is discernible. It is noteworthy that defects in the RNFL are a common occurrence in T2DM, frequently manifesting within the upper half of the retina (12). The aforementioned study initially highlighted a thinning of the RNFL in patients diagnosed with T2DM. The outcomes of our present investigation effectively reinforce this established observation. Nevertheless, it is worth noting that, in our current study, we identified a distinct pattern of RNFL thinning in T2DM patients, specifically manifesting in the temporal region. It is important to acknowledge that this particular finding may be attributed to the inclusion of some patients with early-stage glaucoma that may have been challenging to diagnose accurately. In glaucoma patients, RNFL thinning predominantly occurs in the nasotemporal region. To gain a more comprehensive understanding of this phenomenon, future research endeavors would benefit from an expanded sample size and extended participant follow-up, aimed at providing further clarification on this matter. Crucially, our study represents the inaugural effort to investigate alterations in RNFL thickness

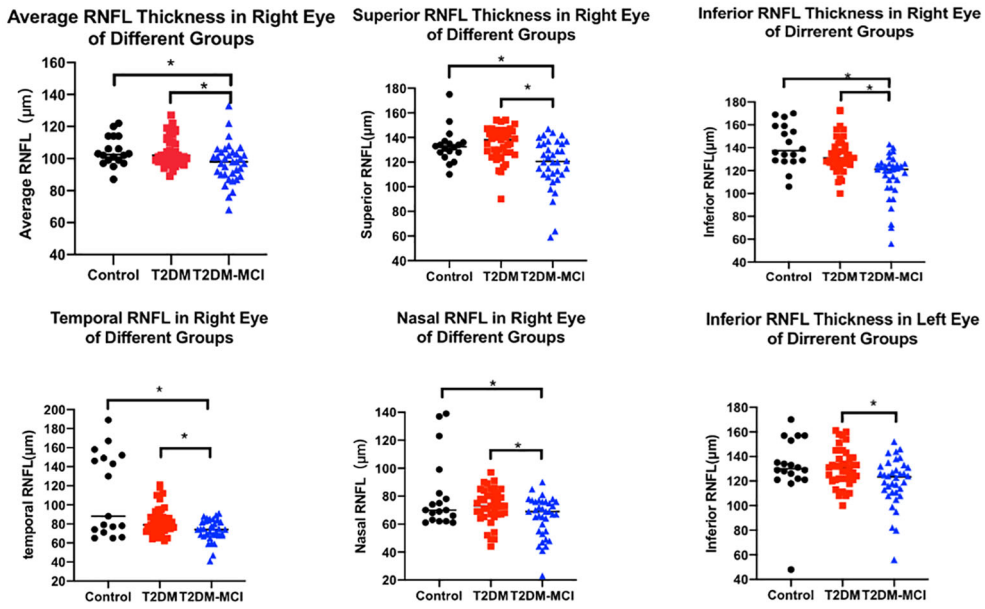

FIGURE 1
RNFL thickness in different groups. The scatter plots accompanied by the mean ± SD present the RNFL thickness in control, T2DM and T2DM-MCI groups. *p<0.05.

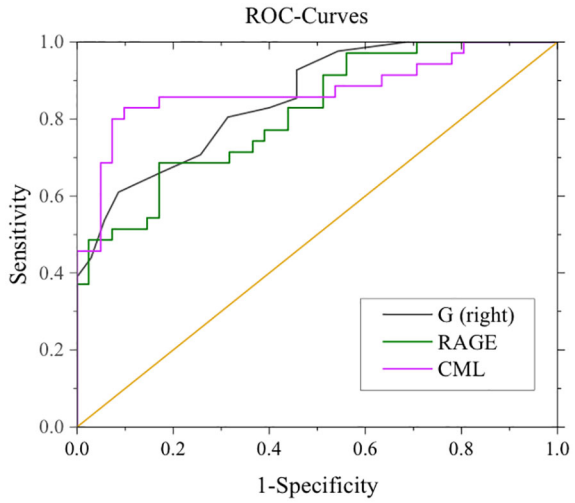

FIGURE 2
ROC curves for mean RNFL in the right eye, CML, RAGE, and MCI. G (right eye): AUC 0.853, sensitivity 91.7%, specificity 61.0%. CML: AUC 0.874, sensitivity: 82.9%, specificity: 90.2%. RAGE: AUC 0.815, Sensitivity 68.6%, Specificity 82.9%.

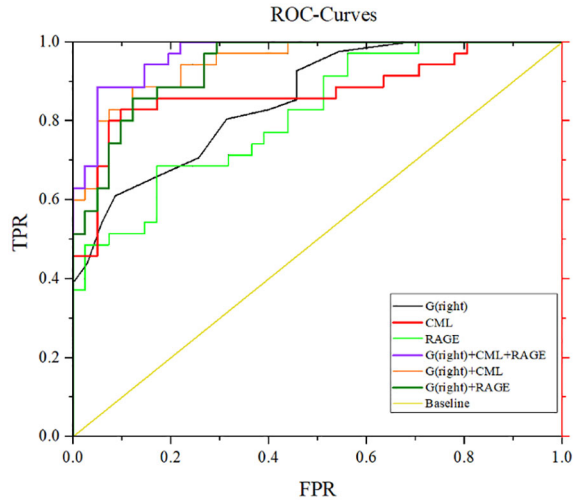

FIGURE 3
ROC curves for mean RNFL thickness, CML, and RAGE diagnosis in the right eye. G (right eye) + CML: AUC 0.948, sensitivity 88.6%, specificity 87.8%. G(right eye)+RAGE:AUC 0.939, sensitivity 82.9%, specificity 87.8%. G(right eye)+CML+RAGE: AUC 0.969, sensitivity 88.6%, specificity 95.1%.

among individuals with both T2DM and MCI. After meticulously accounting for the potential confounder of gender, we ascertained that RNFL thickness was indeed reduced in patients presenting both T2DM and MCI, in comparison to those with T2DM alone. This distinctive change was predominantly evident in the right eye. It is plausible that this observed phenomenon is underpinned by the established correlation between RNFL thickness and hippocampal volume, particularly as MCI patients tend to exhibit initial atrophy in the right hippocampal region (13). Hence, it is evident that alterations in RNFL thickness among patients presenting both T2DM and MCI initially manifest on the right side. Subsequent investigations could incorporate imaging data to provide further substantiation for this assertion. Remarkably, our findings unveil a noteworthy decline in RNFL thickness among patients diagnosed with AD and those grappling with MCI, in contrast to individuals in a normal cognitive state. Notably, we observed a positive correlation between RNFL thickness and cognitive performance scores, specifically the MoCA and MMSE. This correlation implies a potential simultaneous occurrence of retinal nerve fiber degeneration and central nervous system (CNS) degeneration (14).

TABLE 8 Diagnostic efficacy of right eye RNFL thickness, CML, and RAGE in the ROC curve for MCI in patients with T2DM.

Diagnostic indicators	Diagnostic efficacy	Sensitivity	Specificity
G(right)	0.853	91.7%	61.0%
CML	0.874	82.9%	90.2%
RAGE	0.815	68.6%	82.9%
G(right)+CML	0.948	88.6%	87.8%
G(right)+RAGE	0.939	82.9%	87.8%
G(right) +CML+RAGE	0.969	88.6%	95.1%

Neuroinflammation serves as a natural response within the CNS to both external and internal damage, functioning as a protective mechanism for the brain. However, it's important to note that an excessive or prolonged inflammatory response can be detrimental to the CNS. One key player in this intricate inflammatory cascade is IL-18, a pro-inflammatory cytokine categorized within the IL-1 family. Initially recognized as "IFN-γ-inducible factor," IL-18 has garnered substantial attention due to its involvement in a spectrum of infectious, metabolic, and inflammatory conditions. This includes but is not limited to instances such as influenza virus infections, atherosclerosis, diabetes, chronic obstructive pulmonary disease, and Crohn's disease, where there exists ample evidence of IL-18's pivotal roll (15). Chronic cytokine-mediated inflammation is a prominent feature in T2DM, underscoring the pivotal role of the inflammatory mechanism in the onset and progression of T2DM (16). Notably, our study unveiled that T2DM patients exhibited significantly elevated serum levels of Interleukin-18 (IL-18) compared to the control group (17). As a proinflammatory factor, IL-18 assumes a central role in both the inception and progression of T2DM, a finding consistent with previous research results. The upsurge in circulating IL-18 levels may signify a state of chronic inflammation within the brain, and its overexpression could potentially trigger tau protein phosphorylation, consequently contributing to neurodegeneration in Alzheimer's Disease (AD) patients (18). In post-mortem brain samples from AD patients, IL-18 was detected within microglia, astrocytes, and neurons. These brain alterations are known to contribute to cognitive decline (19). However, there has been a lack of research examining serum IL-18 changes in individuals with both T2DM and cognitive impairment, and how these changes correlate with cognitive function. Our study

is pioneering in revealing that IL-18 levels were notably higher in T2DM-MCI patients compared to those with T2DM alone and individuals in the normal control group. Moreover, IL-18 exhibited a negative correlation with MoCA and MMSE scores, shedding new light on the intricate relationship between IL-18 and cognitive function in this specific population.

Irisin, an adipokine secreted by muscle tissue, comprises fibronectin type III domain-containing 5 (FNDC5). Enzymatic cleavage of the carboxyl terminus of FNDC5 by protein hydrolases releases irisin, and this protein is expressed not only in skeletal muscle but also in various other tissues (20). Irisin exhibits the potential to exert multiple beneficial effects on glucose metabolism and insulin sensitivity. It achieves this by stimulating energy expenditure, enhancing glucose uptake, promoting glycogenolysis, and concurrently reducing gluconeogenesis, lipogenesis, and lipid accumulation (21). The majority of clinical investigations have consistently revealed lower irisin levels in individuals with prediabetes or T2DM when compared to their non-diabetic counterparts. This phenomenon is likely attributed to diminished FNDC5 synthesis, resulting in reduced irisin secretion within the muscle tissue of obese individuals and those afflicted with T2DM (22). Current research has consistently demonstrated that irisin exerts a protective influence on T2DM by enhancing insulin sensitivity. Our own investigation corroborates these findings, revealing a significant reduction in serum irisin levels among T2DM patients in comparison to the control group. Furthermore, studies have unveiled the presence of FNDC5 and irisin in both murine and human brains, with particular localization within the hippocampus (23). Irisin, notably, plays a pivotal role in modulating the release of inflammatory factors by astrocytes and promoting neuronal survival (24). Additionally, irisin has been implicated in the transcriptional regulation of Brain-Derived Neurotrophic Factor (BDNF), neurotrophins, and myocytokines. Its deficiency or reduction has been linked to neurodegenerative conditions that have the potential to impact cognitive function. This occurs through the promotion of hippocampal neurogenesis, reduction of oxidative stress, enhancement of glucose metabolism, and augmentation of synaptic plasticity (25). Lourenco and colleagues made a significant discovery, noting reduced levels of FNDC5 in the brains of human Alzheimer's Disease, cerebrospinal fluid, and AD mouse models. Their research further demonstrated that elevating FNDC5 levels within the brain or peripherally had a mitigating effect on synaptic and memory impairments in AD mouse models (26). However, there has been a limited number of studies exploring the role of irisin in patients with T2DM and MCI. In light of this, we have revisited the association between serum irisin levels and T2DM-MCI patients. Our findings reaffirm the significance of irisin as a protective factor in this specific population. We observed that serum irisin levels in T2DM-MCI patients were notably lower in comparison to those with T2DM alone and individuals in the normal control group. Furthermore, we identified a significant negative correlation between irisin levels and MoCA as well as MMSE scores. These results provide further substantiation for the protective role of irisin in T2DM patients with MCI.

Advanced AGEs are isomeric compounds originating from the non-enzymatic reaction between glucose or other sugar derivatives and proteins or lipids. These compounds have been identified in excess of 20 distinct forms within blood, tissues, and various food sources. Notable examples include CML, CEL, pyrrolizidine acid, pentosidine, and methylglyoxal lysine dimer (27). RAGE, an acronym for Receptor for Advanced Glycation End Products, belongs to the immunoglobulin superfamily of cell surface molecules. Positioned within the major histocompatibility complex class III site, RAGE serves as a receptor for ligands known as AGEs. This interaction primarily occurs through its V-type region, which serves as a critical site facilitating intracellular signal transduction. Recent investigations have shed light on the connection between heightened AGE formation within a metabolically perturbed environment and its consequences on cellular responsiveness to insulin. Specifically, this process impacts insulin sensitivity, insulin secretion, and the overall insulin signaling cascade. Ultimately, these disruptions contribute to the development of insulin resistance, which is a pivotal factor in the onset of DM (28). AGEs not only serve as biomarkers indicating hyperglycemia, pro-inflammatory states, or oxidative conditions but also actively participate in the pathogenesis of complications associated with DM. These multifaceted roles are largely mediated through their interactions with the Receptor for RAGE (29). Indyk et al. made a noteworthy discovery by documenting significant elevations in serum AGEs and sRAGE levels among diabetic patients. This finding further reinforces the compelling link between AGEs and their respective receptors in the context of diabetes development (30). In the diabetic state, there is an upsurge in endogenous AGE production, concomitant with the upregulation of RAGE and the downregulation of the AGE scavenger receptor AGR1. This cascade of events potentially contributes to a reduction in the clearance of AGEs, amplifying their impact within the system (31). Research has unveiled compelling evidence indicating that for each 100ng/ml rise in CML levels, there is an associated 35% increase in the risk of developing diabetes (32). Indeed, all the aforementioned studies have consistently demonstrated elevated serum levels of CML and the RAGE in individuals diagnosed with T2DM. Our own investigation corroborates these findings, underscoring significantly higher levels of CML and RAGE among T2DM patients when compared to their normal counterparts. Moreover, our current study has reported that exposure to AGEs leads to an upregulation of APP expression, both *in vitro* and *in vivo* experiments. This elevated APP expression subsequently results in increased levels of β-amyloid, suggesting a potential significant role for AGEs as a risk factor in the pathogenesis of AD (33). Activation of RAGE exacerbates not only Aβ production and aggregation but also the formation of neurofibrillary tangles (NFTs). Additionally, RAGE activation disrupts synaptic transmission and neuronal function, thereby promoting the onset and progression of AD. Inhibition of RAGE has been shown to mitigate Aβ-induced damage in neuronal cells and cerebral vasculature (34). Presently, all available research consistently underscores the pivotal role of the AGEs-RAGE pathways in the pathogenesis of AD. However, there is a notable absence of relevant studies investigating the involvement of AGEs-RAGE in individuals with T2DM combined with MCI. In this investigation, we have made a groundbreaking discovery,

demonstrating for the first time that levels of CML and the RAGE were markedly elevated in patients diagnosed with T2DM combined with MCI, in comparison to those with T2DM alone and individuals in the normal control group. Furthermore, we have established significant negative correlations between serum CML and RAGE concentrations and MoCA as well as MMSE scores. To further substantiate the role of the CML-RAGE pathway in the pathogenesis of T2DM combined with MCI, future animal experiments will be conducted at subsequent stages of this research.

In this study, we made several noteworthy findings. Firstly, we observed elevated serum levels of IL-18, CML, and the RAGE in patients with T2DM. These elevations were positively correlated with MoCA and MMSE scores. Furthermore, we noted significantly lower levels of irisin and a reduction in the average RNFL thickness in the right eye among T2DM patients. Irisin and RNFL measurements also exhibited positive correlations with MoCA and MMSE scores. To ensure the robustness of our findings, we conducted a correction analysis, factoring in potential confounders such as age, years of education, HbA1c, and LDL-C levels. Even after accounting for these variables, the associations between CML, RAGE, RNFL thickness in the right eye, and cognitive impairment remained statistically significant. Our ROC curve analysis further underscored the diagnostic potential of these indicators for MCI in T2DM patients. The average RNFL thickness in the right eye exhibited an impressive area under the curve (AUC) of 0.853, along with a sensitivity of 91.7%, indicating its strong potential as a diagnostic tool for MCI in T2DM patients. Similarly, CML demonstrated an AUC of 0.874 with a specificity of 90.2%, suggesting its effectiveness in minimizing false negatives in MCI diagnosis. RAGE, with an AUC of 0.815 and a specificity of 82.9%, also displayed potential for reducing false negatives in MCI diagnosis. These findings collectively introduce novel avenues for identifying MCI in T2DM patients. However, it is imperative that future research endeavors encompass more centers and larger sample sizes to further validate the utility of these three indicators as a basis for diagnosing MCI in individuals with T2DM.

In comparison to utilizing serological indices or OCT examinations in isolation, the combined diagnostic approach demonstrates superior capability in distinguishing MCI from healthy individuals. Within this study, we identified three key markers for diagnosing MCI in patients with T2DM through regression and correlation analyses. Our findings strongly advocate for the adoption of a combined diagnostic model encompassing average RNFL thickness, CML, and the RAGE in the right eye. This amalgamated model offers enhanced diagnostic efficacy, thus proposing its potential as a supplementary tool in clinical diagnostics. Nevertheless, the study population for this research is derived from a single center, and being a cross-sectional study, it has inherent limitations. Subsequent investigations could involve multi-center, prospective studies for further exploration.

Conclusion

In summary, our study reveals important correlations between cognitive function and specific biomarkers. Notably, the average RNFL thickness in the right eye exhibited a positive correlation with cognitive function. Conversely, serum CML and RAGE levels displayed negative correlations with cognitive function. These findings suggest the potential diagnostic utility of these three indicators in identifying MCI in individuals with T2DM.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the ethics committee of the Second Hospital of Shandong University. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

RL: Data curation, Writing – original draft. FZ: Data curation, Writing – original draft. LL: Formal analysis, Writing – original draft. PX: Investigation, Writing – original draft. YM: Methodology, Software, Writing – original draft. XZ: Funding acquisition, Project administration, Writing – review & editing. SC: Funding acquisition, Project administration, Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Lei Sha,
China Medical University, China

REVIEWED BY
Olga Barca Mayo,
University of Santiago de Compostela, Spain
Yanjun Du,
Hubei University of Chinese Medicine, China

*CORRESPONDENCE
Zhigang Li
lizhigang620@126.com

†These authors have contributed equally to this work and share first authorship

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Electroacupuncture stimulation improves cognitive ability and regulates metabolic disorders in Alzheimer's disease model mice: new insights from brown adipose tissue thermogenesis

Ting Li[1†], Junjian Tian[1†], Meng Wu[1], Yuanshuo Tian[2] and Zhigang Li[1*]

[1]School of Acupuncture-Moxibustion and Tuina, Beijing University of Chinese Medicine, Beijing, China, [2]Dongzhimen Hospital, Beijing University of Chinese Medicine, Beijing, China

Background: Metabolic defects play a crucial role in Alzheimer's disease (AD) development. Brown adipose tissue (BAT) has been identified as a novel potential therapeutic target for AD due to its unique role in energy metabolism. Electroacupuncture (EA) shows promise in improving cognitive ability and brain glucose metabolism in AD, but its effects on peripheral and central metabolism are unclear.

Methods: In this study, SAMP8 mice (AD model) received EA stimulation at specific acupoints. Cognitive abilities were evaluated using the Morris water maze test, while neuronal morphology and tau pathology were assessed through Nissl staining and immunofluorescence staining, respectively. Metabolic variations and BAT thermogenesis were measured using ELISA, HE staining, Western blotting, and infrared thermal imaging.

Results: Compared to SAMR1 mice, SAMP8 mice showed impaired cognitive ability, neuronal damage, disrupted thermoregulation, and metabolic disorders with low BAT activity. Both the EA and DD groups improved cognitive ability and decreased tau phosphorylation ($p<0.01$ or $p<0.05$). However, only the EA group had a significant effect on metabolic disorders and BAT thermogenesis ($p<0.01$ or $p<0.05$), while the DD group did not.

Conclusion: These findings indicate that EA not only improves the cognitive ability of SAMP8 mice, but also effectively regulates peripheral and central metabolic disorders, with this effect being significantly related to the activation of BAT thermogenesis.

KEYWORDS

Alzheimer's disease, metabolic, brown adipose tissue, thermogenesis, tau pathology, electroacupuncture

1 Introduction

It is well known that Alzheimer ‘s disease (AD) is a neurodegenerative disease. The aggregation of extracellular amyloid plaques and formation of neurofibrillary tangles by hyperphosphorylated tau protein in neurons are the main neuropathological features closely related to central metabolic defects (1). Recent research has shown that the basic determinants of metabolism, such as a decline in brain glucose metabolism (2) and central insulin resistance, are the characteristics of AD intrinsic metabolic disorder (3), which is inextricably related to glucose metabolism and insulin sensitivity in the peripheral circulation (4, 5). Based on the widespread application of positron emission tomography (PET) scanning in medical research (6), several studies have confirmed the pathological characteristics of brain glucose metabolism disorders in patients with AD (7). Effective utilization of glucose in the brain is crucial for neuronal function and central activity. Impaired glucose utilization may be a key factor in the pathogenesis and is directly related to cognitive impairment in patients with AD.

Epidemiological studies have shown that AD is highly prevalent in the older adult population after 65 years of age, and its prevalence increases exponentially with age (8, 9). It is conceivable that the incidence of AD will continue to rise (10, 11), with the progress of social aging and the increase of human life expectancy. Theories of aging may explain the origin of AD under systematic considerations. Age also plays an important role in energy metabolism. With an increase in age, the metabolic rate of the body slows down (12), the risk of an imbalance between heat generation and consumption increases (13), and the ability to maintain the core temperature of the body decreases because of the dissipation of more heat due to the reduction of peripheral vascular contraction (14, 15). A previous study showed that, compared to young people, the body temperature of older adults is relatively different, and the amplitude of the circadian rhythm is slightly decreased (16–18). A systematic review found that the normal body temperature of adults over 60 years old was lower than that of the young (19), and the comprehensive temperature data of the rectum, mouth, armpit and ear showed that the average body temperature of older adults was 0.3°C lower than the acceptable normal hypothermia value (20). Studies have suggested that the loss of thermoregulation observed in elderly individuals may be caused to some extent by low BAT activity (21). Although this has not been confirmed in older volunteers, the role of BAT in accelerating thermogenesis cannot be ignored. It is generally believed that aging and thermal regulation defects are common phenomena in older adults and patients with AD (22).

As a uniquely thermogenic tissue in mammals (23–26), BAT decouples mitochondrial ATP synthesis from fuel oxidation via an uncoupling protein (UCP1), releasing and spreading all the energy generated by glucose and triglyceride metabolism in the matrix into the whole body in the form of heat. Thus, it can regulate body temperature, improve glucose (27–29) and lipid metabolism (30), and regulate insulin resistance (31, 32) and has been considered a valuable potential target for the treatment of peripheral and central metabolic disorders (22, 32–36), such as Alzheimer’s disease, in which age-associated thermoregulatory deficits contribute to energy metabolic failure.

Electroacupuncture (EA) is a modern method of acupuncture based on the meridian theory of traditional Chinese medicine (TCM) and is widely used in clinical practice. EA involves microbiological stimulation through the administration of small electric currents at selected acupoints along the meridians to prevent and treat diseases. GV20 and GV29 are specific governor-vessel acupoints. According to the TCM meridian theory, “governor meridian is the sea of yang vessels,” and it regulates the yang qi (vital energy) of the whole body. The role of yang qi is to warm the whole body, a function that is very similar to the BAT thermogenic function of maintaining core body temperature.

Our previous research has proven that electroacupuncture significantly enhanced glucose metabolism in the hippocampus (37, 38) and improved cognitive function by delaying pathological deposition through AKT/GSK3β signaling (39). However, the mechanism by which EA regulates brain glucose metabolism has not yet been elucidated, and it is unclear whether it affects both peripheral and central metabolic reactions. Therefore, considering the unique role of BAT, we visualized and qualitatively analyzed the thermogenic activity of BAT, observed the glucose and triglyceride content in the peripheral serum of SAMP8 mice, analyzed the HOMA-IR index, quantitatively analyzed the key proteins of the central insulin pathway, and performed a correlation analysis to elucidate the effect of EA on BAT thermogenesis and peripheral-central metabolism. The current study reveals the effects of EA on AD from different perspectives and provides a basis for further elucidation of the pathological mechanisms and treatment of AD.

2 Materials and methods

2.1 Experimental animals

Eight-month-old male SAMP8 (accelerated aging mice/prone 8) and homologous anti-aging SAMR1 mice (accelerated aging mice/resistance 1) were purchased from Beijing Huafukang Biotechnology Co. Ltd. (animal lot: SCXK [Beijing] 2019-0008). The mice were raised in a single cage in a barrier environment animal room at the Beijing University of Chinese Medicine. The animals were given adaptive feeding for 5 days before the experiment, temperature at 22 (± 2) °C, humidity at 45 (± 5) %, a 12h/12h day and night cycle, and free access to food and water. All experimental procedures and animal welfare were approved by the Animal Ethics Committee of Beijing University of Chinese Medicine (Ethics number: BUCM-2021110810-4174).

2.2 Animal grouping and intervention

Forty-five SAMP8 mice were randomly divided into three groups: the AD model group (AD group), Donepezil Drug group (DD group), Electro-Acupuncture Group (EA group). Fifteen SAMR1 mice served as the normal control group (NC group).

The NC and AD groups were used as experimental controls under the same feeding conditions without any treatment. However, they were fixed with a self-made mouse sleeve at the same time each day for 28 days.

The mice in the DD group were gavaged with donepezil at a dose of 0.65 mg/kg, crushed when used, and dissolved in 0.9% saline (40).To ensure the same processing conditions, the mice were fixed with a self-made mouse sleeve at the same time each day for 28 days.

The mice in the EA group were fixed inactively with a self-made mouse sleeve that did not affect breathing. Two disposable sterile acupuncture needles (size: 0.25 mm × 13 mm, ZY500, Beijing Zhongyan Taihe Medical Instrument Co. Ltd., China) were obliquely inserted 0.5 cm deep into the GV20 and GV29 acupoints. GV20 was located at the midpoint between the auricular apices, whereas GV29 was located midway between the medial ends of the two eyebrows. The needle handle was connected to an EA instrument (SDZ-V; Suzhou Medical Supplies Factory Co., Ltd. China). The frequency of the instrument was adjusted to 2 Hz and the current intensity was 1 mA. The needle handle vibrated slightly, whereas the mice were quiet and did not struggle. GV26 used the prick method and did not retain needles. EA was administered for 20 min at the same time every day for 28 days.

2.3 Core body temperature monitoring and acquisition of infrared thermal imaging

Core body temperature was monitored before and after the intervention using an Intelligent Digital Rectal Thermometer (TH212, China Communications Construction Technology Development Co., Ltd., China). The thermometer was inserted gently approximately an inch into the rectum, taking care not to insert it too far. It was kept in place for about a minute or until it beeped, indicating the reading was complete. The temperature displayed on the thermometer was recorded, and the thermometer was then cleaned according to the manufacturer's instructions. These steps were repeated two more times to measure the rectal temperature, ensuring that the thermometer was properly cleaned before each subsequent measurement. Each measurement was conducted within the same time range (2:00-4:00 pm) to ensure consistency and accuracy. Finally, the average value obtained from the three recorded temperatures was used as the core temperature value for each mouse.

After the 28-day intervention period, all mice were allowed to move freely above the cage lid. Meanwhile, an infrared camera lens was placed 0.5 m above the mice to shoot thermal images of their backs (hair on the backs was removed one day before shooting).

Infrared thermal imaging is a technique used to determine surface temperature according to the physical law of radiation transfer. Medical infrared thermal imagers focus on the invisible infrared radiation energy emitted by an object and subject it to photoelectric conversion to generate processed electrical signals. The electrical signal is then simulated, amplified, and converted into a digital image signal. An image showing the thermal characteristics of an object was presented on the corresponding display device. A warm (bright) color represents a high-temperature region, whereas a cool (dark) color represents a low-temperature region. The intensity of the infrared radiation emitted by an object is primarily a function of its temperature. Infrared thermal imaging has been used to study surface temperatures in various mammals (41–43), and some researchers have used it in TCM research on acupuncture meridians and acupoints (44–47).

A Testo 865 handheld infrared thermal imager (Germany) was used to display the distribution of the skin surface temperature fields. The imager is a 160×120-pixel infrared pixel detector that can detect objects sized< 0.3 mm. The imager can detect temperatures of −20°C to 280°C. The imager is continuously adjustable, with thermal sensitivity of<0.12°C. The SUPPER infrared superpixel (320×240 pixels) has an image enhancement function that captures images through precise displacement, a built-in visible light shooting component with LED lighting, and an autofocus capacity (minimum focus, 0.5 m). It shows the isothermal and low, high, and average values of the regions. Moreover, the reflectivity and reflection of the detected temperature can be set manually. Infrared thermal images can be accurately analyzed on a computer using the IRSOFT professional infrared analysis software. The emissivity of mouse skin (e = 0.97), room temperature (23–25°C), relative humidity (50–60%), and detection distance (0.5 m) were the same for all groups.

2.4 Morris water maze

The Morris water maze (MWM) trial was conducted for 6 days and the hidden platform trials was conducted for the first 5 days. The platform was placed in the center of the southwest (SW) quadrant, slightly below the water surface. The mouse finds a platform to escape. If a hidden platform was successfully identified, the video acquisition system stopped automatically. If they were not found within 60 s, the mice were guided to find the platform and allowed to stay on it for 10 s. The escape latency and swimming speed were recorded. The hidden circular platform in the SW quadrant was removed for the probe trial. The mice were placed in water gently facing the wall of the pool, and the swimming path of the mice was observed within 60 s. The platform crossover number and percentage of time spent in the target quadrant were analyzed.

2.5 Nissl staining

The mice were anesthetized by an intraperitoneal injection of pentobarbital (80 mg/kg body weight) for the sample collection. The mouse brain tissue was completely removed, fixed with paraformaldehyde, embedded in paraffin, then sliced, dewaxed, and stained with 0.5% toluidine blue aqueous solution (RY-0004, Beijing Zhongke Wanbang Biotechnology Co. Ltd., China). Stained tissues were made transparent, mounted, and observed under a microscope (KF-PRO-120, China).

2.6 Hematoxylin-eosin staining

Brown adipose tissue was embedded in paraffin, sliced, dewaxed, and stained with hematoxylin-eosin (RY-0002, Beijing Zhongke Wanbang Biotechnology Co. Ltd., China). The stained tissues were dehydrated, mounted, and observed under a microscope.

2.7 Immunofluorescence staining

Mouse brain tissues (3-mm thick) were collected, fixed with paraformaldehyde, and dehydrated using an ethanol gradient. The tissues were paraffin embedded and sliced into 4-micron thick slices with a slicing machine (Leica, Germany, model: RM2235). Immunofluorescence staining was performed. The primary antibody used was rabbit polyclonal P-tau (1:200; antibody number: bs-2368R, China). The secondary antibody used was Goat Anti-Rabbit IgG H&L Alexa Fluor 594 (1:500; antibody number: ab150080, China). Finally, the sections were stained with DAPI (YG-0001; Beijing, China). Fluorescence scanning was performed using ImageJ.

2.8 Enzyme-linked immunosorbent assays

Blood samples were collected by eyeball extraction. The centrifuge tube containing blood was centrifuged at 4°C and 10000 rpm for 5 min, and the supernatant was separated into serum. The serum was placed in a cryopreservation tube and stored in a refrigerator at -20°C. Serum glucose, triglyceride, and insulin levels were detected using a mouse enzyme-linked immunosorbent assay kit, according to the manufacturer's instructions, and the absorbance was read.

2.9 Western blotting

Mouse tissues were removed from liquid nitrogen for rapid grinding in a precooled mortar. The ground powder and cell lysates were placed in Eppendorf tubes. After centrifugation at 4°C, tissue proteins were extracted, and protein quantification was performed using the bicinchoninic acid method. A 15% SDS-PAGE separation gel solution was prepared and 5% concentrated gel was added. The treated samples were placed in the sample holes of a concentrated gel in a predetermined order for electrophoresis. After the protein samples were electrotransferred, a polyvinylidene fluoride (PVDF) membrane (Millipore, Germany; product number: IPVH00010, batch number: R9PA20712) was taken out and sealed with 5% TBS-T skimmed milk powder at room temperature for 60 min. Next, the primary antibodies were added and blocked at 4°C overnight. After washing, the secondary antibodies were added and shaken at 37°C for 60 min. Finally, an enhanced chemiluminescence method was used to expose and develop the imprinting. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The density of all WB bands was compared with that of the GAPDH band.

2.10 Statistics analysis

Statistical analyses were performed using SPSS 26.0 (SPSS/IBM 26.0; SPSS Inc., USA). Data are expressed as the mean ± SD ($\bar{X} \pm S$). The hidden platform trial and swimming speed results were analyzed using a repeated-measures analysis. One-way ANOVA followed by the least significant difference (LSD) multiple-range test was used to compare normally distributed data and homogeneity of variance. The level of significance was set at $P< 0.05$. Pearson correlation analysis was used to analyze the correlation between variables.

3 Results

3.1 EA stimulation can improve the spatial learning and memory ability of AD model mice

The results of the MWM test are shown in Figure 1. The escape latency of the NC, EA, and DD group decreased gradually from the second to the fifth day and decreased significantly from the third to the fifth day ($P< 0.05$ or $P< 0.01$). However, no significant change was observed in the AD group from the second day to the third day. Until the fourth day, escape latency in the AD group showed a downward trend. Compared to the AD group, the escape latency of the NC group was significantly lower ($P< 0.05$, $P< 0.01$). The escape latencies of the EA group and DD group were significantly lower than those of the AD group from the second day to the fifth day ($P< 0.01$). No significant differences were observed between the EA and DD groups. No significant differences in swimming speed were observed between groups.

The platform crossover number (Figure 1C) and the percentage of time spent in the target quadrant (Figure 1D) were significantly higher in the NC group than in the AD group ($P< 0.05$, $P< 0.01$, respectively). Compared to the AD group, the platform crossover number and percentage of time spent in the target quadrant in the EA and DD groups were higher by varying degrees ($P< 0.05$, $P< 0.01$). No significant differences in these measurements were observed between the DD and EA groups. This indicated that EA stimulation could improve the learning and memory abilities of SAMP8 mice, and its efficacy was comparable to that of the DD group.

Donepezil (DNP) is a well-established and effective treatment for Alzheimer's disease (48). As an acetylcholinesterase inhibitor, it plays a crucial role in maintaining the activity of neurons involved in learning and cognition by inhibiting the breakdown of acetylcholine at the synapses (49). Additionally, DNP's selective inhibition of central rather than peripheral ChEs is expected to reduce the incidence of adverse events, including peripheral metabolism issues (50). Given its good tolerability and safety profile, DNP was used as a positive control group in our study to examine the effective improvement of EA on the behavior of SAMP8 mice.

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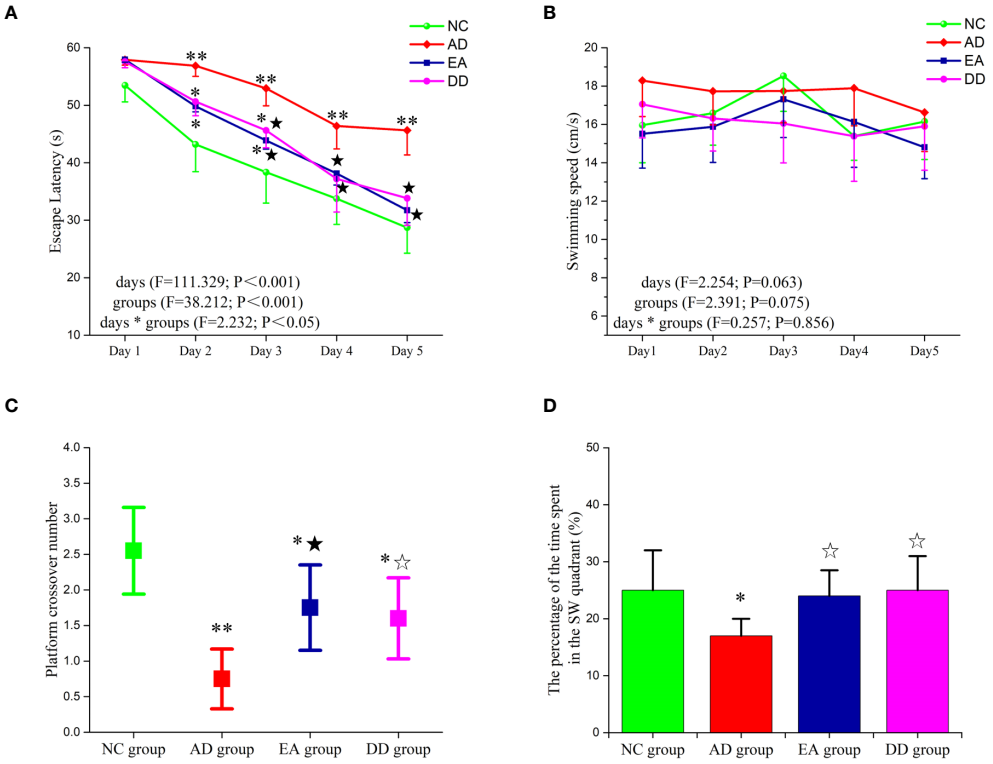

FIGURE 1
Results of MWM trial (n=10, mean ± SD) for each group. **(A, B)** Comparison of the escape latency and swimming speed of each group in the hidden platform trials. **(C, D)** Comparison of the platform crossover number and the percentage of time spent in the target quadrant of each group in the probe trial. Compared to the NC group, **P*<0.05,***P*<0.01 compared to the AD group, ☆*P*<0.05, ★*P*<0.01.

3.2 EA stimulation can improve neuronal morphology and reduce P-Tau levels of AD model mice

The results of the Nissl staining are shown in Figure 2A. In the NC group, the neurons were arranged regularly and densely and a clear and complete cell contour was observed. The neurons were rich in Nissl bodies, and the nucleus was full and satiated. In the AD group, nerve cells were severely damaged and a large number of cells were loosely arranged and disordered. Scattered cell debris, deep nuclear staining, and nuclear disappearance were also observed. The arrangement of nerve cells in the EA and DD groups was more regular than that in the AD group, and the cell contour was clear; however, the cell body and nucleus were still not as complete as those in the NC group were, and there were irregular cell bodies and nuclear staining.

Furthermore, undamaged and morphologically intact neurons are identified and counted, enabling quantitative analysis of their characteristics. The results are displayed in Figures 2C, D. Compared to SAMR1 mice, the number of neurons in SAMP8 mice (AD, EA, DD groups) was significantly reduced, with consistent trends observed in the hippocampus and cortex ($P<0.01$ or $P<0.05$). In comparison to the AD group, the number of neurons in the EA and DD groups showed a relative increase, suggesting that EA and DD interventions can effectively reduce neuronal damage.

We investigated the effect of EA stimulation on pathological tau protein by conducting immunofluorescence staining. Figure 2B demonstrates the merged staining results, where P-tau (Thr231) was labeled with red fluorescence and DAPI was labeled with blue fluorescence. The intensity of the staining corresponds to the level of protein expression. This staining technique allowed us to visualize and analyze the distribution of P-tau as well as assess any changes induced by EA stimulation.

In the cortex of the NC group, there was no significant expression of P-Tau, as indicated by the absence of red fluorescence. Conversely, in the AD group, there was a notable presence of red fluorescence, primarily concentrated in and around the neuronal cell body. When comparing the EA group and DD group to the AD group, the red fluorescence expression appeared relatively weakened in both treatment groups. We specifically analyzed the CA3 area of the hippocampus. The NC group showed no significant red fluorescence expression, indicating the absence of P-tau. In contrast, the AD group displayed a prominent increase in red fluorescence, indicating a high level of P-tau expression. Interestingly, both the EA and DD groups exhibited relatively weakened red fluorescence expression, suggesting that EA stimulation and Donepezil drug treatment may potentially contribute to a reduction in P-tau levels.

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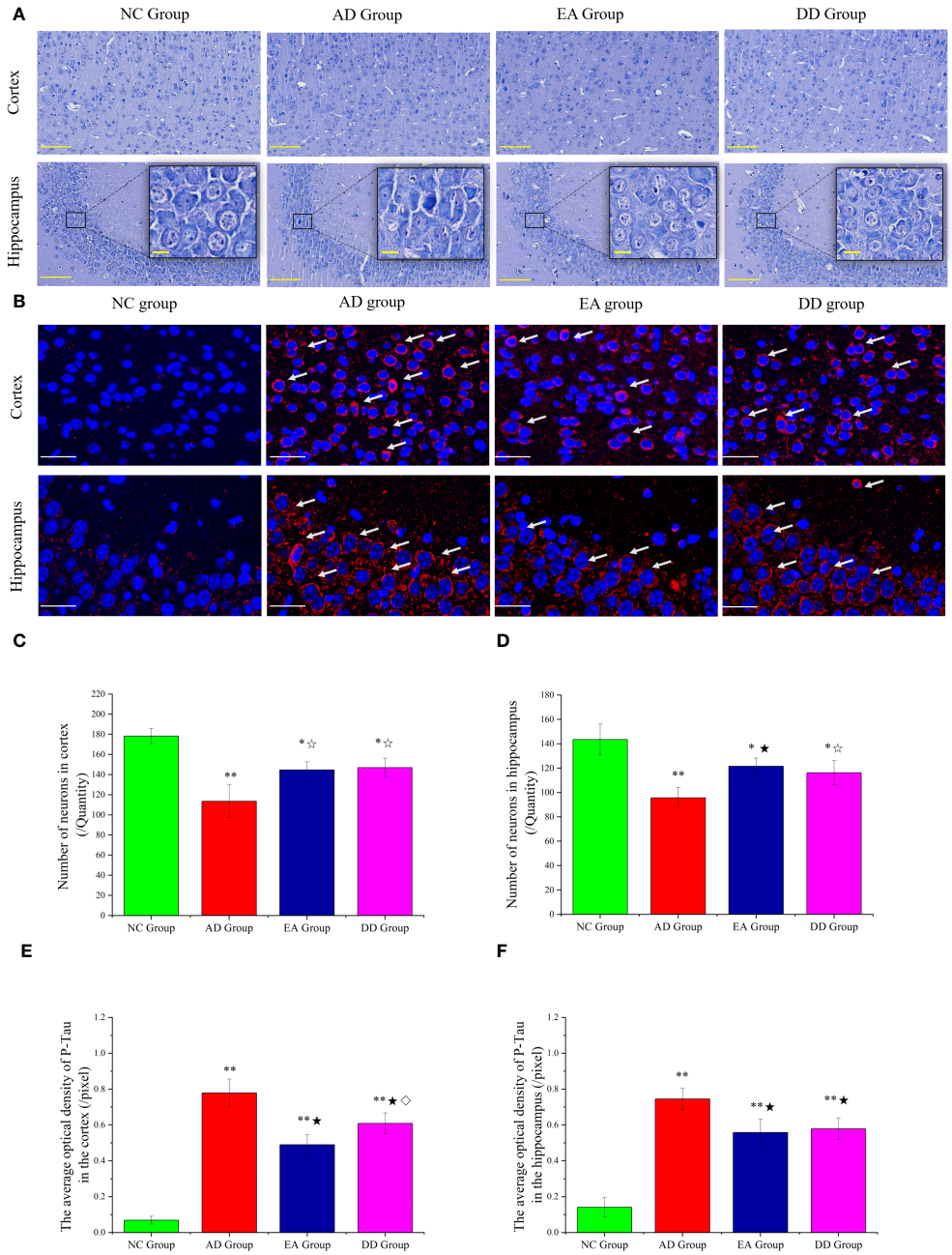

FIGURE 2
(A) Results of Nissl staining in the cortex and hippocampus of mice in each group (n=4) (The scale bar is 100 μm). The scale bar in the detailed enlarged picture is 25 μm. **(B)** Representative images of IF staining of P-tau (Thr231). P-tau (Thr231) are labeled with red fluorescence. The scale bar is 25 μm. **(C, D)** Comparison of the number of neurons in the cortex and hippocampus of each group (n=4). **(E, F)** Comparison of the mean optical density of P-tau in the cortex and hippocampus of each group (n=4). Supplement: Compared to the NC group, *P<0.05, **P<0.01 compared to the AD group, ☆P<0.05, ★P<0.01; compared to the EA group, ⋄P<0.05.

Upon further analysis (Figures 2E, F), we found that the average optical density in the cortex and hippocampus was significantly higher in the AD, EA, and DD groups compared to the NC group ($P< 0.01$). Furthermore, when comparing the AD group with the EA and DD groups, we observed a significant decrease in both the content and average optical density of P-tau in these groups($P< 0.01$). Remarkably, the EA group displayed a more pronounced effect in reducing cortical P-tau levels compared to the DD group ($P< 0.05$). In summary, our findings indicate that EA stimulation has a substantial impact on reducing P-tau levels, particularly in the cortex.

3.3 AD model mice have peripheral metabolic disorders, which can be regulated by EA stimulation

Core body temperature is the most intuitive indicator of metabolic reactions. There was no significant difference in the core

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body temperature of each group before the intervention. However, the distribution range of the core temperature values of each group was compared. As shown in Figure 3A, the core body temperature of the NC group was concentrated at about 37°C, while the distribution of the AD, EA, and DD groups was irregular and discrete. The temperature was as high as 38°C and as low as 35.3°C, but most were distributed between 36-36.5°C. The overall core temperature distribution was lower than that in the NC group. After the intervention, as shown in Figure 3B, there was still no significant difference in the core temperature between the groups (Supplementary Material). However, the body temperature distribution of the AD and DD groups was still discrete, whereas the distribution of the EA group showed a concentrated trend, close to the distribution trend of the NC group. Figure 3C shows the serum glucose (GLU) levels in each group. The GLU level of the NC group was within the ideal range, whereas the AD group was higher than that of the NC group ($P< 0.01$). Compared to the AD group, the serum GLU level in the EA group was significantly lower ($P< 0.01$), but there was no significant change in the DD group. Serum triglycerides (TG) in each group are shown in Figure 3D. Serum TG levels in the AD group were significantly higher than those in the NC group. Compared to the AD group, the serum TG content in the EA group was slightly reduced, but the difference was not statistically significant. Serum insulin (INS) levels are shown in Figure 3E. It was

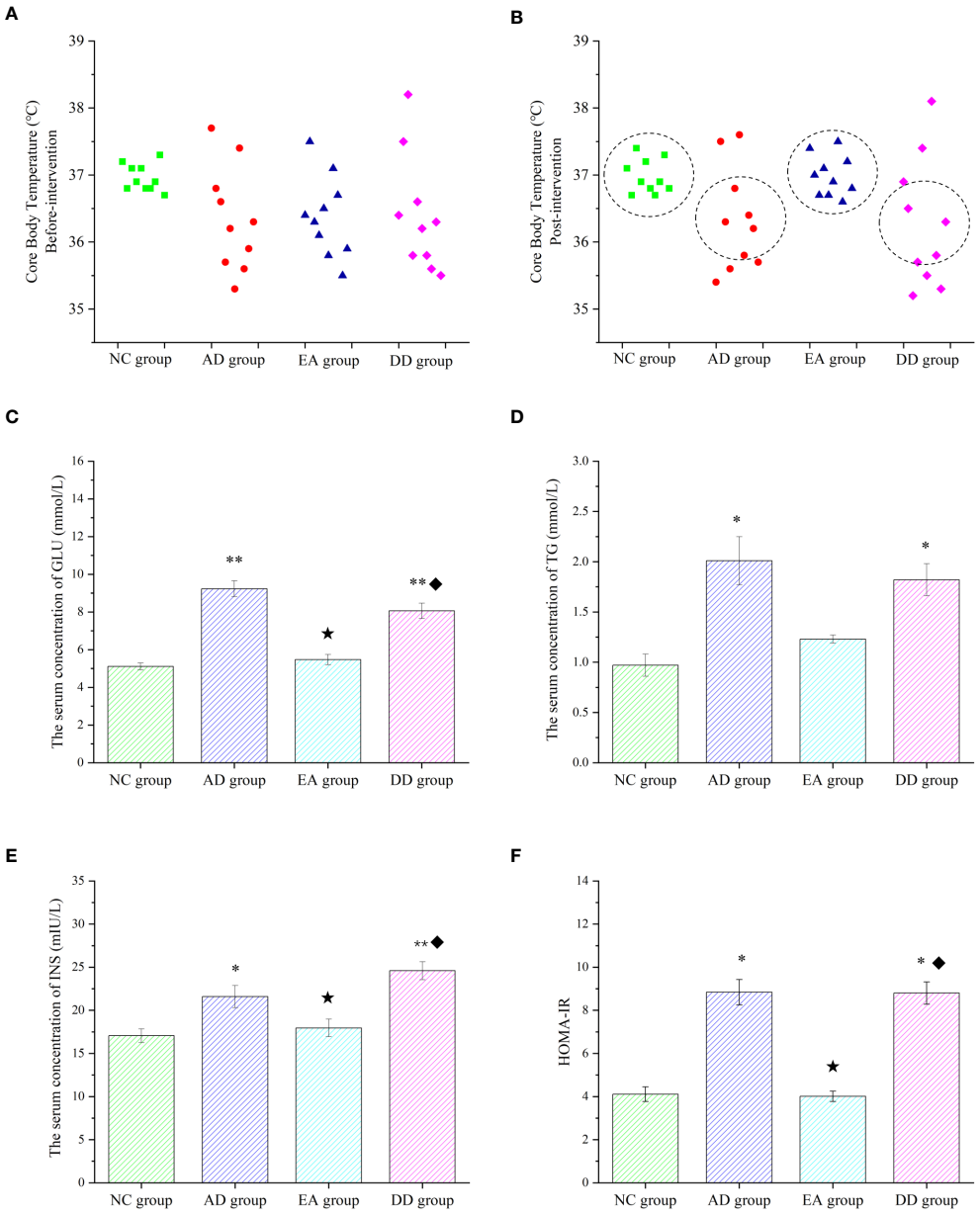

FIGURE 3
Peripheral metabolic results of each group. **(A, B)** Comparison of core body temperature before and after intervention in each group (n=10). **(C–F)** ELISA results of glucose, lipid, insulin, and HOMA-IR in peripheral blood (n=6). Compared to the NC group, *P<0.05,**P<0.01 compared to the AD group, ★P<0.01; compared to the EA group, ◆P<0.01.

found that, compared with the NC group, the INS content of the AD group was higher, and the INS content of the EA group was not significantly different from that of the NC group. The INS in the EA group was significantly lower than that in the AD group ($P< 0.01$).

Insulin resistance (IR) was analyzed by evaluating the homeostasis model assessment (HOMA) index, and HOMA-IR levels were calculated using the following equation (51): (fasting blood insulin [mIU/L] × blood glucose [mmol/L])/22.5. The HOMA-IR values in each group are shown in Figure 3F. Compared with the NC group, the AD group was significantly higher ($P< 0.05$). The HOMA-IR values were significantly lower in the EA group than in the AD group ($P< 0.01$). However, the levels of serum GLU, TG, INS, and HOMA-IR in the DD group were higher than those in the NC group were, but not different from those in the AD group. This indicates that AD model mice have peripheral metabolic disorders, including thermoregulation disorders, which can be regulated by EA stimulation rather than by donepezil.

3.4 EA stimulation could promote BAT thermogenesis

Brown adipose tissue (BAT) is the only non-shivering thermogenic organ in mammals and plays an irreplaceable role in regulating glucose and lipid metabolism and maintaining energy homeostasis (52). To explore whether the improvement of thermoregulation and peripheral metabolism by EA stimulation is related to BAT, we analyzed the skin temperature of the BAT area by infrared thermography and the hematoxylin and eosin (HE) morphology of BAT tissue, and quantitatively studied the thermogenic protein UCP1. Figure 4A represents an infrared thermal image of each group. The small elliptical circle refers to the area in which the BAT is located. The warm (bright) and cold (dark) colors represent the high-temperature and low-temperature regions, respectively. The infrared thermal image shows that the small elliptical circle was red in the AD group, white in the NC group, and white and bright in the EA group, indicating that the skin temperature in the BAT area of the AD group was lower than that of the NC and EA groups. We statistically analyzed the maximum, minimum, and average skin temperatures in this small elliptical circular area. The results are shown in Figure 4C. Compared to the NC group, the highest and lowest temperatures in the BAT region of the AD and DD groups were significantly lower ($P< 0.01$), whereas the highest temperature in the EA group was higher. Compared with the AD group, the lowest, highest, and average temperatures in the EA group were significantly higher ($P< 0.01$), whereas there was no significant difference in the DD group.

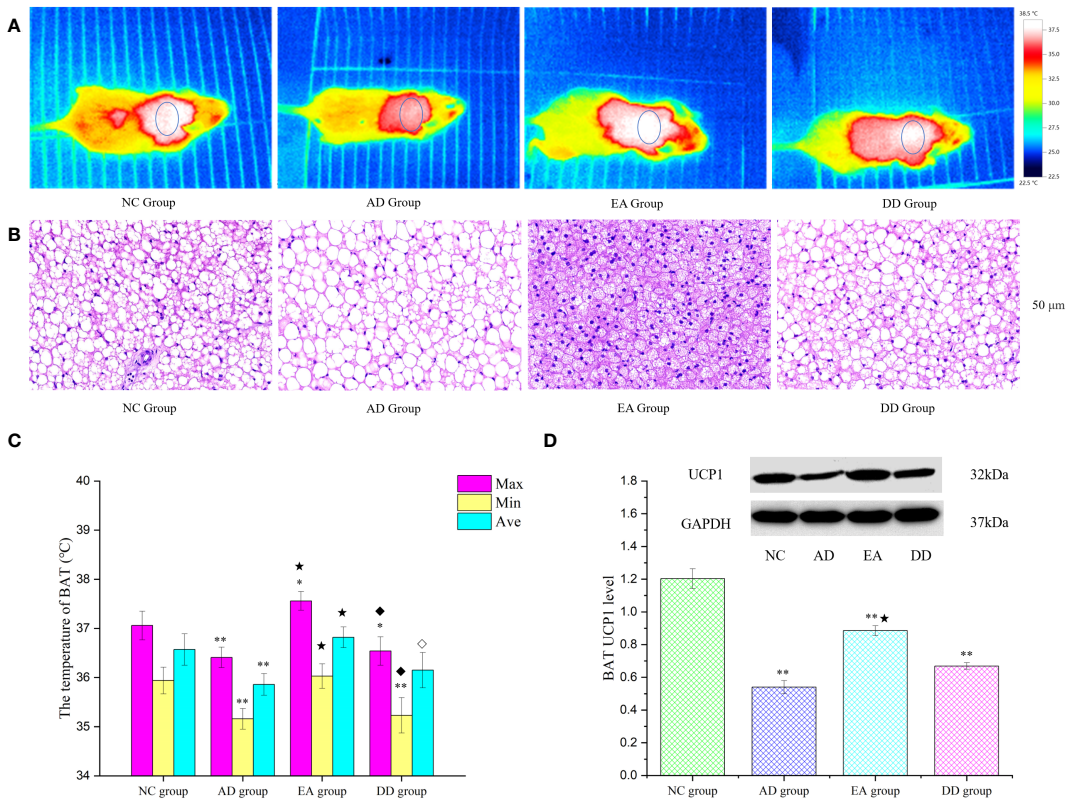

FIGURE 4
The results of BAT thermogenesis in each group. **(A)** The representative infrared thermal image of each group (n=10). **(B)** BAT HE results of each group (n=4) (50 μm). **(C)** Comparison of BAT skin temperature in each group (n=10). **(D)** Western Blot representative bands and statistical results of UCP1 protein in BAT (n=6). Compared to the NC group, *P<0.05,**P<0.01 compared to the AD group, ★P<0.01; compared to the EA group, °P<0.05; ◆P<0.01.

The BAT HE staining results are shown in Figure 4B. H&E staining of the NC group revealed single, small lipid droplets in BAT and larger lipid droplets in the AD group. The BAT HE group showed a multilocular stimulation morphology. However, the DD group was similar to the AD group. Figure 4D displays representative bands and statistical results of UCP1 protein in BAT through Western Blot analysis. The UCP1 content in the AD, EA, and DD groups was found to be significantly lower than that in the NC group ($P< 0.01$). However, a notable increase in UCP1 content was observed in the EA group compared to the AD group. These findings support the notion that EA stimulation promotes BAT thermogenesis.

3.5 EA stimulation up-regulated the content of central insulin receptor protein (IRS-1), promoted the phosphorylation of AKT

To further investigate whether the mechanism of EA stimulation in AD is related to the central insulin pathway, we analyzed the relative expression of key proteins in the cortical insulin pathway. As shown in Figure 5, compared with the NC group, the relative expression of IRS1, P-AKT, in the AD, EA, and DD groups were significantly lower than those in the NC group ($P< 0.01$ or $P< 0.05$), while the relative expression of GSK3β was higher than that in NC group ($P< 0.01$). There was no difference in the relative expression of AKT between groups. Compared to the AD group, EA stimulation significantly upregulated the relative expression of IRS1, P-AKT and P-GSK3β ($P< 0.01$). This indicates that EA stimulation upregulates the content of central IRS-1 and promotes AKT and GSK3β phosphorylation.

3.6 Effects of peripheral metabolism on key proteins of the central insulin pathway and their relationship with UCP1

Based on the above experimental results and the theory that peripheral and central metabolisms are closely related (4), we analyzed the Pearson correlation between peripheral glucose, lipid, HOME-IR, and key central insulin pathway proteins (Figure 6). We found a significant negative correlation between serum GLU and TG levels and central IRS-1 and P-AKT protein levels, especially with IRS-1 and HOMA-IR. This suggests that peripheral hyperglycemia, hyperlipidemia, and insulin resistance may be associated with decreased activity of central IRS-1 and P-AKT proteins. Based on these findings, we can infer that peripheral metabolism affects key proteins of the central insulin pathway, thereby influencing central insulin sensitivity.

Furthermore, the correlation analysis reveals a negative correlation between UCP1 and peripheral glucose, lipid, and HOMA-IR levels. This suggests that the activation of brown adipose tissue is associated with the utilization of glucose and lipids. Additionally, UCP1 shows a positive correlation with central IRS-1, indicating that the activation of brown adipose tissue has a positive effect on IRS-1 expression. Moreover, UCP1 exhibits a negative correlation with GSK3β and a positive correlation with P-GSK3β, suggesting a potential association between UCP1 and GSK3β activity. These correlation analysis results provide important insights into understanding the impact of peripheral metabolism on the central insulin pathway and lay the foundation for further research.

4 Discussion

The MWM test is an important means of evaluating the learning and spatial memory abilities of experimental animals. It is the main method used for behavioral testing in biological research because of its high reliability (53). In this study, the MWM test was used to evaluate the effect of EA on cognitive ability in an AD mouse model. The results of the hidden platform experiment revealed no significant differences in the swimming speed of each group, indicating that the swimming ability of each group was similar. The escape latency of the NC group was always lower than those of the AD, EA, and DD groups, indicating that the spatial learning ability of SAMR1 mice was higher than that of SAMP8 mice, which is consistent with the pathological changes in cognitive impairment in SAMP8 mice (54). The escape latency of the EA and DD groups was significantly lower than that of the AD group from the second to the fifth day, but the difference between the groups was not significant, indicating that donepezil and EA intervention could improve the spatial learning ability of SAMP8 mice; however, the difference in therapeutic effectiveness was not significant. The results of the probe trial showed that EA had an effect similar to that of donepezil in improving the spatial memory of SAMP8 mice. The cognitive deficits of AD are directly related to neuronal damage (55). Nissl staining showed a large amount of neuronal damage in the cortex and hippocampus of SAMP8 mice, whereas EA and donepezil reduced neuronal damage.

Several studies have highlighted a strong relationship between AD and metabolic disorders. A study conducted by researchers from the University of Oxford involved a 15-year follow-up on 176,000 participants without dementia. The study found that metabolic syndrome was associated with a 12% increased risk of developing dementia (56). These findings were corroborated by a research team from South Korea who also observed a connection between metabolic disturbances and an elevated risk of dementia (57). Recent studies have shown that a decline in brain glucose metabolism and central insulin resistance are considered characteristic features of metabolic disruption in AD patients (3) Disrupted brain glucose metabolism is a persistent feature throughout the pathological process of AD (5), and it may be a key factor in the disease's mechanisms, triggering a cascade of reactions leading to neuronal degeneration and cognitive impairment in AD patients. Furthermore, this disruption is closely associated with peripheral glucose metabolism and insulin sensitivity (4).

Aging is widely considered as one of the principal risk factors to the pathological changes in AD (22, 58). Age plays a significant role in energy metabolism, as metabolic rates tend to slow down as

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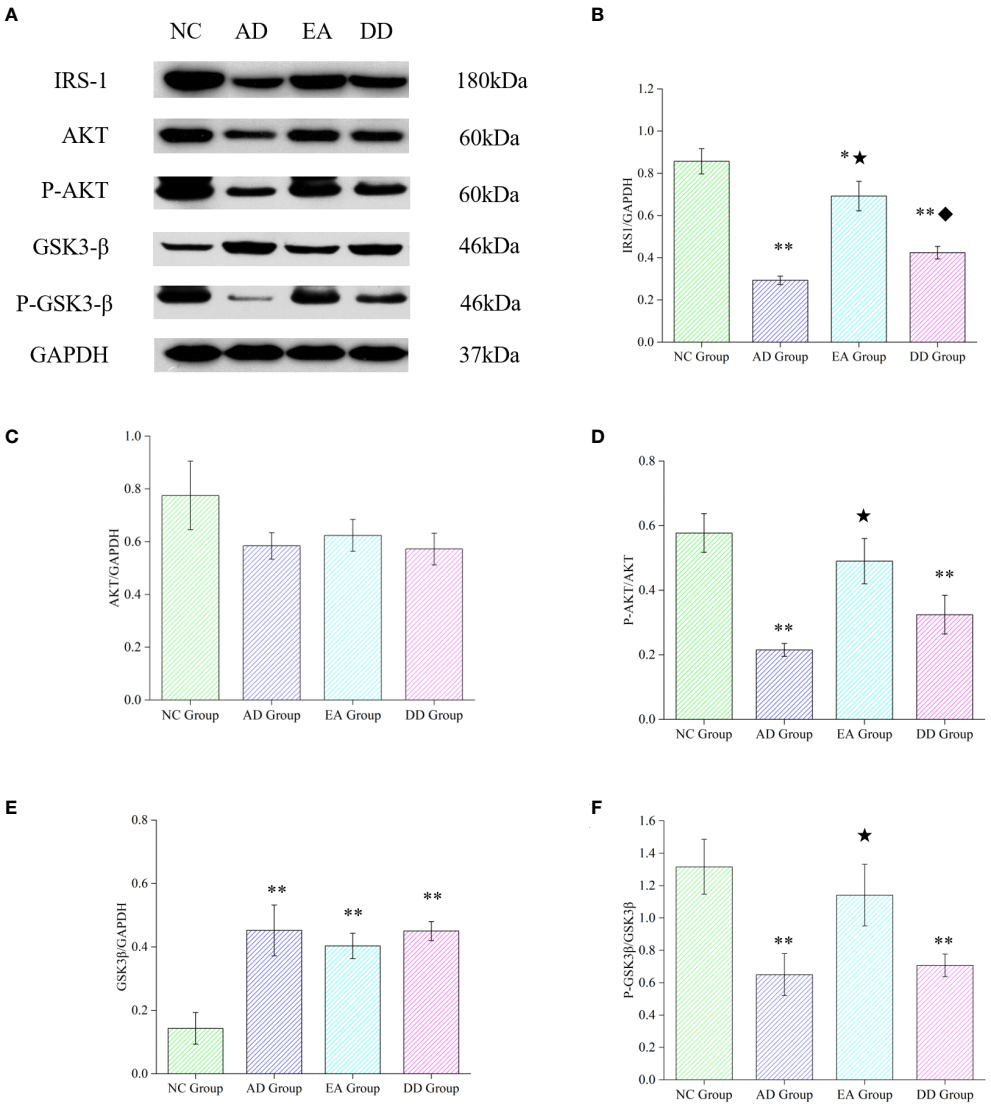

FIGURE 5
Results of key protein levels of the central insulin pathway and pathological tau protein in each group. **(A)** Western Blot representative bands of key proteins of the central insulin pathway. **(B-F)** Comparison of the relative expression of key proteins of the central insulin pathway in each group (n=6). Compared to the NC group, **P*<0.05,***P*<0.01 compared to the AD group, ★*P*<0.01; compared to the EA group, ◆*P*<0.01.

individuals grow older (12).This imbalance in energy production and expenditure disrupts the equilibrium, leading to a decline in the ability to maintain core body temperature (14).The defect of the body temperature regulation mechanism in older adults is accompanied by a sharp increase in the incidence of AD (19, 59). This suggests the possibility that age-related thermoregulatory defects lead to energy failure during AD pathogenesis (22, 58). Therefore, AD is a complex age-related neurodegenerative disease, associated with central and peripheral metabolic anomalies, such as impaired glucose utilization and insulin resistance (22). Therefore, aging is the principal risk factor and the most significant physiological feature of AD. It is accompanied by a decline in metabolism. On the other hand, metabolic diseases, such as diabetes, are characterized by glucose and insulin dysfunctions, and have been documented to be associated with an increased risk of developing AD.

To investigate the role of EA in AD energy metabolism, we conducted an in-depth study. Our study examined the core body temperature of each group and found that the mean value was not significantly different between SAMR1 and SAMP8 mice before and after the intervention. However, analysis of the body temperature distribution in each group showed that the distribution of SAMP8 mice was discrete before the intervention. Following the intervention, the distribution trend in the EA group showed clear changes, and the overall trend was similar to that in the NC group. These results indicate that SAMP8 mice have thermoregulation disorders that mainly manifest as overall hypothermia, but individual differences are large (16). Body temperature is the

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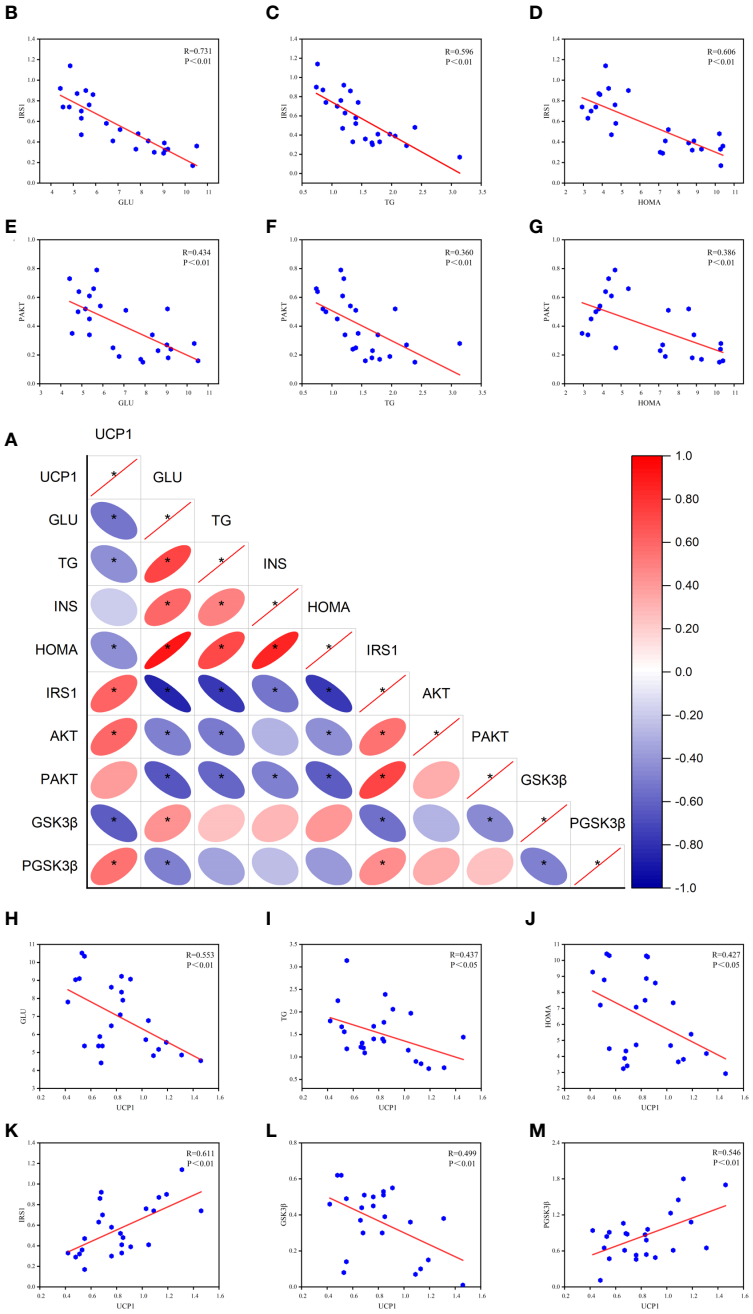

FIGURE 6
Results of Pearson correlation between peripheral metabolism and key proteins of the central insulin pathway. **(A)** Correlation heat map. **(B-D)** Pearson correlation analysis of GLU, TG, HOMA, and IRS-1. **(E-G)** Pearson correlation analysis of GLU, TG, HOMA, and P-AKT. **(H-J)** Pearson correlation analysis of GLU, TG, HOMA, and UCP1. **(K-M)** Pearson correlation analysis of UCP1 and IRS-1, GSK3β, P-GSK3β (R value and p-value given). *$P<0.05$.

oldest known human metabolic parameter. To date, no consensus has been reached on the association between the pathological diagnosis of AD and the mean body temperature. It has been reported that there is no difference in average body temperature between patients with AD and normal controls (60). Some studies have suggested that patients with AD have a lower core body temperature (61), whereas others have reported opposite results (62). These inconsistencies depend on how and when the temperature is measured, as well as the stage of the disease and the associated motor or neuropsychological symptoms. Patients with AD often experience agitation and behavioral disorders that may affect their body temperature. The discrete distribution of core body temperature in SAMP8 mice also illustrates an obstacle to its thermoregulatory mechanism, which is more closely related to AD than to simple temperature differences.

A study of GLU, TG, and INS in peripheral serum found that SAMP8 mice had obvious metabolic disorders, manifested as hyperglycemia, hyperlipidemia, and hyperinsulinemia. These

results are also consistent with a previous study in which SAMP8 mice showed age-related insulin resistance (63). This suggests that the SAMP8 strain is the best rodent model for studying age-related metabolic complications. It is also associated with energy metabolism disorders. EA regulated these disorders, whereas DD showed no effect on peripheral metabolic disorders.

When talking about thermoregulation and peripheral glucose and lipid metabolism, the role of BAT must be considered. BAT is a key component of mammalian non-shivering thermogenesis that maintains core temperature and energy consumption (23–26). Previous studies in rodents have shown (22) that the regulation of the internal body temperature by exposing BAT to cold directly affects AD pathology. Although cold adaptation is not suitable for elderly individuals, this study provides the first evidence that chronic BAT stimulation may be a valuable strategy for treating AD. Subsequent studies have found that tau phosphorylation is extremely sensitive to temperature (64) and that BAT stimulation can antagonize tau phosphorylation (65, 66). In this study, we used infrared thermal imaging technology to evaluate the effect of EA on BAT thermogenic activation and quantitatively analyzed UCP1 thermogenic protein. Our results support the idea that EA stimulation promotes BAT thermogenesis, although no statistically significant increase in core body temperature was observed in mice. UCP1 is considered the main molecular driving force behind thermal regulation and energy homeostasis of BAT (67). Our results showed that although the content of UCP1 in the EA group was up-regulated, the content of UCP1 in the BAT of SAMP8 mice was still much lower than that in SAMR1 mice ($P< 0.01$), which also suggests that thermal regulation and metabolic disorders in SAMP8 mice may be related to low BAT activity.

Evidence suggests that reduced phosphorylation of insulin receptors and downstream substrates leads to IR in the brain. The PI3K/AKT-dependent pathway can improve cerebral insulin resistance and regulates tau phosphorylation in AD (68). Based on our immunofluorescence results for P-tau, we found that EA could inhibit the phosphorylation of tau in the cortex and hippocampus. Therefore, we quantitatively analyzed key proteins in the insulin pathway and found that EA significantly upregulated the content of IRS-1 and promoted AKT phosphorylation. This was consistent with the results of our previous acupuncture experiments based on APP/PS1 mice (39).

AD is a central peripheral metabolic disease with thermoregulation deficiency (22). Studies have shown that peripheral blood-based interventions can improve or even reverse related brain dysfunctions (69). In this study, we found a significant negative correlation between peripheral glucose, lipid, HOMA-IR, IRS-1, and p-AKT levels, indicating that peripheral metabolic disorders may influence the sensitivity of the central insulin pathway. EA can regulate peripheral metabolic disorders, significantly increase IRS-1 levels, promote AKT phosphorylation, and enhance insulin sensitivity. Further correlation analysis showed that the activation of BAT thermogenesis promotes peripheral glucose and lipid metabolism, regulates insulin resistance, and has a positive effect on IRS-1 upregulation. GSK3β is a downstream target of the PI3K/AKT signaling pathway and is involved in tau phosphorylation as a major tau kinase (70). The positive correlation between UCP1 and P-GSK3β suggests a potential connection between the activation of BAT thermogenesis and the activity of GSK3β. This relationship could potentially have a beneficial effect, helping to alleviate the negative effects caused by high levels of GSK-3β on the brain. Therefore, the mechanism by which EA reduces the abnormal phosphorylation of tau is likely to be attributed to the effect of BAT thermogenesis on overall metabolism. This suggests that intervention in peripheral metabolism may be an effective therapeutic strategy for AD.

5 Conclusion

In this study, we confirmed that EA improves cognitive function and neuronal morphology in SAMP8 mice. This is the first report that EA regulates thermoregulatory dysfunction and peripheral metabolic disorders in SAMP8 mice. We demonstrated for the first time that EA has an overall regulatory effect on peripheral-central metabolism, and proposed that this effect may be related to BAT thermogenesis.

Furthermore, AD is not a simple brain pathological disease but also a closely related peripheral and central metabolic disease. Therefore, a new perspective on the pathology of AD is required. From a holistic perspective, the periphery and center are viewed as a whole that can interact with each other for better understanding and treatment. EA is a method for understanding and treating diseases based on a holistic view of traditional Chinese medicine. This may explain why EA has a multitarget effect compared to donepezil.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The animal study was approved by Animal Ethics Committee of Beijing University of Chinese Medicine (Ethics number: BUCM-2021110810-4174). The study was conducted in accordance with the local legislation and institutional requirements.

Author contributions

TL: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. JT: Data curation, Formal analysis, Methodology, Writing

– original draft. MW: Data curation, Formal analysis, Methodology, Project administration, Writing – review & editing. YT: Project administration, Writing – review & editing. ZL: Conceptualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fendo.2023.1330565/full#supplementary-material

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EDITED BY
Joana Nicolau,
Son Llatzer Hospital, Spain

REVIEWED BY
Ian James Martins,
University of Western Australia, Australia
Kourosh Zarea,
Ahvaz Jundishapur University of Medical Sciences, Iran
Rosmeri Rivera Irigoin,
Son Llatzer Hospital, Spain

*CORRESPONDENCE
Xiaofeng Zhu
zxiaof@jnu.edu.cn
Ronghua Zhang
tzrh@jnu.edu.cn

†These authors have contributed equally to this work

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Development and validation of a cognitive dysfunction risk prediction model for the abdominal obesity population

Chun Lei[1†], Gangjie Wu[1†], Yan Cui[2], Hui Xia[1], Jianbing Chen[2], Xiaoyao Zhan[2], Yanlan Lv[2], Meng Li[2], Ronghua Zhang[3,4,5*] and Xiaofeng Zhu[2,6*]

[1]General Practice, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong, China, [2]School of Traditional Chinese Medicine, Jinan University, Guangzhou, Guangdong, China, [3]College of Pharmacy, Jinan University, Guangzhou, Guangdong, China, [4]Cancer Research Institution, Jinan University, Guangzhou, Guangdong, China, [5]Guangdong Provincial Key Laboratory of Traditional Chinese Medicine Informatization, Jinan University, Guangzhou, Guangdong, China, [6]Traditional Chinese Medicine Department, The First Affiliated Hospital of Jinan University, Guangzhou, Guangdong, China

Objectives: This study was aimed to develop a nomogram that can accurately predict the likelihood of cognitive dysfunction in individuals with abdominal obesity by utilizing various predictor factors.

Methods: A total of 1490 cases of abdominal obesity were randomly selected from the National Health and Nutrition Examination Survey (NHANES) database for the years 2011–2014. The diagnostic criteria for abdominal obesity were as follows: waist size ≥ 102 cm for men and waist size ≥ 88 cm for women, and cognitive function was assessed by Consortium to Establish a Registry for Alzheimer's Disease (CERAD), Word Learning subtest, Delayed Word Recall Test, Animal Fluency Test (AFT), and Digit Symbol Substitution Test (DSST). The cases were divided into two sets: a training set consisting of 1043 cases (70%) and a validation set consisting of 447 cases (30%). To create the model nomogram, multifactor logistic regression models were constructed based on the selected predictors identified through LASSO regression analysis. The model's performance was assessed using several metrics, including the consistency index (C-index), the area under the receiver operating characteristic (ROC) curve (AUC), calibration curves, and decision curve analysis (DCA) to assess the clinical benefit of the model.

Results: The multivariate logistic regression analysis revealed that age, sex, education level, 24-hour total fat intake, red blood cell folate concentration, depression, and moderate work activity were significant predictors of cognitive dysfunction in individuals with abdominal obesity ($p < 0.05$). These predictors were incorporated into the nomogram. The C-indices for the training and validation sets were 0.814 (95% CI: 0.875-0.842) and 0.805 (95% CI: 0.758-0.851), respectively. The corresponding AUC values were 0.814 (95% CI: 0.875-0.842) and 0.795 (95% CI: 0.753-0.847). The calibration curves demonstrated a satisfactory level of agreement between the nomogram model and the observed data. The DCA indicated that early intervention for at-risk populations would provide a net benefit, as indicated by the line graph.

Conclusion: Age, sex, education level, 24-hour total fat intake, red blood cell folate concentration, depression, and moderate work activity were identified as predictive factors for cognitive dysfunction in individuals with abdominal obesity. In conclusion, the nomogram model developed in this study can effectively predict the clinical risk of cognitive dysfunction in individuals with abdominal obesity.

KEYWORDS

abdominal obesity, cognitive dysfunction, NHANES, risk factor, nomogram

1 Introduction

According to the latest World Obesity Map 2023, the whole prevalence of overweight and obesity is projected to reach 51% by 2035, affecting over 4 billion individuals in the world. This upward trend is attributed to modifications in dietary habits and lifestyle choices (1). The consequences of obesity extend beyond personal health, posing significant challenges to public health and economic productivity (2). [Urgent measures are required to address this growing concern and alleviate its burden on individuals, communities, and healthcare systems.

Cognitive functioning encompasses executive functioning, memory, perceptual-motor functioning, verbal functioning, complex attention, and social cognitive functioning. Cognitive dysfunction, which refers to impairment in one or more aspects of cognitive function, has become a social burden and significantly diminishes people's life experiences (3). In China, there are studies on the risk factors related to cognitive dysfunction. Research by Huilian Duan et al. (4) has shown that unhealthy lifestyles, apolipoprotein E (APOE) ϵ4 genotype, and methyltetrahydrofolate reductase (MTHFR) TT genotype are significantly associated with cognitive dysfunction in Chinese elderly people. Among lifestyle factors, healthy diet and physical exercise play a crucial role in preventing cognitive decline. In a longitudinal study (5) in China, age, BMI, blood pressure, cholesterol, and depression were identified as important predictors of cognitive dysfunction. However, there has been limited research in China investigating the relevant factors influencing cognitive dysfunction in the population affected by abdominal obesity. Research from Europe indicates that obesity affects cognitive abilities such as working memory, language, and executive function (6). A meta-analysis revealed a clear association between abdominal obesity and cognitive dysfunction, with abdominal obesity potentially increasing the likelihood of cognitive impairment (7). Mendelian randomization studies have established a causal link between abdominal obesity and cognitive performance, demonstrating that abdominal obesity negatively impacts cognitive function (8). Understanding these risk factors could enable the identification of high-risk groups at an earlier stage and facilitate the assessment of disease severity and prognosis.

A Nomogram is a tool that calculates a total score by summing up the scores of all Influencing factors. It utilizes these scores to predict the probability of a clinical event occurrence, offering individualized analysis and prediction. Taking into account a number of risk factors, the nomogram can aid in diagnosing or predicting the prognosis of diseases (9). Prior research has not explored risk prediction models specifically associated with cognitive dysfunction and abdominal obesity. Our objective is to identify risk factors for cognitive dysfunction in individuals with abdominal obesity and incorporate them into a nomogram prediction model. This model will help clinicians in early diagnosis, as well as the development of personalized preventive measures and treatment plans.

2 Materials and methods

2.1 Study design and participants

The data for this study was obtained from the National Health and Nutrition Examination Survey (NHANES) website. NHANES is a comprehensive and nationally representative survey conducted to evaluate and understand the health, well-being, and nutrition of individuals residing in America. It collects data through various methods, including interviews, physical examinations, and laboratory tests, aiming to provide a comprehensive understanding of the health and nutritional characteristics of the population. The survey can be accessed online at https://wwwn.cdc.gov/nchs/nhanes/. For our analysis, we selected the data from 2011 to 2014, which included 19,931 individuals in total.

Sampling method: The NHANES sample design consists of a multiyear, stratified, clustered four-stage sample, with data released on a 2-year cycle, and the four-stage sample is divided primarily into (a) primary sampling units (PSUs) (counties, groups of areas within counties, or combinations of adjacent counties), (b) segments (census tracts or combinations of tracts) within PSUs, (c) dwelling units (DUs) (households) within segments, and (d) individuals within households. The sample represents the noninstitutionalized civilian population residing in the 50 states

and the District of Columbia. The specific NHANES sample design, including specifications for clustering, stratification, and oversampling population subgroups, changed over time. Specific methods are available on the NHANES database website. (https://wwwn.cdc.gov/nchs/nhanes/).

Participants who met the diagnostic criteria for abdominal obesity in NHANES from 2011 to 2014 were included in this study. Waist circumference has been identified as a reliable indicator of visceral fat accumulation and adverse metabolic characteristics (10), hence we used waist circumference as a diagnostic measure for abdominal obesity. Inclusion criteria: waist size ≥ 102 cm for men and waist size ≥ 88 cm for women (11). Exclusion criteria: Participants with missing information on cognitive function and covariates.

2.2 Ethics statement

Participants who were included in the NHANES database were required to sign an informed consent form. This form has been reviewed and approved by the National Center for Health Statistics Ethics Review Board.

2.3 Data selection and measurements

NHANES administered a battery of cognitive performance assessments to participants from 2011 to 2014. This battery of tests included the Consortium to Establish a Registry for Alzheimer's Disease (CERAD), Word Learning subtest, Delayed Word Recall Test, Animal Fluency Test (AFT), and Digit Symbol Substitution Test (DSST) (12). These tests have been commonly used in large-scale screenings and clinical investigations aimed at assessing the cognitive abilities of older individuals (13–15). The overall score for the CERAD exam was calculated using three Word Learning subtests and a delayed memory test.

The criteria for cognitive dysfunction were based on previous studies (16), which averaged Z-scores for CERAD, AFT, and DSST to generate a composite Z score representing overall cognitive performance. The Z-score formula was as follows: $Z = (x-u)/\sigma$, where x represented the test score for each participant, u referred to the mean test score across all participants, and σ referred to the standard deviation of the test scores across participants. Cognitive dysfunction was defined as a Z-score below the 25th percentile, corresponding to a value of -0.56 in our sample.

We selected several potential predictors that may have an impact on cognitive development based on contemporary clinical practice and relevant academic research (17, 18). These predictors included sociodemographic characteristics, lifestyle factors (such as 24-hour nutrient intake, physical activity intensity, sleep time, smoking, etc.), anthropometric variables, laboratory examination variables, and disease status.

Sociodemographic characteristics of the participants included the age, sex, race, education, and marital status. The 24-hour total nutrient intake data were obtained through a 24-hour dietary recall method conducted by two nutritionists. Dietary review interviews took place at a mobile physical examination center, and the calorie and nutrient content of each food and drink were calculated based on the quantity and corresponding nutrients reported by the United States Department of Agriculture. The following nutrient intake variables were included in the research: total energy (kcal), protein (g), carbohydrate (g), dietary fiber (g), fat (g), and alcohol intake (g). Physical activity intensity included vigorous work activity, moderate work activity, vigorous recreational activity, moderate recreational activity, and sedentary minutes. Work and recreational activities were considered if engaged in for more than ten minutes. Sleep time referred to the number of hours slept on weekday nights. A respondent was considered a smoker if they had smoked more than 100 cigarettes in their lifetime. Anthropometric variables included body mass index (BMI) calculated as kg/m^2. The laboratory tests included red blood cell folate concentration (RBC folate) (nmol/L), glycosylated hemoglobin (%), plasma albumin (g/L), and serum creatinine (mmol/L). Prevalence of hypertension, diabetes, asthma, arthritis, gout, angina, stroke, liver disease, and depression were also considered. Depression was assessed using the Patient Health Questionnaire-9 (PHQ-9), with a score ≥ 5 indicating depression (19). The remaining chronic diseases were based on questionnaire survey data.

2.4 Statistical analysis

R software (version 4.2.3) was used for statistical analysis. Shapiro-Wilk tests were performed to check for normal distribution of continuous data. Independent samples t-tests were used to evaluate differences between two normally distributed datasets, and the variables were described with mean ± standard deviation (SD). Mann-Whitney U test was used to analyze differences between the two datasets without normal distribution, and the variables were described with the median and interquartile range (IQR). Meanwhile, chi-square test or Fisher's exact test was utilized for comparison of categorical variables. Besides, the variables were described with percentages.

The data was randomized into either training set (n = 1043) or validation set (n = 447) using a 7:3 ratio. Lasso regression analysis (20) was applied to the training sample data to select predictors of cognitive dysfunction in abdominal obesity, and the appropriate lambda (λ) value was determined through 10-fold cross-validation. The selected variables were then incorporated into multifactor logistic regression analysis to further filter the variables. The predicted variables with p values < 0.05 were incorporated into the nomogram model.

Once the nomogram was constructed, we assessed its performance using various metrics. The consistency index (C-index), the area under the receiver operating characteristic (ROC) curve (AUC), and calibration curves were used to evaluate the predictive ability. Decision curve analysis (DCA) was adopted to assess the clinical applicability of the nomogram.

3 Results

3.1 Baseline characteristics

Between 2011 and 2014, NHANES included a total of 6,222 individuals with abdominal obesity. After excluding participants

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with missing information on cognitive dysfunction and covariates, 1,490 participants in total were included in the study. The baseline characteristics of these subjects are presented in Table 1. Among them, 882 (59.19%) were female and 608 (40.81%) were male. Approximately 25.23% of the participants reported cognitive dysfunction. Randomly chosen participants with abdominal obesity were split into a training group (n=1,043) and a validation group (n=447).

TABLE 1 Baseline characteristics of the study population.

Variable	Total (n=1490)	Non-cognitive dysfunction (n=1114)	cognitive dysfunction (n=376)	P-value
Age (year)	68.00[63.00, 75.00]	68.00[63.00, 73.00]	71.00[65.75, 78.00]	<0.001
Sex (%)				
Male	608(40.81)	423(37.97)	185(49.20)	
female	882(59.19)	691(62.03)	191(50.80)	0.001
Race (%)				
Mexican American	131(8.79)	85(7.63)	46(12.24)	
Other Hispanic	142(9.53)	80(7.18)	62(16.49)	
Non-Hispanic White	811(54.43)	668(59.96)	143(38.03)	
Non-Hispanic Black	343(23.02)	230(20.65)	113(30.05)	
Other Race	63(4.23)	51(4.58)	12(3.19)	<0.001
Education (%)				
Less than 9th grade	140(9.40)	48(4.31)	92(24.47)	
9-11th grade	208(13.96)	114(10.23)	94(25.00)	
High school graduate	380(25.50)	279(25.04)	101(26.86)	
Some college graduate	459(30.81)	399(35.82)	60(15.96)	
College graduate Or above	303(20.33)	274(24.60)	29(7.71)	<0.001
Marital (%)				
Married/Living with Partner	852(57.18)	648(58.17)	204(54.25)	
Widowed/Divorced/Separated	553(37.11)	404(36.27)	149(39.63)	
Never married	85(5.71)	62(5.56)	23(6.12)	0.415
BMI (kg/m^2)	30.65[27.70, 34.20]	30.60[27.60, 34.10]	30.80[28.10, 34.40]	0.232
24-hour total nutrient intake				
Total energy (kcal)	1675.00[1240.75, 2195.50]	1750.50 [1292.25, 2255.25]	1456.50[1088.5, 2007.0]	<0.001
Protein (g)	64.16 [46.50, 88.37]	66.66[48.85, 89.75]	58.67[42.10, 82.95]	<0.001
Carbohydrate (g)	200.69[148.80, 271.49]	204.80[153.79, 272.96]	186.19[133.62, 261.97]	<0.001
Dietary fiber (g)	14.30[9.60, 20.90]	14.80[10.00, 21.30]	12.80[8.30, 19.00]	<0.001
Total fat (g)	62.19[42.00, 90.87]	67.00[44.11, 93.93]	52.41[35.63, 77.96]	<0.001
Alcohol (g)	0.00[0.00, 0.00]	0.00[0.00, 0.00]	0.00[0.00, 0.00]	0.001
Laboratory tests				
RBC folate (nmol/L)	1260.00[908.25, 1727.50]	1290.00(927.25, 1730.00]	1195.00[877.25, 1720.00]	0.030
glycosylated hemoglobin (%)	5.80[5.50, 6.40]	5.80[5.50, 6.20]	6.00[5.60, 6.70]	<0.001
Albumin (g/L)	42.00[40.00, 44.00]	42.00[40.00, 44.00]	41.00[39.75, 43.00]	0.001
Serum creatinine (mmol/L)	80.44[68.07, 99.01]	79.56[67.18, 96.36]	83.98[69.62, 110.50]	<0.001
Hypertension	998(66.98)	736(66.07)	262(0.67)	0.197

(Continued)

TABLE 1 Continued

Variable	Total (n=1490)	Non-cognitive dysfunction (n=1114)	cognitive dysfunction (n=376)	P-value
Diabetes (%)	412(27.65)	269(24.15)	143(38.03)	<0.001
Asthma	220(14.77)	165(14.81)	55(14.63)	0.930
Arthritis	811(54.43)	581(52.15)	230(61.17)	0.002
Gout	132(8.86)	88(7.90)	44(11.70)	0.024
Angina	86(5.77)	57(5.12)	29(7.71)	0.061
Stroke	90(6.04)	52(4.67)	38(10.11)	<0.001
Liver disease	87(5.84)	58(5.21)	29(7.71)	0.073
Depression	400(26.85)	252(22.62)	148(39.36)	<0.001
Physical activity (%)				
Vigorous work activity	161(10.81)	141(12.66)	20(5.31)	<0.001
Moderate work activity	439(29.46)	363(32.59)	76(20.21)	<0.001
Vigorous recreational activity	113(7.58)	102(9.16)	11(2.93)	<0.001
Moderate recreational activity	558(37.45)	460 (41.29)	98(26.06)	<0.001
Sedentary(min)	360.00[240.00, 480.00]	360.00[240.00, 480.00]	360.00[240.00, 480.00]	0.270
Sleep time(h)	7.00[6.00, 8.00]	7.00[6.00, 8.00]	7.00[6.00, 8.00]	0.775
Smoking (%)	747(50.13)	554(49.73)	193(51.33)	0.591

3.2 Results of Lasso regression and logistic regression analysis

Lasso regression analysis was performed to identify optimal predictors (Figure 1). These predictors were then used in multivariate logistic regression analysis, which revealed that age ($p < 0.001$), sex ($p < 0.001$), education level ($p < 0.001$), 24-hour total fat intake ($p < 0.001$), RBC folate ($p = 0.007$), depression ($p < 0.001$), and moderate work activity ($p = 0.008$) were related with cognitive dysfunction in individuals with abdominal obesity with statistical significance (Table 2).

3.3 Development of nomogram

According to the findings of Lasso and logistic regression analyses, a prediction model consisting of seven predictors (age, sex, education, total fat intake, RBC folate, depression, and

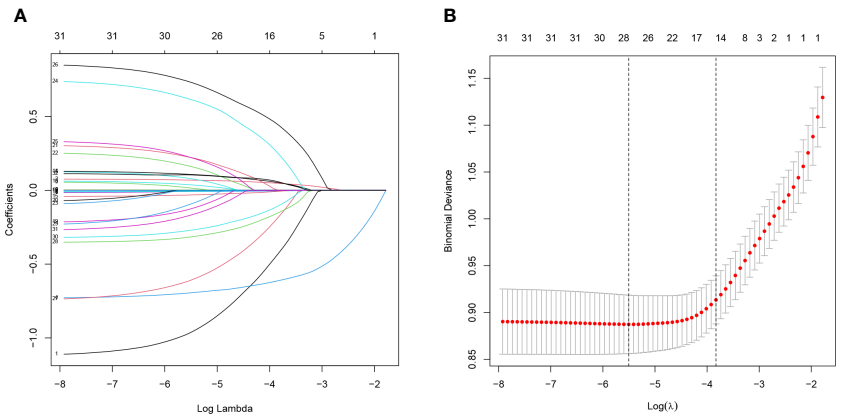

FIGURE 1
Lasso regression analysis of risk factors for cognitive dysfunction in abdominal obesity. **(A)** coefficient profile was created using the logarithmic (lambda) sequence, and the optimal lambda yielded non-zero coefficients. **(B)** Tenfold cross-validation was used to identify the ideal parameter (lambda) in the LASSO model utilizing minimal requirements. Plotting was done with the partial likelihood deviation (binomial deviation) curve in relation to log (lambda). A virtual vertical line at the optimal value was drawn using one SE of minimum criterion (the 1-SE criterion).

TABLE 2 Multivariate Logistic regression analysis of the risk of cognitive dysfunction in people with abdominal obesity.

Variable	Multivariate analysis	95%CI	P-value
	Odds ratio		
Age	1.07	1.04-1.10	<0.001
Sex			
Male	Reference		
Female	0.36	0.23-0.55	<0.001
Education			
Less than 9th grade	Reference		
9-11th grade	0.54	0.28-1.05	0.070
High school graduate	0.24	0.13-0.45	<0.001
Some college graduate	0.10	0.05-0.20	<0.001
College graduate or above	0.08	0.04-0.18	<0.001
Total fat	0.99	0.98-0.99	<0.001
RBC folate	1.00	0.99-1.00	0.007
Depression			
Yes	2.34	1.56-3.53	<0.001
No	Reference		
Moderate work activity			
Yes	0.56	0.36-0.86	0.009
No	Reference		

moderate work activity) was constructed. This model was represented as a nomogram (Figure 2). According to different variables, a vertical line is drawn at the top of the nomogram to obtain the corresponding score. The scores of each variable are added together to obtain the total score, and the corresponding total risk score is obtained at the bottom of the nomogram. For example, if a 72-year-old male graduated from high school at a young age with a total fat intake of 80g in 24 hours, a red blood cell folate concentration of 1500 nmol/L, no history of depression, and no moderate physical activity, the total score is 237.5 points (28 points for gender, 31 points for age, 44 points for high school graduation, 72 points for total fat intake in 24 hours, 42.5 points for red blood cell folate concentration, 0 point for history of depression, and 20 points for moderate physical activity). For the mentioned patient, the total score is 237.5, and the probability corresponding to this total score on the nomogram will be between 0.3 and 0.4. The probability of cognitive dysfunction in this elderly person is estimated to be between 30% and 40%.

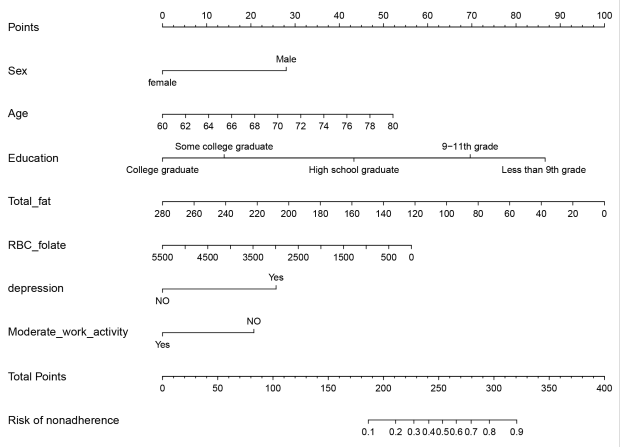

FIGURE 2
Nomogram prediction model for cognitive dysfunction in abdominal obesity.

3.4 Assessment of predictive nomogram

The validity and discriminative ability of the nomogram were evaluated using various metrics. The C-index for the training set was 0.814 (95% CI: 0.875-0.842), indicating good discriminability. The C-index for the validation set was 0.805 (95% CI: 0.758-0.851), further confirming the model's discriminative power. The sensitivity, specificity, and AUC of the training group were 0.814, 0.659, and 0.822, respectively. For the validation group, the AUC was 0.795, specificity was 0.761, and sensitivity was 0.718 (Figure 3). These results demonstrated the strong discriminative and predictive capabilities of the nomogram. The calibration curves for both sets were nearly straight lines with a slope of 1, indicating good agreement between predicted probabilities and actual outcomes (Figure 4). DCA was applied to assess the clinical validity of the model (Figure 5). The DCA showed that the prediction model provided net benefits for both the training and validation sets, indicating superior net benefits and prediction accuracy of the nomogram model.

4 Discussion

Over the past few years, there has been a consistent rise in the prevalence of obesity (21), highlighting a concerning trend in public health. This alarming rise in obesity rates has captured the attention of individuals, healthcare professionals, and governments alike. The prevalence and mortality of dementia are also increasing due to aging and population growth (22). One study, which followed up individuals for 42 years, found that obesity was associated with dementia later in life (23). Other studies have investigated the relationship between pre-dementia BMI and subsequent dementia development, suggesting it may be a predictor (24). Obesity can impair cognitive functions such as memory, speech learning, and executive ability (25). Some studies have shown that not only the volume but also the location of fat deposition is associated with disease, particularly the deposition of visceral fat in the abdomen, which plays a significant role independent of total fat (26). A Mendelian randomized study involving nearly 10,000 Asian individuals found that visceral fat and BMI may contribute to cognitive decline. Each 0.27 kg increase in abdominal fat was equivalent to 0.7 years of cognitive decline (27). Another cohort

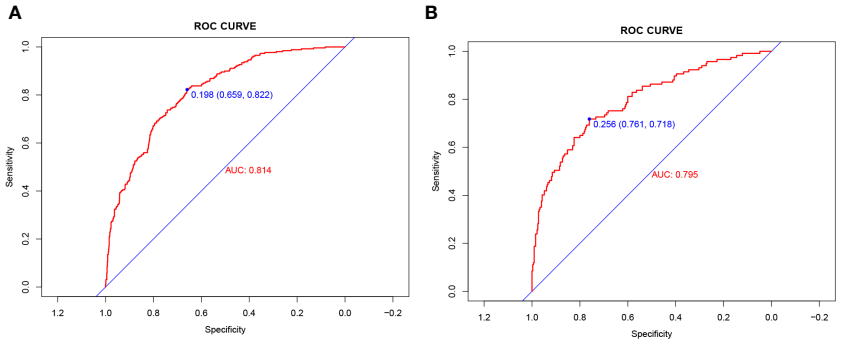

FIGURE 3
(A) Nomogram ROC curves generated from the training dataset. **(B)** Nomogram ROC curves generated using the validation dataset.

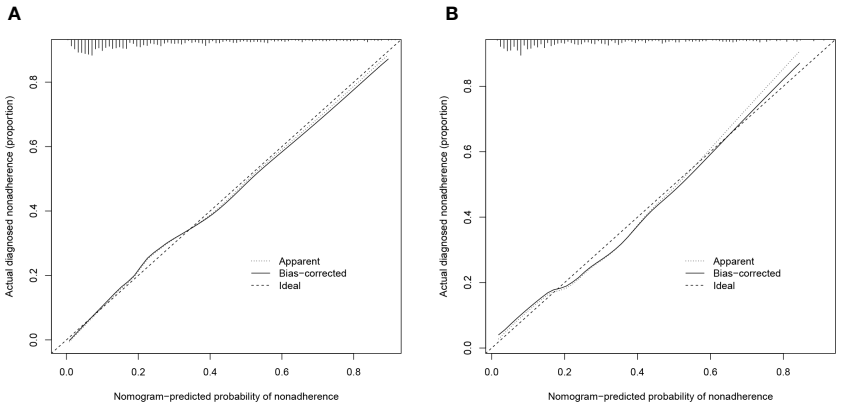

FIGURE 4
(A) Calibration plot for the training dataset. **(B)** Calibration plot for the validation dataset.

study, which included 9,189 participants and excluded those with clinically diagnosed cardiovascular disease while correcting for confounding factors such as educational attainment, arrived at a similar conclusion. It was found that for every 9.2% increase in body fat percentage or 36 milliliters of visceral fat, there was an accelerated aging of 1 year in terms of corresponding cognitive functions (28). Therefore, it is crucial to understand the risk predictors of cognitive dysfunction in abdominal obesity and take steps to reduce its incidence.

The results of this study revealed that age, sex, education, total fat intake, RBC folate, depression, and moderate work activity were important predictors of cognitive dysfunction in individuals with

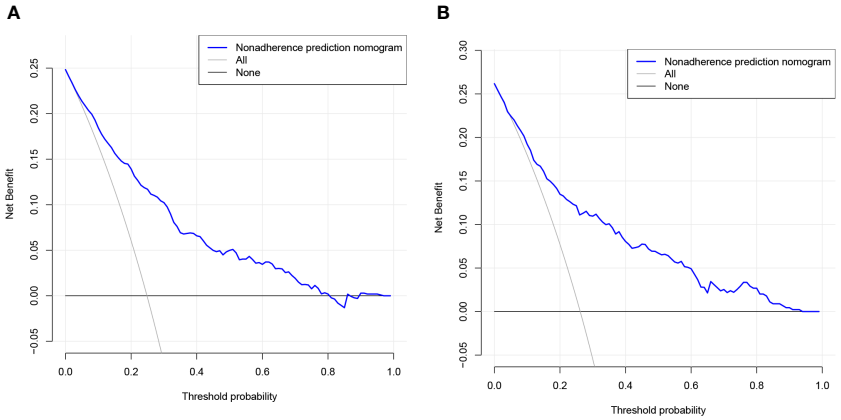

FIGURE 5
(A) DCA curves for the training dataset. **(B)** DCA curves for the validation dataset.

abdominal obesity. Age is a significant factor affecting cognition (29), which gradually declines with increasing age. Dario Bachmann et al. (30) showed that age is a major factor in cognitive variability, significantly correlating with white matter hyperintensities (WMH) and lateral ventricular volumes. Age also showed a strong correlation with cortical thickness in a large neocortical ROI (NEOcomp ROI) and hippocampal volume, leading to cognitive decline.

Our study found that males were more likely to have cognitive dysfunction. Research shows that men are more prone to developing mild cognitive impairment (31) compared to women. In individuals with the apolipoprotein E genotype $\epsilon 2/\epsilon 3$, males have a higher risk of developing Alzheimer's disease than females (32). Among older adult males, cognitive dysfunction is more likely to occur in those who are older, have higher education levels, are nulliparous, have depressive symptoms, and are socially inactive (33). Therefore, interventions should be tailored to different populations.

We also found that as education levels increased, the odds of cognitive dysfunction decreased in individuals with abdominal obesity. The mechanisms by which education improves cognitive abilities have not been fully understood yet. It may be related to the quality of education, occupational complexity, and participation in cognitive intellectual activities. Additionally, individuals with lower education levels may have less knowledge about diseases and receive less health education. Higher education and early access to education may, therefore, be beneficial in delaying cognitive decline in later life (34).

Dietary nutrient intake also has an impact on cognitive function (35). However, this study found that 24-hour total fat consumption was a risk indicator for cognitive dysfunction in individuals with abdominal obesity. These findings may be associated with the type of fat consumed; for example, consuming a high amount of polyunsaturated fatty acids may reduce the risk of cognitive dysfunction (36). Monounsaturated and polyunsaturated fatty acids can help lower LDL cholesterol levels and increase HDL cholesterol level (37). A prospective observational study by Gustafson et al. (38) also found that individuals who consumed higher amounts of total polyunsaturated fat had a lower risk of Alzheimer's disease and dementia. Therefore, further investigation is needed to understand the relationship between different types of dietary fat and cognitive function in obese individuals with abdominal obesity.

Furthermore, there is a significant link between folic acid intake and cognitive performance, with research showing that higher folic acid intake is associated with improved cognitive function when vitamin B12 intake is normal (39), which was consistent with our findings. Folate deficiency can lead to elevated homocysteine levels and affect cognitive function (40). In a controlled study, participants who received 800ug of oral folic acid daily experienced a 53.9% increase in serum folate concentration after three years, and improvements in cognitive abilities such as memory, information processing speed, as well as sensory motor speed were much better compared to the placebo group (41). Therefore, folic acid supplementation may help delay cognitive deterioration.

Depression is common in individuals with cognitive dysfunction. A 30-year study found that individuals with mental disorders in adolescence were more likely to develop dementia later in life compared to those without mental disorders (42). Additionally, Holly Elser et al. found that patients with depression had an increased cumulative risk of dementia compared to controls, with an overall hazard ratio of 2.41. A diagnosis of depression at an early, middle, or late age was linked to a higher dementia risk (43). Thus, paying attention to mental health at an early age may reduce or delay the burden of dementia later in life.

Moderate work activity, defined as work involving moderate physical activity like brisk walking or lifting light objects for 10 minutes continuously, resulting in a mild increase in respiration or heart rate, is consistent with most studies showing that moderate physical activity reduces the risk of dementia (44). Encouraging individuals with abdominal obesity to engage in moderate physical activity at work is therefore important.

The nomogram has been extensively used in various clinical trials and is a commonly employed predictive model in clinical practice (45, 46). However, no nomogram has been reported to predict the development of cognitive dysfunction based on data from individuals with abdominal obesity. This study used nine predictors preselected by LASSO regression analysis to build a nomogram for predicting the probability of cognitive dysfunction in individuals with abdominal obesity. The nomogram models were well calibrated and clinically relevant, enabling effective identification of cognitive dysfunction in individuals with abdominal obesity.

Although the C-index, ROC curves, calibration curves, and clinical utility of the nomogram have been well validated, the study does have a few limitations. Firstly, it is a cross-sectional study with potential selection bias, thus requiring validation through prospective and multi-center studies. Secondly, the diagnosis of cognitive impairment was assessed using various scales, which may introduce bias.

5 Conclusion

This study employs Lasso regression and multivariable logistic regression to select potential predictive factors. Ultimately, seven predictive variables, including age, sex, education, total fat intake, RBC folate, depression, and moderate work activity, are integrated into the nomogram. The data for these variables are readily accessible, and the predictive model is easy for clinical use. It is clinically significant for primary healthcare workers or primary care physicians to rapidly assess the risk of cognitive impairment in individuals with abdominal obesity. This helps control the development of cognitive impairments, thus reducing the socioeconomic burden and caregiving pressure.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/supplementary material.

Ethics statement

The studies involving humans were approved by the National Center for Health Statistics Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not

required from the participants or the participants' legal guardians/ next of kin in accordance with the national legislation and institutional requirements.

Author contributions

CL: Data curation, Formal analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing. GW: Data curation, Formal analysis, Investigation, Project administration, Writing – original draft, Writing – review & editing. YC: Methodology, Resources, Visualization, Writing – review & editing. HX: Methodology, Resources, Visualization, Writing – review & editing. JC: Software, Writing – review & editing. XYZ: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. YL: Validated, Writing – review & editing. ML: Validated, Writing – review & editing. RZ: Conceptualization, Investigation, Project administration, Supervision, Writing – review & editing. XFZ: Software, Writing – review & editing.

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Conflict of interest

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Lei Sha,
China Medical University, China

REVIEWED BY
Bing Zhang,
Nanjing Drum Tower Hospital, China
Luca Steardo,
University Magna Graecia of Catanzaro, Italy

*CORRESPONDENCE
Dongsheng Zhang
yunyun122424@126.com

†These authors have contributed equally to this work and share first authorship

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Analysis of white matter tract integrity using diffusion kurtosis imaging reveals the correlation of white matter microstructural abnormalities with cognitive impairment in type 2 diabetes mellitus

Jie Gao[1†], Peichun Pan[2†], Jing Li[3], Min Tang[1], Xuejiao Yan[1], Xin Zhang[1], Man Wang[1], Kai Ai[4], Xiaoyan Lei[1], Xiaoling Zhang[1] and Dongsheng Zhang[1*]

[1]Department of MRI, Shaanxi Provincial People's Hospital, Xi'an, China, [2]Department of Graduate, Shaanxi University of Chinese Medicine, Xianyang, China, [3]Department of Graduate, Xi'an Medical University, Xi'an, China, [4]Department of Clinical Science, Philips Healthcare, Xi'an, China

Background: This study aimed to identify disruptions in white matter integrity in type 2 diabetes mellitus (T2DM) patients by utilizing the white matter tract integrity (WMTI) model, which describes compartment-specific diffusivities in the intra- and extra-axonal spaces, and to investigate the relationship between WMTI metrics and clinical and cognitive measurements.

Methods: A total of 73 patients with T2DM and 57 healthy controls (HCs) matched for age, sex, and education level were enrolled and underwent diffusional kurtosis imaging and cognitive assessments. Tract-based spatial statistics (TBSS) and atlas-based region of interest (ROI) analysis were performed to compare group differences in diffusional metrics, including fractional anisotropy (FA), mean diffusivity (MD), axonal water fraction (AWF), intra-axonal diffusivity (D_{axon}), axial extra-axonal space diffusivity ($D_{e,//}$), and radial extra-axonal space diffusivity ($D_{e,\perp}$) in multiple white matter (WM) regions. Relationships between diffusional metrics and clinical and cognitive functions were characterized.

Results: In the TBSS analysis, the T2DM group exhibited decreased FA and AWF and increased MD, $D_{e,\parallel}$, and $D_{e,\perp}$ in widespread WM regions in comparison with the HC group, which involved 56.28%, 32.07%, 73.77%, 50.47%, and 75.96% of the mean WM skeleton, respectively ($P < 0.05$, TFCE-corrected). $D_{e,\perp}$ detected most of the WM changes, which were mainly located in the corpus callosum, internal capsule, external capsule, corona radiata, posterior thalamic radiations, sagittal stratum, cingulum (cingulate gyrus), fornix (stria terminalis), superior longitudinal fasciculus, and uniform fasciculus. Additionally, $D_{e,\perp}$ in the genu of the corpus callosum was significantly correlated with worse performance in TMT-A ($\beta = 0.433$, $P < 0.001$) and a longer disease duration ($\beta = 0.438$, $P < 0.001$).

Conclusions: WMTI is more sensitive than diffusion tensor imaging in detecting T2DM-related WM microstructure abnormalities and can provide novel insights into the possible pathological changes underlying WM degeneration in T2DM. $D_{e,\perp}$ could be a potential imaging marker in monitoring disease progression in the brain and early intervention treatment for the cognitive impairment in T2DM.

KEYWORDS

type 2 diabetes mellitus, diffusion kurtosis imaging, microstructure, white matter tract integrity, neuroimaging

1 Introduction

Type 2 diabetes (T2DM) is the most common metabolic disease with a high incidence worldwide. According to the latest data from the International Diabetes Federation in 2019, the number of people with T2DM worldwide was 463 million, and the global prevalence rate of T2DM was 9.3% (1). Brain damage caused by persistent hyperglycemia, also defined as diabetic encephalopathy, which includes changes in neurophysiology and brain structures as well as the resultant cognitive impairment involving memory, attention and executive functions (2, 3), has recently attracted increasing attention. Cognitive impairment in T2DM is usually not necessarily accompanied by subjective cognitive complaints (4). Furthermore, with the increasing prevalence of diabetes and an aging population, the incidence of cognitive impairment is expected to increase gradually, posing great challenges for future health effects. However, the neuropathological basis of the cognitive impairment associated with T2DM is still unclear.

A series of recent neuroimaging studies have revealed that T2DM is accompanied by cerebral microstructural impairments related to cognitive decline. In addition to gray matter atrophy, white matter (WM) microstructural abnormalities are also believed to play a prominent role in T2DM-induced cognitive impairment (5–8). Diffusion tensor imaging (DTI) can provide an effective and quantitative method to delineate WM microstructural changes. The integrity of the WM may be inferred on the basis of changes in DTI-derived parameters, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), potentially providing evidence to evaluate pathological changes in T2DM. The existing studies based on DTI have confirmed extensive WM microstructural abnormalities in T2DM, and some important regions of the WM have also been demonstrated to be correlated with neuropsychological performance (9–11). Furthermore, the WM changes in T2DM mainly manifested as decreased FA and increased MD and RD, which may reflect axonal degeneration, myelin breakdown, or other factors. However, due to the non-specificity of DTI measures, this approach showed limitations in determining the altered structures underlying these diffusional abnormalities. In addition, since the DTI model assumes a Gaussian distribution of water-diffusion processes with a mono-exponential signal decay, it cannot easily characterize crossing fibers, leading to limited information.

Diffusional kurtosis imaging (DKI) is a clinically feasible extension of DTI that examines the additional contribution of non-Gaussian diffusion effects to provide additional information on brain microstructural complexity (12). The derived metrics from DKI, including mean kurtosis (MK), axial kurtosis ($K_{\parallel}$), and radial kurtosis ($K_{\perp}$), can measure the complexities of structures and can be used to assess white matter and gray matter simultaneously. Although effective, DKI metrics can only probe voxel-level diffusion that mingles the effects of heterogeneous microenvironments but are not specific to features of microstructure, since they are calculated based on a model of brain tissue microstructure involving a single compartment. This limitation makes it difficult to interpret potential neuropathological changes. For example, the reduced MK, $K_{\perp}$ and $K_{\parallel}$ could be caused by a reduction in axons, demyelination and an increase of free water in the outer space of the axon (or both). Subsequently, a two-compartment non-exchange diffusion model based on DKI has been proposed to identify compartment-specific white matter tract integrity (WMTI) in the intra-axonal spaces and extra-axonal spaces (13). Notably, the derived metrics, including the axonal water fraction (AWF), intra-axonal diffusivity (D_{axon}), and extra-axonal axial and radial diffusivities ($D_{e,//}$and $D_{e,\perp}$), offer enhanced insights into the microstructural features. D_{axon} serves as a biomarker with high sensitivity and specificity for detecting axonal abnormalities. $D_{e,//}$and $D_{e,\perp}$indicates a relatively free water content in the extra-axonal space, as well as the integrity of myelin sheath. AWF quantifies the proportion of water within the axons relative to the total water fraction encompassing both intra- and extra-axonal water. These metrics greatly improve the characterization of subtle tissue microstructural changes, and have been demonstrated to deepen the understanding of WM alterations in various neurological disorders, such as Alzheimer's disease (14), multiple sclerosis (15), aging (16), and mild traumatic brain injury (17).Since WMTI metrics can reveal more specific diffusivity changes from water inside the axons (potentially glial processes) and outside axons (excluding water from myelin sheath and interstitial space), this study aims to (1) determine whether WMTI metrics are more sensitive than DTI metrics in investigating WM microstructural changes in patients with T2DM; (2) further speculate the possible neuropathological changes associated with cognitive impairment in

T2DM patients based on additional information provided by WMTI metrics; and (3) investigate the correlations between altered WMTI metrics and clinical/cognitive variables to identify the imaging biomarkers of cognitive decline in patients with T2DM.

2 Materials and methods

2.1 Participants

The study was conducted in accordance with the Helsinki declaration. The Human Ethics Committee of Shaanxi Provincial Peoples Hospital approved all experimental procedures after receiving informed consent (the ethical committee protocol number: 2022K101).

Seventy-three patients with T2DM were recruited from the Department of Endocrinology of Shaanxi Provincial People's Hospital, and 57 healthy controls (HCs) matched for age, sex, and education level were obtained from our health examination center. All the participants were between 40 and 70 years of age, right-handed, and educated for at least six years. The T2DM patients met the standard criteria proposed by the American Diabetes Association (18) and without a history of hypoglycemia or hyperglycemia. The other inclusion criteria in the HC group were as follows: (1) no symptoms or a family history of diabetes; (2) fasting glucose < 7.0 mmol/L; and (3) glycosylated hemoglobin A1c (HbA1c) level < 6.0%. The exclusion criteria for both groups were as follows: (1) history of neurological disorders, such as cerebral infarctions, brain tumors, vascular malformations, or traumatic brain injury; (2) illicit substance abuse, alcohol abuse, or psychiatric disorders; (3) any other systemic disease irrelevant to diabetes and affecting cognition; (4) periventricular or deep WM hyperintensities with a Fazekas score > 1 on T2-weighted fluid-attenuated inversion recovery (FLAIR) images; and (5) inability to complete MRI examinations or unsatisfactory MRI images.

2.2 Clinical data and neuropsychological test results

All participants' clinical data, including information regarding their age, sex, educational level, blood pressure, height, weight, and body mass index (BMI), were obtained from medical records and questionnaires. Laboratory tests, including blood biochemical analysis and evaluation of fasting plasma glucose (FPG) and HbA1c levels were performed and their results were recorded. All participants underwent the following series of neuropsychological assessments: Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were used to assess general cognitive function; Trail-Making Test A (TMT-A) reflected cognitive processing skills (psychomotor speed, processing speed, and visuospatial skills) and attention; Trail-Making Test B (TMT-B) provided information regarding executive function; Auditory Verbal Learning Test (AVLT) was used to evaluate episodic memory; and Clock Drawing Test (CDT) was performed to evaluate visuospatial skills. Neuropsychological tests were performed by a psychiatrist trained in systematic testing.

2.3 Image acquisition

Conventional MRI and DKI data were obtained using a 3.0 T MR scanner (Ingenia, Philips Medical Systems, the Netherlands) equipped with a 16-channel phase-array head coil. Conventional MRI, including sagittal three-dimensional T1-weighted imaging (repetition time [TR]/excitation time [TE] = 7.5/3.5 ms, field of view [FOV] = 250 × 250 mm^2, matrix = 256 × 256, slice thickness = 1 mm) and T2 FLAIR (TR/TE = 6000/150 ms, FOV = 230 × 230 mm^2, matrix = 320 × 320, slice thickness = 6 mm) were used to identify visible brain lesions. DKI was performed using a spin-echo echo-planar imaging sequence with the following parameters: TR/TE = 6000/150 ms, slice thickness = 6 mm, FOV = 224 × 224 mm^2, matrix = 112 × 112, number of excitations = 1, b value = 0, 1000, 2000 s/mm^2, 32 diffusion encoding directions for each nonzero b value with the same distribution. The gradient directions were uniformly distributed by performing the electro-static repulsion method (19, 20).

2.4 Data processing

Data pre-processing included the following steps. First, the image format was converted from DICOM to NIFTI. Second, non-brain tissues, such as the scalp and skull, were removed using the Brain Extraction Tool (BET), which was performed in FMRIB's Software Library (FSL). Third, the diffusion-weighted images were realigned to the non-weighted b0 images to correct head motion and eddy current-induced distortions (also a tool of FSL). Finally, artifact-corrupted diffusion-weighted imaging scans were excluded by using an automated method (21). Only two b values (0 and 1000 s/mm^2) were used for DTI fitting, which was performed with the FMRIB diffusion toolbox (FDT), and four DTI parameters, including FA and MD, were obtained. The kurtosis tensor was estimated by using a constrained weighted linear least squares method (CWLLS) (22, 23) in MATLAB (MathWorks, Natick, Massachusetts). Then, the WMTI metrics, including AWF, D_{axon}, $D_{e,\parallel}$, and $D_{e,\perp}$, were estimated using the algorithms of the WMTI model (13) with an in-house program implemented in MATLAB.

2.5 TBSS analysis

FSL with tract-based spatial statistics (TBSS, part of FSL) was used to analyze all of the above DTI and WMTI metrics and compare intergroup differences in WM. All participants' FA images were first aligned to a common target FMRIB58_FA in the MNI 152 standard space using nonlinear registration. Then, the mean FA image and its skeleton were generated. The aligned FA image of each participant was projected onto the mean FA skeleton (threshold = 0.2). The resulting FA skeleton images were fed into

voxel-wise cross-subject statistics to identify intergroup differences in major WM tracts. The number of permutations was set at 5000. The results were corrected for multiple comparisons by controlling the family-wise error rate after threshold-free cluster enhancement (TFCE). TBSS analysis was also performed for other DTI and WMTI metrics parameters. The results of all tests were considered to be significant at $P < 0.05$. The Johns Hopkins University (JHU) WM ICBM-DTI-81 WM labels atlas in FSL (http://fsl.fmrib.ox.ac.uk/fsl/fslwiki/Atlases) was used to label significant tracts.

2.6 ROI analysis

ROI analysis was also performed based on the Johns Hopkins University WM label atlas, in which the entire WM was parceled into 48 ROIs. The FA, MD, and WMTI metrics were measured in these ROIs and reported as mean ± standard deviation. The ROI-based comparisons between the T2DM and the HC groups were further performed in regions that showed significant differences in the TBSS analysis described above.

The correlations between DTI and WMTI metrics within the resultant ROIs and the neuropsychological assessment scores, disease duration, and HbA1c level in T2DM patients were analyzed by using the multiple linear regression analyses, with age, sex, and years of education as the covariates. The Bonferroni correction was applied to correct for multiple comparisons. The regression coefficients (β) and *P* values were calculated.

2.7 Statistical analysis

The demographic, clinical, and neuropsychological data were analyzed by using SPSS (*SPSS version 20.0*, IBM, USA). The measurement data were reported as mean ± standard deviation, and categorical data were presented as frequencies and percentages. Intergroup comparisons of these data were performed using Student's *t* tests or χ^2 tests as appropriate. All tests were taken to be significant at $P < 0.05$.

3 Results

3.1 Clinical and neuropsychological data

Clinical and neuropsychological data are presented in Table 1. The T2DM and control groups showed no significant differences in sex, age, years of education, BMI, blood pressure, and the total cholesterol, triglyceride, and low-density lipoprotein levels. The T2DM group showed higher HbA1c and FPG levels and lower high-density lipoprotein levels than the HC group ($P < 0.001$). Of the 73 patients with T2DM, 32 were receiving insulin treatment, and the remaining 41 patients were receiving oral hypoglycemic agents. The T2DM group showed poorer performance in general cognitive function (MMSE, $P = 0.016$; MoCA, $P < 0.001$). In addition, the T2DM group showed significantly worse results in the cognitive domains of attention, executive function, and episodic memory than the HCs.

3.2 WM skeleton voxel-wise TBSS comparisons

Figure 1 shows the derived FA, MD, AWF, D_{axon}, $D_{e,\parallel}$ and $D_{e,\perp}$ maps from an HC for illustration. In TBSS analysis, the T2DM group exhibited significantly deceased FA and AWF and increased MD, $D_{e,\parallel}$, and $D_{e,\perp}$ over widespread WM regions (as shown in Figure 2), which involved 56.28% (33874/60190 voxels), 32.07% (19303/60190 voxels), 73.77% (44404/60190 voxels), 50.47% (30380/60190 voxels), and 75.96% (45721/60190 voxels) of the mean WM skeleton, respectively ($P < 0.05$, TFCE-corrected). Additionally, increased D_{axon} values were only found in some discrete WM regions in the T2DM group compared with HC group. To recognize the more robust and severely impaired WM tracts in T2DM patients, additional investigations with more strict criteria ($P < 0.01$, TFCE-corrected) were performed in the TBSS analysis. These results also showed that $D_{e,\perp}$ exhibited more involved WM regions (43.83%) than FA and MD (39.66% and 29.45%, respectively), which were mainly located in the whole corpus callosum (CC), internal capsule (IC), external capsule (EC), corona radiata (CR), posterior thalamic radiations (PTR), sagittal stratum (SS), cingulum (cingulate gyrus), fornix (stria terminalis), superior longitudinal fasciculus (SLF), and uncinate fasciculus (UF).

3.3 Group differences in the atlas-based tract ROIs

In the ROI-based quantitative analysis, the T2DM patients again showed similar changes in FA, AWF, MD, D_{axon}, $D_{e,\parallel}$, and $D_{e,\perp}$ in most WM regions as in the TBSS analysis. In the 48 WM tracts defined in the JHU WM ICBM-DTI-81 WM labels, more regions had increased $D_{e,\perp}$ (28/48 regions) than decreased FA (18/48 regions) in the T2DM group (shown in Tables 2, 3). Figure 3 summarizes the FA and $D_{e,\perp}$ differences in some WM fiber tracts. Notably, only $D_{e,\perp}$ differences were found in some crossing fibers, including the pontine crossing tract, bilateral PTR, right SLF, and bilateral UF.

3.4 Correlations between ROI-wise diffusion metrics and cognitive and clinical data

Based on the WM fiber tracts showing significant intergroup differences, we explored the relationships between diffusion metrics (FA and $D_{e,\perp}$) of these tracts and the neuropsychological scores, disease duration, and HbA1c level in T2DM patients. The results showed that higher $D_{e,\perp}$ values in the genu of the CC (GCC) were

TABLE 1 Clinical characteristics of participants.

Clinic information	T2DM (n = 73)	HC (n =57)	T/χ^2 value	*P* value
Gender (male/female)	55/18	38/19	1.183	0.277[#]
Age (years)	55.64 ± 7.65	54.18 ± 5.64	-1.260	0.210
Formal education (years)	13.77 ± 2.40	14.05 ± 2.52	-0.914	0.361
BMI (kg/m^2)	25.16 ± 2.73	24.46 ± 2.72	-1.462	0.146
Systolic pressure (mmHg)	127.36 ± 15.92	125.70 ± 13.41	-0.342	0.732
Diastolic pressure (mmHg)	79.08 ± 11.14	82.09 ± 10.73	1.551	0.123
Total cholesterol (mmol/L)	4.44 ± 1.15	4.75 ± 0.77	1.799	0.074
Triglycerides (mmol/L)	2.05 ± 1.35	1.73 ± 1.07	-1.432	0.154
HDL (mmol/L)	1.04 ± 0.27	1.31 ± 0.29	5.572	<0.001
LDL (mmol/L)	2.61 ± 0.81	2.82 ± 0.67	1.595	0.113
Fasting glucose (mmol/L)	7.81 ± 2.28	5.07 ± 0.56	-9.931	<0.001
HbA1c (%)	7.85 ± 2.05	5.44 ± 0.43	-9.011	<0.001
Diabetes duration (year)	8.34 ± 5.93	/	/	/
Insulin use(n/%)	32/43.84	/	/	/
MMSE	28.09 ± 1.64	28.82 ± 1.14	-2.407	0.016
MoCA	24.81 ± 2.64	27.59 ± 0.76	-6.624	<0.001
TMT-A(s)	82.59 ± 25.27	73.09 ± 25.14	-2.132	0.035
TMT-B(s)	163.15 ± 49.02	137.19 ± 56.79	-2.794	0.006
CDT	25.77 ± 5.92	26.04 ± 3.12	-1.699	0.089
AVLT-total	40.44 ± 6.67	43.09 ± 5.65	2.401	0.018
AVLT-delay recall	8.19 ± 2.82	8.51 ± 2.32	0.686	0.494

Data are presented as mean ± standard deviation or number (%) unless otherwise indicated. BMI, body mass index; HDL, high density lipoprotein; LDL, low density lipoprotein; HbA1c, glycated hemoglobin; MMSE, Mini-Mental State Examination; MoCA: Montreal Cognitive Assessment; TMT-A, Trail Making Test A; TMT-B, Trail Making Test B; CDT, clock drawing test; AVLT, Auditory Verbal Learning Test. [#]The P value was obtained using a χ^2 test. / means there is no data in the corresponding cells.

significantly correlated with worse performance in the TMT-A (β = 0.433, $P < 0.001$) and longer disease duration (β = 0.438, $P < 0.001$), as shown in Figure 4. Subsequently, we conducted additional analysis employing bootstrap methodology to examine the mediating influence of disease duration on the relationship between $D_{e,\perp}$ values in the GCC and TMT-A. The findings indicated that the indirect impact of disease duration was not statistically significant (β = 0.041, 95%[CI] = [-.171, 0.253]). The comprehensive statistical outcomes are presented in Table 4. $D_{e,\perp}$ in the resultant ROIs was not significantly correlated with the HbA1c level ($R < 0.4$ or $P > 0.05$). FA was not significantly correlated with any the neuropsychological scores, disease duration, or HbA1c level ($R < 0.4$ or $P > 0.05$).

4 Discussion

The present study is the first to investigate WM microstructural changes in T2DM patients by using WMTI models based on DKI. This study demonstrated decreased FA and AWF and increased MD, $D_{e,//}$, and $D_{e,\perp}$ in widespread WM regions in T2DM patients. Additionally, our findings suggest that among all diffusional metrics, $D_{e,\perp}$ is the most sensitive metric for detecting WM disruptions. In particular, the T2DM and HC groups showed only $D_{e,\perp}$ differences in some crossing fibers, including the pontine crossing tract, bilateral PTR, right SLF, and bilateral UF. Additionally, $D_{e,\perp}$ showed significant correlations with disease duration and cognitive performance, potentially providing valuable imaging evidence for predicting cognitive impairment.

Chronic brain damage in patients with T2DM has been a topic of concern, and understanding the pathogenesis of WM impairment in patients with T2DM has become increasingly important, since WM is believed play a prominent role in T2DM-induced cognitive impairment. Previous DTI studies employing TBSS, tractography, and voxel-based methods have consistently shown a reduction in FA and an elevation in MD within the WM of individuals with T2DM (7, 24). DTI is an effective modality for detecting the directional diffusion patterns of water molecules within tissues. FA and MD are two diffusion metrics commonly employed to quantify the directional preference of water molecules and average displacement within the WM (25), and have been widely recognized as significant composite metrics in the assessment of WM microstructural abnormalities. The observed decrease in FA and increase in MD may indicate compromised

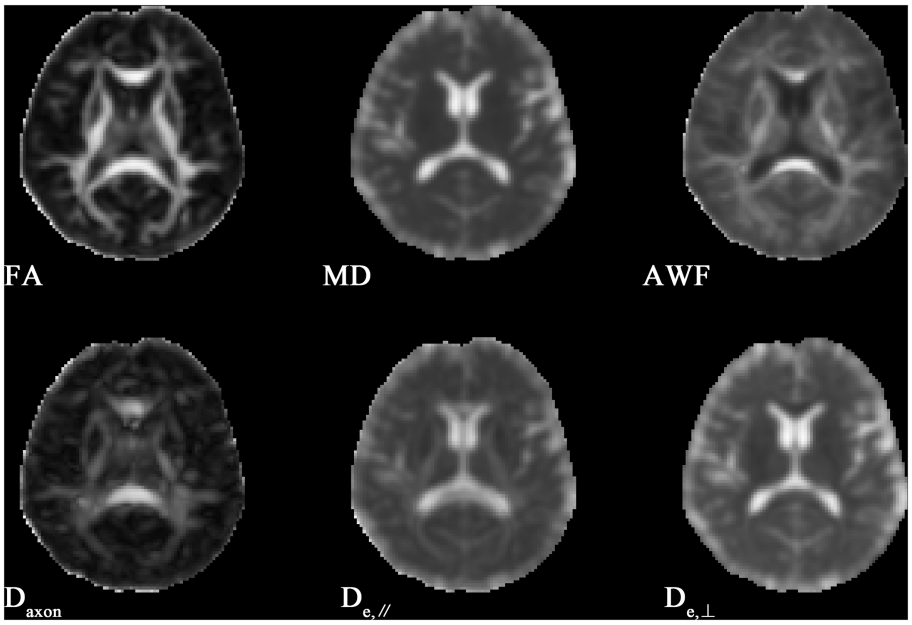

FIGURE 1
Illustrations of FA, MD and WMTI metrics in slice of basal ganglia of a healthy control.

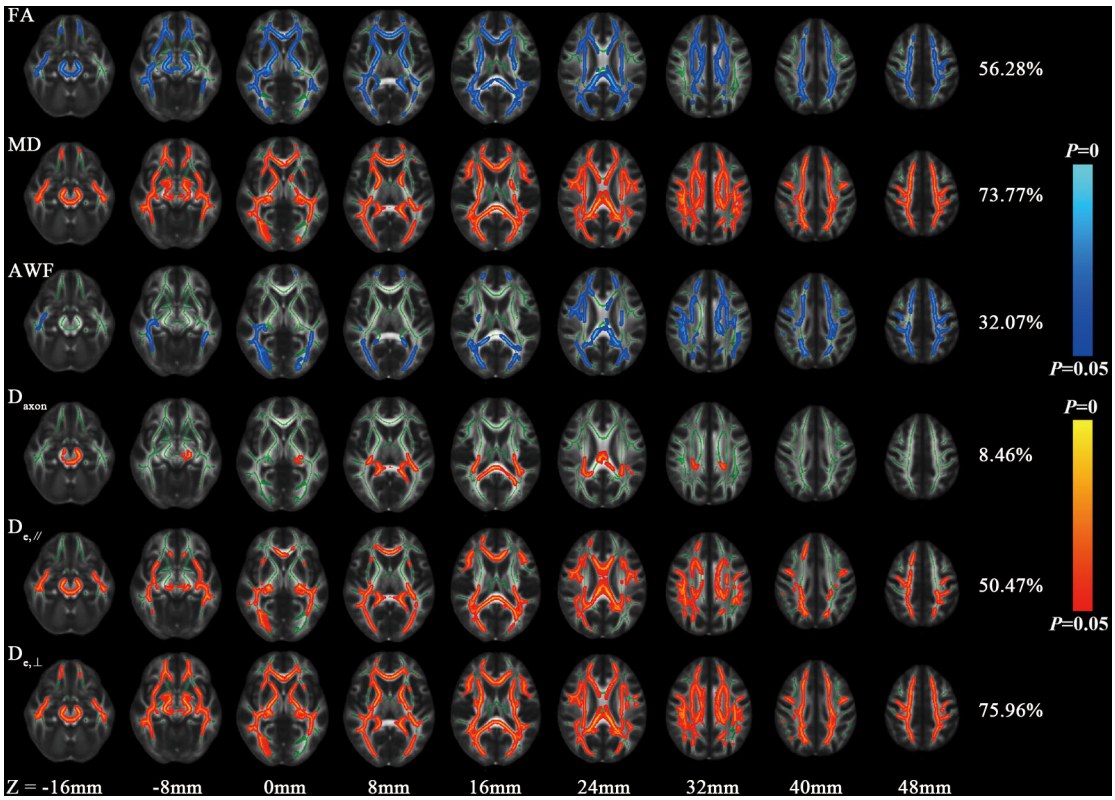

FIGURE 2
Differences of FA, MD and WMTI metrics between HC and T2DM group by voxel-wise TBSS analysis. Regions colored light blue-blue represent significantly decreased voxels ($P< 0.05$) in the T2DM group compared with the HC group, while regions colored yellow-red represent significantly increased voxels ($P< 0.05$). These have been overlaid on the mean FA template with a mean skeleton (regions colored green). Numbers on the right of each row are percentage of voxels that changed in the whole brain.

TABLE 2 White matter integrity differences of FA and $D_{e,\perp}$ in disrupted WM tracts between T2DM and HC group during ROI-based statistical analysis.

Fiber tract	FA	$D_{e,\perp}$
PCT	–	YES
GCC	YES	YES
BCC	–	YES
SCC	YES	YES
CST	Bilateral	Bilateral
ML	Bilateral	Bilateral
CP	Bilateral	Bilateral
ALIC	R	R
PLIC	R	R
ACR	R	R
SCR	Bilateral	Bilateral
PCR	–	Bilateral
PTR	R	R
Sagittal stratum	R	Bilateral
EC	R	R
Cingulum (cingulate gyrus)	Bilateral	Bilateral
FORNIX	–	Bilateral
SLF	–	R
UF	–	Bilateral
Number of ROIs	18	28

YES means diffusional metrics of this ROI showed significant difference between T2DM and HC groups, since it is a whole region which can't be divided into left and right sides. - means these is no difference between the two groups. R means the right side of ROI region, and bilateral means both the right and left sides. PCT, pontine crossing tract; GCC, genu of corpus callosum; BCC, body of corpus callosum; SCC, splenium of corpus callosum; CST, corticospinal tract; ML, medial lemniscus; CP, cerebral peduncle; ALIC, anterior limb of internal capsule; PLIC, posterior limb of internal capsule; ACR, anterior corona radiata; SCR, superior corona radiata; PCR, posterior corona radiata; PTR, posterior thalamic radiation; EC, external capsule; SLF, superior longitudinal fasciculus; UF, uncinate fasciculus.

microstructural integrity of the WM (26, 27), which can be potentially attributed to various pathological processes such as ischemia, demyelination, axonal damage, inflammation, and edema (28). However, the ability of DTI models to accurately differentiate between these distinct pathological changes is limited in terms of specificity. To elaborate further, empirical diffusion measures solely offer indirect assessments of microstructure, thereby introducing uncertainty regarding their physical interpretation in relation to microscopic tissue parameters.

The WMTI model, based on DKI, provides more accurate and specific estimates of the microstructural brain environment by characterizing the intra-axonal and extra-axonal spaces. This allows for a comprehensive understanding of white matter changes from a biophysical perspective. In the model, the intra-axonal space represents impermeable myelinated axons, described by D_{axon}, while the extra-axonal space represents the permeable medium of glial cells and myelin sheath, described by $D_{e,//}$and $D_{e,\perp}$. In addition, AWF, which characterizes the water fraction inside axons compared to total water fraction, maps axonal packing density and has been validated by electron microscopy images in mice (29). The utilization of WMTI metrics has proven advantageous in investigating WM alterations and potential pathogenesis in various clinical studies involving Alzheimer's disease (14), multiple sclerosis (15), normal aging (16), and mild traumatic brain injury (17). In the present study, alongside the observed reductions in FA and increases in MD, significantly decreased AWF and increased $D_{e,//}$and $D_{e,\perp}$ were also identified in widespread WM regions of patients with T2DM. In terms of the interpretation of AWF, the decreased AWF could potentially be attributed to a reduction in intra-axonal water or an increase in the overall water content. Given that D_{axon} did not exhibit a decrease in this study, it is highly likely that the widespread decrease in AWF is primarily attributable to heightened diffusivity originating from the extra-axonal space. Moreover, it can be postulated that the elevated $D_{e,\perp}$ observed in T2DM patients aligns with demyelination, while the increased $D_{e,//}$signifies augmented permeability within the glial cellular environment, induced by hyperglycemia or inflammation. In addition, increased D_{axon} values were also found in some discrete WM regions, which may simultaneously reflect axonal injury in limited regions.

The aforementioned diffusion findings align with the histological characteristics observed through light and electron microscopy studies (30, 31), which indicated degenerative alterations in glial cells, disruption of the myelin sheath, fragmentation of neurofilaments, and abnormalities in oligodendrocytes in rats with T2DM (31, 32). These WM microstructural abnormalities can be explained by several possible mechanisms. Hyperglycemia is known to cause widespread impairment of microvascular function, including the brain's microvasculature (33). This impairment may lead to inflammatory responses and reduced blood flow, ultimately resulting in chronic cerebral ischemia (34). Moreover, the elevated oxidative stress resulting from hyperglycemia plays a significant role in the development of endothelial dysfunction, facilitating the pro-inflammatory process (35). These pathogenic factors and pathways are highly likely to contribute to the dysregulation of cerebral blood supply. Notably, the WM is particularly susceptible to inadequate blood supply (36), and disruption of WM microstructural integrity (28) can be predominantly attributed to a combination of hyperglycemia-induced inflammation, hypoperfusion, oxidative stress, and endothelial dysfunction (36). We speculate that the initial pathologic change is mainly characterized by demyelination, and thus seemingly increased the $D_{e,//}$, $D_{e,\perp}$ and AWF across widespread WM regions. As neurodegeneration progresses, the loss of axons occurs secondary to demyelination, leading to the increased D_{axon} in limited WM regions.

This study showed a large degree of overlap in group differences between WMTI and DTI metrics. Consequently, this outcome serves as evidence of the dependability of the employed metrics in the identification of T2DM-associated microstructural abnormalities. Nevertheless, the DTI model assumes of a Gaussian distribution of water-diffusion processes with a mono-exponential signal decay. This assumption poses challenges in characterizing complex fibers and consequently limits the amount of information that can be obtained. In contrast, DKI allows for the

TABLE 3 Differences of FA and $D_{e,\perp}$ (means ± SDs) between T2DM and HC group during ROI-based statistical analysisROI.

	FA				$D_{e,\perp}(\times10^{-3}mm/s^2)$			
	HC	T2DM	t	p	HC	T2DM	t	p
PCT	0.30 ± 0.03	0.29 ± 0.03	0.964	0.337	1.52 ± 0.12	1.58 ± 0.11	-2.780	0.006
GCC	0.39 ± 0.04	0.37 ± 0.03	2.675	0.009	1.55 ± 0.17	1.62 ± 0.13	-2.846	0.005
BCC	0.37 ± 0.04	0.35 ± 0.03	1.551	0.124	1.69 ± 0.18	1.76 ± 0.12	-2.546	0.013
SCC	0.43 ± 0.04	0.42 ± 0.03	2.133	0.035	1.58 ± 0.15	1.65 ± 0.12	-2.995	0.003
CST.R	0.31 ± 0.04	0.29 ± 0.03	3.495	0.001	1.67 ± 0.17	1.77 ± 0.16	-3.584	<0.001
CST.L	0.33 ± 0.03	0.31 ± 0.03	3.394	0.001	1.61 ± 0.13	1.69 ± 0.14	-3.588	<0.001
ML.R	0.37 ± 0.03	0.35 ± 0.03	3.688	<0.001	1.53 ± 0.12	1.61 ± 0.12	-3.722	<0.001
ML.L	0.34 ± 0.03	0.33 ± 0.02	3.784	<0.001	1.57 ± 0.13	1.65 ± 0.12	-3.690	<0.001
CP.R	0.38 ± 0.03	0.36 ± 0.03	3.547	0.001	1.49 ± 0.13	1.57 ± 0.11	-3.619	<0.001
CP.L	0.39 ± 0.03	0.37 ± 0.02	3.194	0.002	1.50 ± 0.12	1.57 ± 0.10	-3.936	<0.001
ALIC.R	0.32 ± 0.03	0.31 ± 0.03	3.183	0.002	1.35 ± 0.13	1.41 ± 0.10	-2.894	0.004
PLIC.R	0.42 ± 0.04	0.40 ± 0.03	3.057	0.003	1.29 ± 0.12	1.33 ± 0.09	-2.374	0.019
ACR.R	0.26 ± 0.03	0.24 ± 0.02	4.209	<0.001	1.44 ± 0.13	1.49 ± 0.10	-2.667	0.009
SCR.R	0.29 ± 0.03	0.28 ± 0.02	3.404	0.001	1.42 ± 0.12	1.47 ± 0.09	-2.654	0.009
SCR.L	0.30 ± 0.03	0.29 ± 0.02	2.837	0.005	1.43 ± 0.12	1.47 ± 0.09	-2.256	0.026
PCR.R	0.27 ± 0.03	0.27 ± 0.02	1.211	0.229	1.60 ± 0.14	1.65 ± 0.11	-2.304	0.023
PCR.L	0.26 ± 0.03	0.26 ± 0.03	1.390	0.167	1.61 ± 0.14	1.65 ± 0.10	-2.088	0.039
PTR.R	0.31 ± 0.03	0.30 ± 0.02	2.311	0.022	1.54 ± 0.13	1.59 ± 0.12	-2.573	0.011
SS.R	0.30 ± 0.03	0.28 ± 0.02	2.973	0.004	1.54 ± 0.13	1.60 ± 0.10	-2.731	0.007
SS.L	0.29 ± 0.03	0.28 ± 0.02	1.910	0.058	1.55 ± 0.13	1.59 ± 0.11	-2.204	0.029
EC.R	0.26 ± 0.03	0.25 ± 0.02	2.048	0.043	1.41 ± 0.12	1.46 ± 0.10	-2.394	0.018
Cingulum.R	0.26 ± 0.02	0.25 ± 0.02	2.262	0.025	1.42 ± 0.11	1.48 ± 0.08	-3.083	0.003
Cingulum.L	0.27 ± 0.03	0.26 ± 0.02	2.581	0.011	1.43 ± 0.12	1.48 ± 0.08	-3.011	0.003
FORNIX.R	0.28 ± 0.03	0.27 ± 0.02	1.727	0.087	1.55 ± 0.14	1.63 ± 0.12	-3.375	0.001
FORNIX.L	0.29 ± 0.03	0.28 ± 0.02	1.242	0.217	1.58 ± 0.14	1.66 ± 0.12	-3.458	0.001
SLF.R	0.28 ± 0.03	0.28 ± 0.02	1.673	0.097	1.46 ± 0.11	1.51 ± 0.09	-2.299	0.023
UF.R	0.26 ± 0.03	0.25 ± 0.03	1.619	0.108	1.48 ± 0.12	1.52 ± 0.11	-2.309	0.023
UF.L	0.25 ± 0.03	0.24 ± 0.02	1.367	0.174	1.48 ± 0.12	1.53 ± 0.11	-2.509	0.013

PCT, pontine crossing tract; GCC, genu of corpus callosum; BCC, body of corpus callosum; SCC, splenium of corpus callosum; CST, corticospinal tract; ML, medial lemniscus; CP, cerebral peduncle; ALIC, anterior limb of internal capsule; PLIC, posterior limb of internal capsule; ACR, anterior corona radiata; SCR, superior corona radiata; PCR, posterior corona radiata; PTR, posterior thalamic radiation; SS, sagittal stratum; EC, external capsule; SLF, superior longitudinal fasciculus; UF, uncinate fasciculus; R, right; L, left.

estimation of the non-Gaussian distribution of water molecules and enables calculation of kurtosis parameters such as mean kurtosis, radial kurtosis, and axial kurtosis, which can provide insights into the structural complexity. For patients with T2DM, two studies have investigated brain microstructural integrity using DKI and have confirmed its greater sensitivity than DTI in detecting changes in brain microstructure (37, 38). Since WMTI metrics are derived from DKI, we hypothesized that they could offer supplementary insights into WM microstructural abnormalities. As expected, among the WMTI and DTI metrics, $D_{e,\perp}$ detected more WM region differences in group comparisons, thereby exhibiting higher sensitivity and specificity for identification of subtle brain changes. Additionally, consistent with previous studies utilizing DKI, WMTI metrics also demonstrated remarkable capacity for imaging complex crossing fibers, such as the pontine crossing tract, PTR, SLF, and UF. While DKI metrics are sensitive to microstructural features, they lack specificity. Conversely, the $D_{e,\perp}$ metric derived from the WMTI model provides a specific measurement of the extra-axonal compartments. Neurite orientation dispersion and density imaging (NODDI) is another multicompartment model utilized to distinguish the signal originating from extra- and intra-axonal compartments. A recent

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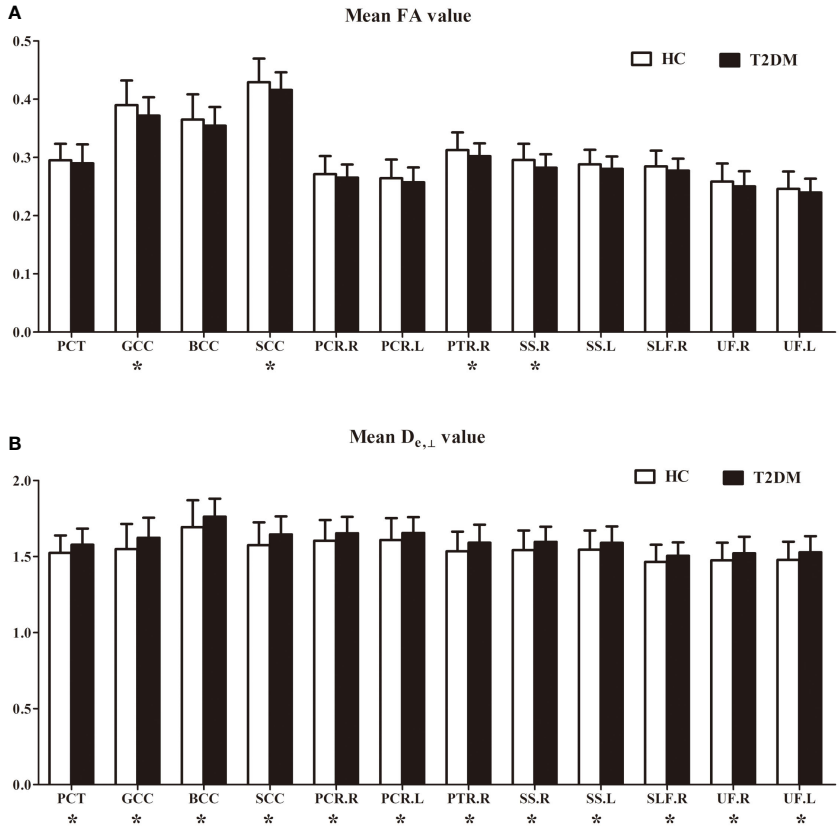

FIGURE 3
FA **(A)** and $D_{e,\perp}$ **(B)** differences in some white matter fiber tracts. PCT, pontine crossing tract; GCC, genu of corpus callosum; BCC, body of corpus callosum; SCC, splenium of corpus callosum; PCR, posterior corona radiata; PTR, posterior thalamic radiation; SS, sagittal stratum; SLF, superior longitudinal fasciculus; UF, uncinate fasciculus; R, right; L, left. *indicates a significant difference between the two groups at a significance level of 0.05.

investigation employed NODDI to examine microstructural modifications in individuals with T2DM, and revealed a diminished intracellular volume fraction (Vic) in T2DM patients experiencing cognitive decline (39). A reduced Vic suggests a decrease in axon density, potentially indicating degeneration of axons and myelin. This is also consistent with our speculation regarding pathological changes. However, this study did not compare the sensitivity and specificity of the WMTI and NODDI metrics in detecting and monitoring microstructural alterations, since it would prolong the scan time and increase the burden for T2DM patients.

Furthermore, this study demonstrated that the $D_{e,\perp}$ values in the GCC were correlated with cognitive function (TMT-A) scores and disease duration. To further analyze the role of disease duration as a possible mediator between the $D_{e,\perp}$ values in the GCC and TMT-A scores, a mediational model was constructed. However, disease duration did not act as a mediator in this model, which contradicts previous beliefs that longer disease duration leads to more severe neurodegeneration and cognitive decline (5). This may be due to the relatively young age (55.64 ± 7.65 years) and short duration of illness (8.34 ± 5.93) in the current study. Diabetes-associated WM impairments have been observed to manifest prior

TABLE 4 The mediating role of disease duration between $D_{e,\perp}$ values in the GCC and TMT-A in T2DM patients.

Effect	Model 1			Mdoel 2			Mdoel 3		
	β	t	95%[CI]	β	t	95%[CI]	β	t	95%[CI]
$D_{e,\perp}$ in GCC	0.433	3.978***	[0.216, 0.651]	0.438	3.557***	[0.192, 0.684]	0.415	3.488***	[0.178, 0.653]
Disease duration							0.041	0.387	[-.171, 0.253]
R^2	0.391			0.220			0.392		
F	22.454***			9.896***			14.837***		

Model 1 represents the effect of $D_{e,\perp}$ in GCC on TMT-A; Model 2 represents the effect of $D_{e,\perp}$ in GCC on disease duration; Model 3 represents the effect of $D_{e,\perp}$ in GCC and disease duration on TMT-A. ***P<0.001.

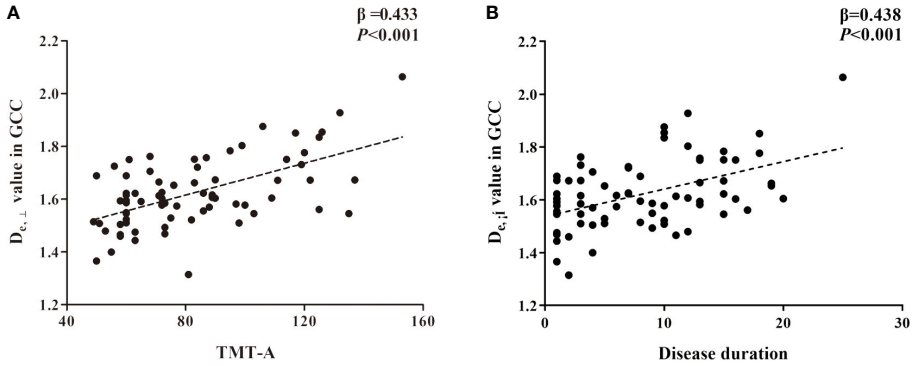

FIGURE 4
The significant correlations between $D_{e,\perp}$ metrics in genu of corpus callosum (GCC) and cognitive **(A)** and clinical data **(B)** in T2DM patients based on ROI-wise analysis.

to cognitive decline, even in individuals with prediabetes (40). Thus, in T2DM patients, impairment of WM microstructural integrity in various regions, particularly the frontal lobe, has been hypothesized to affect both structural and functional connectivity. This disruption may consequently lead to network disturbances and a subsequent reduction in neuronal signal transmission, resulting in slower information processing speed and cognitive decline. While T2DM is associated with a broad spectrum of WM microstructural disruptions, only a subset of these abnormalities appears to be linked to cognitive impairments, indicating the existence of important targets of cognitive impairment in the brain. The GCC, which serves as a principal commissural WM bundle connecting the left and right prefrontal lobes, is closely associated with cognitive function and plays a crucial role in the maintenance of cognitive processes such as attention and execution (41). Additionally, a previous study conducted on patients with prediabetes also indicated that the CC is particularly susceptible to hyperglycemia and may be one of the regions most severely affected by diabetes-related damage (40). Therefore, based on our findings, we propose that the observed increase in $D_{e,\perp}$ within the GCC could potentially serve as an early imaging marker for assessing disease progression and monitoring cognitive function.

The findings in this study also have implications for the clinical management and risk prediction of cognitive decline in patients with T2DM. It has been widely accepted that T2DM was a risk factor associated with mild cognitive impairment and dementia. Cognitive decline starts slowly but can accelerate quickly. Therefore, tracking cognitive performance and changes of WMTI metrics over time and identifying a cut-off value for MCI could help clinicians identify patients with high risk of cognitive impairment early. These patients will receive personalized care, including strict glycemic control and management of cardiovascular risk factors. In addition, they can also benefit from early treatment with medications and cognitive behavioral therapy to prevent or delay cognitive decline.

This study had several limitations. First, this was a single-center study based on a Chinese population and only recruited inpatients with T2DM. Our results require validation in a larger and more diverse population. Second, this was a cross-sectional study, and the follow-up phase is still ongoing. Thus, longitudinal studies have not yet been performed due to the limited number of currently enrolled cases. Longitudinal studies evaluating the effectiveness of WMTI metrics would be useful in this regard. Additionally, patients with T2DM received medications, and we overlooked the effects of these medications on the brain. A partial reflection of medication effects cannot be ruled out in our results. Finally, we only focused on the WM impairments in patients with T2DM. In fact, WMTI metrics based on DKI should also have great potential for mapping changes in gray matter, which is worth further study in the future.

5 Conclusion

This study provides evidence that WMTI metrics exhibit greater sensitivity than DTI metrics in the detection of T2DM-related white matter microstructural abnormalities. The observed changes in WMTI metrics in T2DM indicate varying degrees of degeneration in the myelin sheath and axon. The WMTI model, as a noninvasive and sensitive technique, allows for early assessment of disruptions in white matter integrity among T2DM patients, potentially offering valuable insights into the underlying mechanisms of T2DM-related brain injury. Additionally, the correlation between $D_{e,\perp}$ values in the genu of the corpus callosum and cognitive function and disease severity implies their potential role as imaging markers for monitoring disease progression and cognitive function in patients with T2DM.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving humans were approved by The Human Ethics Committee of Shaanxi Provincial Peoples Hospital. The

studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

JG: Data curation, Formal analysis, Funding acquisition, Methodology, Writing – original draft, Writing – review & editing. PP: Formal analysis, Methodology, Data curation, Writing – review & editing. JL: Formal analysis, Software, Writing – review & editing, Validation. MT: Data curation, Formal analysis, Writing – review & editing, Validation. XY: Validation, Writing – review & editing, Data curation. XZ: Software, Validation, Visualization, Writing – review & editing, Data curation. MW: Formal analysis, Software, Validation, Writing – review & editing. KA: Methodology, Software, Writing – review & editing. XL: Resources, Supervision, Writing – review & editing. XiaonlingZ: Funding acquisition, Resources, Supervision, Writing – review & editing. DZ: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

Author KA was employed by the company Philips Healthcare.

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EDITED BY
Lei Sha,
China Medical University, China

REVIEWED BY
Ian James Martins,
University of Western Australia, Australia
Dorota Formanowicz,
Poznan University of Medical Sciences, Poland

*CORRESPONDENCE
Naifei Chen
chennaifei@jlu.edu.cn
Jiuwei Cui
cuijw@jlu.edu.cn
Wei Li
liwei66@jlu.edu.cn

†These authors have contributed equally to this work and share first authorship

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Revisiting the mechanisms linking blood glucose to cognitive impairment: new evidence for the potential important role of klotho

Xiangliang Liu[1†], Yuguang Li[1†], Xinqiao Chen[1†], Hongmei Yin[2], Fangqi Li[1], Naifei Chen[1*], Jiuwei Cui[1*] and Wei Li[1*]

[1]Cancer Center, The First Affiliated Hospital of Jilin University, Changchun, China, [2]Department of General Practice, The First Affiliated Hospital of Jilin University, Changchun, China

Background: The association between blood glucose and cognition is controversial. Klotho is an anti-aging protein with neural protective effects. This study aimed to use a population-based study to disentangle the relationship between blood glucose levels and cognitive function in older adults, and to explore the role of klotho in it.

Methods: A total of 1445 eligible participants from National Health and Nutrition Examination Survey (NHANES) 2011-2014 were included in our study. Cognitive function was assessed by Digit Symbol Substitution Test (DSST) and categorized into four quartiles (Q1-Q4). General characteristics and laboratory test results including serum klotho concentration and blood glucose levels were collected. Associations of cognitive function and klotho levels with blood glucose concentrations were explored through multivariate linear regression models. Mediation models were constructed to figure out the mediating role of klotho.

Results: All three multivariate linear regression models showed a negative correlation between blood glucose and cognitive function. (Model 1, β=-0.149, 95%CI: -0.202,-0.096, p=0.001; Model 2, β=-0.116, 95%CI: -0.167,-0.065, p=0.001; Model 3, β=-0.007, 95%CI: -0.118,-0.023, p=0.003). Mediation analysis showed that klotho mediated the statistical association between blood glucose level and cognitive function with proportions (%) of 12.5.

Conclusion: Higher blood glucose levels are associated with poorer cognitive performance in non-diabetic older adults, partially mediated through lower klotho levels.

KEYWORDS

glucose, klotho, cognitive function, cognitive impairment, hyperglycemia

1 Introduction

With global population aging, cognitive impairment has become an important public health issue (1). Numerous epidemiological studies have demonstrated that diabetes mellitus is one of the key risk factors for cognitive decline (2–4). Moreover, increased blood glucose levels are thought to be associated with cognitive impairment even among people without diabetes (5–7). For example, a prospective study found that a reverse U-shaped relationship was observed between fasting glucose and cognitive function, identifying a threshold for highest cognitive performance at 3.97–6.20 mmol/L fasting glucose (5)., indicating impaired fasting glucose may be associated with cognitive impairment. However, associations between blood glucose and cognition are inconsistent across different populations. A study among older Koreans showed that higher blood glucose was only associated with lower memory but not other cognitive domains (7), suggesting potential ethnic differences. Therefore, the relationship between glucose and cognition merits investigation across different ethnicities in the U.S.

The mechanisms underlying the impact of glucose on cognition remain elusive. Some studies have proposed that abnormalities in insulin signaling may be a key pathway (8, 9). In recent years, klotho, an aging-related protein, has gained considerable attention as a key regulator of the insulin/IGF pathway (10). Klotho increases insulin receptor affinity and enhances insulin sensitivity (11). Moreover, klotho exerts neuroprotective effects (12, 13). Hence, klotho may mediate the detrimental effects of glucose on cognition, but relevant evidence is scarce. Given the controversies over the relationship between blood glucose and cognition across populations and the uncertain mechanisms, research leveraging a representative U.S. cohort to examine the impact of glucose on cognition and the potential intermediary role of klotho is warranted. This will facilitate mechanistic understanding of the importance of glucose control for cognitive health.

2 Methods

2.1 Study population

The data analyzed in this study were from the National Health and Nutrition Examination Survey(NHANES, https://www.cdc.gov/nchs/nhanes/about_nhanes.htm). population. NHANES collects health and nutrition related data through interviews and physical examinations of a representative sample of the civilian non-institutionalized population. Written informed consent was obtained from each participant before participation in this study.

Cognitive function of the older population was measured in two cycles of NHANES, 2011-2012 and 2013-2014. A total of 3472 participants were included in the analysis after excluding participants under 60 years of old (n = 14,906). Subsequently, eligible participants needed to have complete data on cognitive function and blood glucose. 458 and 1569 participants who did not participate in the cognitive function test and did not participate in the blood glucose test were excluded, respectively. This resulted in an analytic sample of 1445 participants (see Figure 1).

2.2 Cognitive function

NHANES performed the Digit Symbol Substitution Test (DSST) for cognitive performance among participants aged 60 years or older. Completing the Digit Symbol Substitution Test (DSST) requires the integrity of executive function, processing speed, attention, spatial perception, and visual scanning cognitive abilities. The exercise is conducted using a paper form that has a key at the top containing 9 numbers paired with symbols. Participants have 2 minutes to copy the corresponding symbols in the 133 boxes that adjoin the numbers. The score is the total number of correct matches. A sample practice test is administered before the participants begin the main test. In NHANES, participants who could not correctly match the symbols with the numbers on their own during the pretest practice did not continue. Details on scoring can be found in the 1999-2000 NHANES CFQ questionnaire data file documentation https://wwwn.cdc.gov/Nchs/Nhanes/1999-2000/CFQ.htm.

2.3 Measurement of serum soluble klotho

Prior to analysis, all samples were stored at -80°C. Quantification of Klotho concentrations was performed using a commercially available enzyme-linked immunosorbent assay (ELISA) kit manufactured by IBL International (Japan). The laboratory methodology employed as well as quality assurance and quality control procedures have been described previously in the NHANES study documentation.

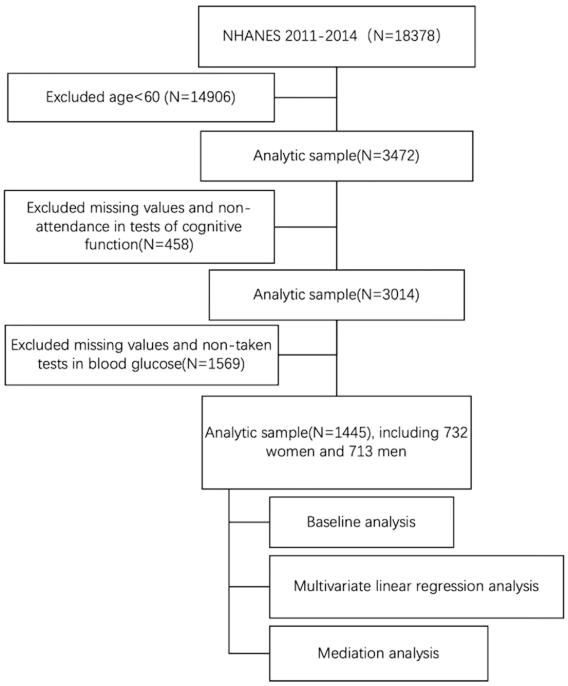

FIGURE 1
Flowchart of the study design and participants excluded from the study.

2.4 Measurement of blood glucose

After a 9-hour overnight fast, fasting blood glucose levels were measured in participants the following morning. These examinations were performed in adherence to established protocols for assessing fasting blood glucose.

2.5 Covariates

Covariates evaluated in this analysis included sex (male, female), race/ethnicity (Mexican American, Non-Hispanic Black, Non-Hispanic White, Other), educational attainment (less than high school, high school graduate, college graduate or above), socioeconomic status assessed by Poverty Income Ratio (cite SES source), smoking status (never, former, current), body mass index (<25.0, 25.0-29.9, ≥30.0 kg/m2), and drinking status (never, former, mild, moderate, heavy drinking) based on established categorizations.

2.6 Statistical analysis

To obtain population-representative statistics, participants were stratified into quartiles based on the median score of the Digit Symbol Substitution Test (DSST). Categorical variables were summarized as frequencies and percentages, with p-values from chi-squared tests reported. Continuous variables were expressed as mean ± standard deviation (SD), with p-values from Student's t-tests presented. Associations of DSST scores and Klotho levels with blood glucose concentrations were explored through multivariate linear regression models.

Direct and indirect effects were evaluated to ascertain the mediating influence of Klotho levels on the relationship between blood glucose and DSST performance. Bootstrapping methodology was employed to determine the statistical significance of the mediation pathway. The magnitude of the mediation effect was quantified as the mediation effect percentage, calculated as (mediation effect/total effect) x 100. Statistical analyses were conducted using R statistical software (version 4.2.2, released 2022-10-31, http://www.r-project.org).

3 Results

Weighted characteristics of the 1445 participants included in the analyses are shown in Table 1. The weighted cognitive function stratified by sex, race, socioeconomic status, education, smoking status, alcohol drinking status, and glucose was statistically significantly different ($P < 0.05$).

Three multivariate linear regression models were constructed to explore the relationship between glucose and cognitive function (Table 2). In the crude model, the standardization coefficient of high glucose and cognitive function is -0.149(95%CI(-0.202,-0.096), p=0.001). After adjusting for sex and race (model 1), the standardization coefficient of high glucose and cognitive function is -0.116(95%CI(-0.167,-0.065), p=0.001). After further adjustment of education attainment, poverty income ratio, smoking status, and alcohol drinking status (model 2), the standardization coefficient of high glucose and cognitive function is -0.070(95%CI(-0.118,-0.023), p=0.003).

We further investigated the association between klotho and cognitive function (Table 3). After adjusting for all covariates, the results showed that the standardization coefficient between the highest quartile of klotho and cognitive function was 0.091(95% CI 0.031,0.152; p=0.003).

Furthermore, mediation analyses were conducted to explore the mediating effect of klotho. Figure 2 shows the mediating role of klotho in the relationship between glucose and sleep cognitive function. Klotho explained 12.5% of the association($p < 0.001$).

4 Discussion

4.1 Main findings

In this nationally representative U.S. sample, we validated the association between higher blood glucose levels and poorer cognitive performance measured by DSST even within non-diabetic range. The linear association was independent of potential confounders including socio-demographics, health behaviors, and adiposity. Our findings corroborate results from several previous epidemiological studies showing dose-response relationships between fasting or postprandial blood glucose levels and cognitive deficits in non-diabetic elderly adults (5–7). The persistent adverse impact of elevated glucose on cognition across the spectrum highlights the need for early screening and preventive interventions even before the diagnosis of diabetes.

Importantly, our study provides novel clinical evidence for the intermediary role of klotho in linking chronic hyperglycemia to cognitive decline. We found that higher serum klotho concentrations were independently associated with better DSST scores. Klotho partially mediated the association between elevated glucose and lower DSST performance. To our knowledge, this is the first population-based study discussing the involvement of klotho in glucose-associated cognitive impairment. The findings align with emerging preclinical evidence indicating neuroprotective properties of klotho against neuronal insulin resistance and oxidative stress (13–15). Our study extends the current literature by identifying klotho as a key intermediary linking circulatory glucose disturbance to cognitive aging in a general elderly population.

4.2 Potential mechanisms and clinical implications

4.2.1 Hyperglycemia-induced neuronal damage

Hyperglycemia can inflict neuronal damage through multiple molecular cascades relevant to cognitive decline. Elevated extracellular glucose enhances the formation of advanced glycation end-products (AGEs), which elicit inflammatory responses and oxidative stress in neurons (16). Excessive reactive oxygen species (ROS) production surpasses endogenous antioxidant capacity, resulting in oxidative damage to lipids, proteins and nucleic acids (17). ROS overproduction also activates stress-related signaling

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TABLE 1 Weighted characteristics of the study population by Cognitive function.

Variable	level	Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*-value
n		363	367	364	351	
sex (%)						
	Female	173 (47.66)	150 (40.87)	188 (51.65)	221 (62.96)	<0.0001
	Male	190 (52.34)	217 (59.13)	176 (48.35)	130 (37.04)	
Race/ethnicity (%)						
	Mexican American	50 (13.77)	38 (10.35)	22 (6.04)	16 (4.56)	<0.0001
	Non-Hispanic Black	108 (29.75)	78 (21.25)	75 (20.60)	38 (10.83)	
	Non-Hispanic White	112 (30.85)	179 (48.77)	209 (57.42)	222 (63.25)	
	Other race/ethnicity	93 (25.62)	72 (19.62)	58 (15.93)	75 (21.37)	
Poverty (%)						
	<1	111 (34.47)	56 (16.62)	37 (10.95)	22 (6.79)	<0.0001
	[1, 3)	160 (49.69)	178 (52.82)	145 (42.90)	94 (29.01)	
	≥3	51 (15.84)	103 (30.56)	156 (46.15)	208 (64.20)	
Education (%)						
	low high school	206 (54.21)	192 (55.65)	171 (47.50)	151 (42.18)	0.0017
	High school	94 (24.74)	71 (20.58)	92 (25.56)	90 (25.14)	
	College or above	80 (21.05)	82 (23.77)	97 (26.94)	117 (32.68)	
Smokers						
	former smoker	126 (34.81)	158 (43.05)	148 (40.66)	124 (35.43)	0.0022
	Never smoker	184 (50.83)	157 (42.78)	173 (47.53)	199 (56.86)	
	Current smoker	52 (14.36)	52 (14.17)	43 (11.81)	27 (7.71)	
Alcohol drinkers						
	Former drinker	140 (40.58)	97 (27.02)	91 (25.14)	59 (17.00)	<0.0001
	Heavy drinker	21 (6.09)	42 (11.70)	15 (4.14)	23 (6.63)	
	Mild drinker	83 (24.06)	129 (35.93)	160 (44.20)	184 (53.03)	
	Moderate drinker	22 (6.38)	34 (9.47)	34 (9.39)	44 (12.68)	
	Never drinker	79 (22.90)	57 (15.88)	62 (17.13)	37 (10.66)	
BMI (kg/m2) (%)						
	<25	96 (27.12)	102 (27.87)	93 (25.76)	103 (29.51)	0.8627
	[25, 30)	125 (35.31)	133 (36.34)	123 (34.07)	115 (32.95)	
	≥30	133 (37.57)	131 (35.79)	145 (40.17)	131 (37.54)	
blood sugar (mmol/L) (mean (SD))		6.85(2.57)	6.48(1.93)	6.24(1.59)	6.08(1.54)	<0.0001
klotho (pg/mL) (mean (SD))		818.172 (271.577)	851.201 (304.541)	868.537 (347.222)	864.512 (240.838)	0.2059

Mean ± SE for continuous variables: P-value was calculated by the weighted T test.
% (SE) for categorical variables: P-value was calculated by the weighted chi-square test.

molecules including p38 mitogen-activated protein kinase (p38 MAPK) and c-Jun N-terminal kinase (JNK) (18). The activation of p38 MAPK/JNK pathways leads to aberrant hyperphosphorylation of tau, a hallmark of Alzheimer's disease (19).

Additionally, hyperglycemia disrupts cellular proteostasis by inducing endoplasmic reticulum (ER) stress and mitochondrial dysfunction (20–22). ER stress can elicit neuronal apoptosis through caspase activation. Mitochondrial dysfunction caused by

TABLE 2 The associations between glucose and Cognitive function.

	Crude model[a]		Model 1[b]		Model 2[c]	
	Standardization coefficientβ(95% CI)	P-value	Standardization coefficientβ(95% CI)	P-value	Standardization coefficientβ(95% CI)	P-value
Glucose (mmol/L)						
<= 6.1	Reference		Reference		Reference	
(6.1,7]	-0.082(-0.135,-0.029)	0.002	-0.069(-0.120,-0.018)	0.008	-0.028(-0.075,0.018)	0.236
>= 7	-0.149(-0.202,-0.096)	0.001	-0.116(-0.167,-0.065)	0.001	-0.070(-0.118,-0.023)	0.003

CI, confidence intervals.
[a]Crude model: no covariates were adjusted.
[b]Model 1: sex and race/ethnicity were adjusted.
[c]Model 2: sex, race/ethnicity, education attainment, poverty income ratio, smoking status, and alcohol drinking status were adjusted.

hyperglycemia not only reduces ATP production but also exacerbates ROS generation, establishing a vicious cycle to aggravate oxidative damage in neurons (23). Hyperglycemia also downregulates insulin signaling in the brain by attenuating insulin receptor substrates and Akt signaling, leading to impairments in glucose metabolism and plasticity (24).

These molecular mechanisms represent potential therapeutic targets. Pharmacological agents alleviating oxidative stress, neuroinflammation, ER stress, mitochondrial dysfunction, and insulin resistance could help mitigate hyperglycemia-induced neuronal injury. Lifestyle interventions including exercise, cognitive training, and caloric restriction may also counteract these pathological processes underlying glucose-associated cognitive decline (25–27).

4.2.2 Neuroprotective mechanisms of klotho

Klotho may counteract the detrimental effects of hyperglycemia on cognition through diverse mechanisms (Figure 3). By increasing insulin receptor affinity, klotho can enhance insulin sensitivity and restore neuronal insulin signaling (28, 29). Through regulating redox systems, klotho suppresses ROS generation and inhibits ROS-induced activation of p38 MAPK/JNK pathways, curbing aberrant phosphorylation of tau (11). Previous studies have shown Sirtuin 1 is involved in insulin release and the regulation of klotho. Klotho may antagonize oxidative stress, activate cellular autophagy, and inhibit neuroinflammation to reduce cognitive impairment by affecting the AMPK/SIRT1 signaling pathway, which plays an important role in glucose regulation, neuron proliferation, oxidative stress, and cognition (30–32).. Klotho also upregulates antioxidant enzymes like superoxide dismutase to bolster neuronal defenses against oxidative damage (33).

Moreover, klotho protects against ER stress-induced apoptosis by blocking ASK1 signaling and inducing autophagy (11). Autophagy degrades misfolded proteins accumulated during ER stress to maintain proteostasis. Klotho preserves mitochondrial homeostasis by inhibiting stress-related signaling molecules and apoptosis pathways (34–36). By alleviating hyperglycemia-induced oxidative, ER and mitochondrial stress, klotho maintains neuronal integrity and function.

Our clinical findings complement these experimental studies to support the neuroprotective role of klotho against glucose toxicity. Further research is needed to elucidate the precise molecular events and signaling pathways linking klotho to neuronal resilience. Uncovering these mechanisms may unveil new possibilities for klotho-based therapies.

Strategies to enhance klotho activity, such as caloric restriction, could offer innovative avenues for mitigating cognitive aging related to glucose dysregulation (37). Exercise training increases klotho

TABLE 3 The associations between klotho and Cognitive function.

	Standardization coefficient β	95% CI	*P*-value
Klotho (pg/mL)			
Q1	Reference		
Q2	0.067	(0.006,0.127)	0.030
Q3	0.095	(0.034,0.155)	0.002
Q4	0.091	(0.031.0.152)	0.003

Adjusted for sex, race/ethnicity , education attainment, poverty income ratio, smoking status, and alcohol drinking statuss.
CI, confidence intervals.

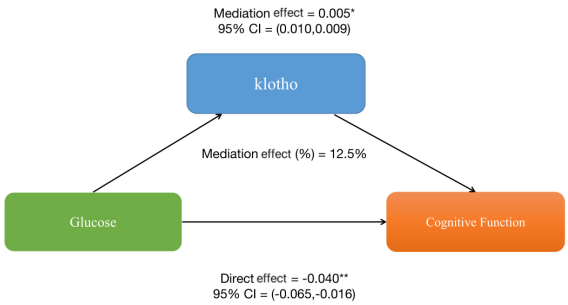

FIGURE 2
The mediation analysis of klotho on the relationship between glucose and cognitive function.

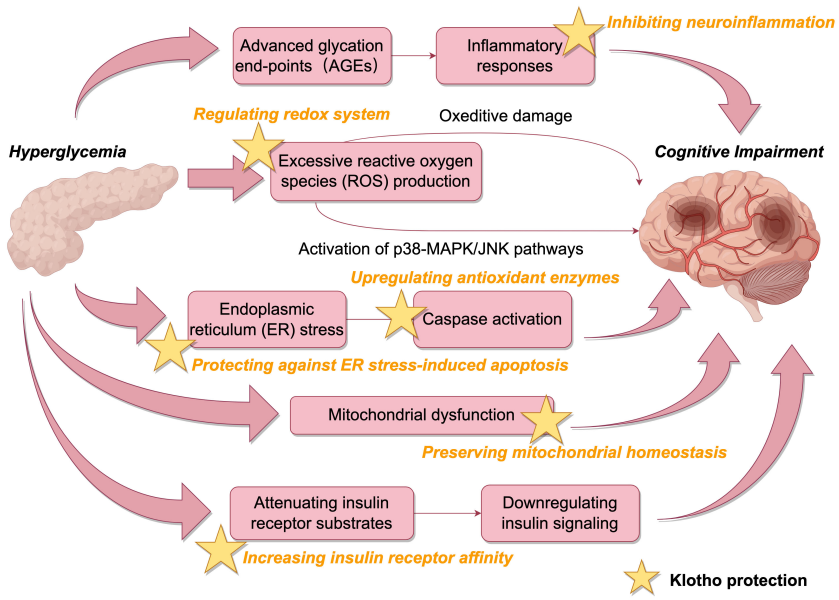

FIGURE 3
Neuroprotective mechanisms of klotho (By Figdraw).

levels and improves cognition in older adults, likely through a klotho-dependent pathway (38). Some phytochemicals like curcumin and resveratrol have demonstrated ability to upregulate klotho expression (39, 40). Developing nutritional or pharmacological approaches to boost klotho function warrants investigation for dementia prevention and treatment.

4.3 Research strengths, limitations and future directions

Major strengths of the study include the population-based national sample, exploration of a novel intermediary mechanism, and rigorous statistical approach adjusting for potential confounders. However, several limitations should be acknowledged. The cross-sectional nature precludes causal inference. While we selected instrumental variables meeting stringent criteria, residual confounding cannot be excluded. Generalizability to other populations requires further verification. Owing to data constraints, we did not have comprehensive cognitive assessments or neuroimaging biomarkers to enable detailed investigation across cognitive domains.

Future large-scale longitudinal studies incorporating multi-domain cognitive test batteries, neuroimaging, and biomarker assessments are warranted to validate the interrelationships between glucose, klotho, and domain-specific cognitive trajectories. Clinical trials are needed to establish causal impacts of interventions modulating klotho on cognition. Animal models could help elucidate precise molecular mechanisms underlying the cognitive protection afforded by klotho. Elucidating the role of klotho in neuronal maintenance and resilience may uncover innovative prevention opportunities against cognitive aging and dementia related to metabolic disturbance.

5 Conclusion

In summary, our study provides novel clinical evidence that higher blood glucose levels are associated with poorer cognitive performance in non-diabetic older adults, partially mediated through lower klotho levels. Our findings underscore the importance of early glycemic control and highlight klotho as a potential intermediary linking glucose disturbance to cognitive aging. Further research into the links between glucose, klotho and cognition may open promising translational opportunities for dementia prevention.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by National Center for Health Statistics Ethics Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

XL: Conceptualization, Investigation, Writing – original draft, Data curation, Software. YL: Data curation, Software, Writing –

original draft, Formal Analysis, Validation. XC: Formal Analysis, Writing – original draft, Conceptualization, Investigation, Methodology. HY: Resources, Visualization, Writing – review & editing. FL: Data curation, Formal Analysis, Investigation, Software, Writing – review & editing. NC: Investigation, Methodology, Resources, Supervision, Writing – review & editing. JC: Project administration, Resources, Supervision, Validation, Writing – review & editing. WL: Project administration, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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EDITED BY
Vijaya Juturu,
Independent Researcher, Flemington, United States

REVIEWED BY
Faizul Jaafar,
Monash University, Malaysia
Maharshi Bhaswant,
Tohoku University, Japan

*CORRESPONDENCE
Pilar Sanchis
pilar.sanchis@uib.es

†These authors have contributed equally to this work and share first authorship

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Oral phytate supplementation on the progression of mild cognitive impairment, brain iron deposition and diabetic retinopathy in patients with type 2 diabetes: a concept paper for a randomized double blind placebo controlled trial (the PHYND trial)

Antelm Pujol[1†], Pilar Sanchis[1,2*†], María I. Tamayo[1], Joana Nicolau[1], Félix Grases[2], Ana Espino[3], Ana Estremera[4], Elena Rigo[5], Guillermo J. Amengual[4], Manuel Rodríguez[4], José L. Ribes[6], Isabel Gomila[6], Olga Simó-Servat[7] and Lluís Masmiquel[1†]

[1]Vascular and Metabolic Diseases Research Group, Endocrinology Department, Son Llàtzer University Hospital, Health Research Institute of the Balearic Islands (IdISBa), Palma de Mallorca, Spain, [2]Laboratory of Renal Lithiasis Research, University of Balearic Islands, Research Institute of Health Science (IUNICS) Health Research Institute of Balearic Islands, (IdISBa), Palma de Mallorca, Spain, [3]Neurology Department, Son Llàtzer University Hospital, Palma de Mallorca, Spain, [4]Neuroradiology Unit, Son Llàtzer University Hospital, Palma de Mallorca, Spain, [5]Neuroopthalmology Unit, Son Llàtzer University Hospital, Palma de Mallorca, Spain, [6]Biochemistry Department, Son Llàtzer University Hospital, Palma de Mallorca, Spain, [7]Diabetes and Metabolism Research Unit, Vall d'Hebron Research Institute, Universitat Autònoma de Barcelona, Barcelona, Spain

Type 2 diabetes mellitus has a worldwide prevalence of 10.5% in the adult population (20–79 years), and by 2045, the prevalence is expected to keep rising to one in eight adults living with diabetes. Mild cognitive impairment has a global prevalence of 19.7% in adults aged 50 years. Both conditions have shown a concerning increase in prevalence rates over the past 10 years, highlighting a growing public health challenge. Future forecasts indicate that the prevalence of dementia (no estimations done for individuals with mild cognitive impairment) is expected to nearly triple by 2050. Type 2 diabetes mellitus is a risk factor for the development of cognitive impairment, and such impairment increase the likelihood of poor glycemic/metabolic control. High phytate intake has been shown to be a protective factor against the development of cognitive impairment in observational studies. Diary phytate intake might reduce the micro- and macrovascular complications of patients with type 2 diabetes mellitus through different mechanisms. We describe the protocol of the first trial (the PHYND trial) that evaluate the effect of daily phytate supplementation over 56 weeks with a two-arm double-blind placebo-controlled study on the progression of mild cognitive impairment, cerebral iron deposition, and retinal involvement in patients with type 2 diabetes mellitus. Our hypothesis proposes that phytate, by inhibiting advanced glycation end product formation and chelating transition

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metals, will improve cognitive function and attenuate the progression from Mild Cognitive Impairment to dementia in individuals with type 2 diabetes mellitus and mild cognitive impairment. Additionally, we predict that phytate will reduce iron accumulation in the central nervous system, mitigate neurodegenerative changes in both the central nervous system and retina, and induce alterations in biochemical markers associated with neurodegeneration.

KEYWORDS

type 2 diabetes mellitus, cognitive impairment, phytate, Mediterranean diet, mild cognitive impairment

1 Introduction

The treatment and prevention of increasing numbers of patients with type 2 diabetes mellitus (T2DM) and mild cognitive impairment (MCI) due to demographic and lifestyle changes are important challenges faced by healthcare systems. MCI is a milder form of cognitive dysfunction that precedes dementia and affects almost one in two people over the age of 65 with T2DM (1). People with MCI are at high risk of dementia (DE); the majority of studies indicate a progression rate to DE ranging from 20% to 40%, with an annual rate ranging between 5% and 17% (2). In addition, people with T2DM have a very high risk of MCI in comparation to the non-diabetic population. In these sense, it has been recognized that T2DM acts as an important accelerator of DE in patients with MCI (3, 4). Cognitive impairment makes self-care difficult and increases the risk of hypoglycemia, which increases comorbidity, hospital admissions, and the costs of T2DM to healthcare systems (5, 6).

T2DM has been correlated with an accelerated decline in cognitive function among the elderly and an increased likelihood of developing mild cognitive impairment and a higher risk of dementia, including both Alzheimer's disease (AD) and vascular dementia (7). In fact, T2DM doubles the risk of developing AD. This increase is maintained even after adjusting for vascular risk factors (8, 9). The mechanism connecting diabetes to AD is multifactorial and not well-defined. However, its pathogenesis is known to involve a decrease in brain-derived neurotrophic factor (BDNF) and an increase in oxidative stress due to the accumulation of advanced non-enzymatic glycation products (AGEs), systemic inflammation via tumor necrosis alpha (TNF-a), interleukin 1beta (IL-1b) and interleukin 6 (IL-6), increased lipid peroxidation, and decreased superoxide dismutase (SOD) activity (7, 10). In AD, it has been seen that AGEs, through their receptor (RAGE), increase the expression of tau and Aß (11).

Concomitantly, people with T2DM have a higher prevalence of sarcopenia and dynapenia in comparison with an age-matched population without T2DM. This prevalence could be a consequence of chronic inflammation and oxidative stress associated with this condition (12). In addition, sarcopenia and dynapenia have been associated with cognitive impairment (13). The preservation of the muscle tissue could be an effective strategy for improving not only metabolic state but also cognitive performance.

Investigating the relationship between iron homeostasis and mild cognitive impairment (MCI) is pivotal, considering the emerging evidence suggesting a potential link between disrupted iron metabolism and cognitive decline. Iron plays a crucial role in various cellular processes, including oxygen transport, energy metabolism, and neurotransmitter synthesis. However, dysregulation of iron homeostasis can lead to the accumulation of excess iron in the brain, contributing to oxidative stress, neuroinflammation, and neuronal damage (14). Ferroptosis is a unique form of iron-mediated programmed cell, which is distinct from apoptosis, necrosis, autophagy, and other forms of cell death (14). Iron deposits in neurons are closely tied to neurodegeneration and cognitive decline, although the specific mechanisms driving these connections remain uncertain (14). Various factors can alter iron homeostasis, causing the gradual accumulation of iron in various tissues, including the central nervous system (CNS) (15–17). Aging itself and diseases such as AD, Parkinson's

Abbreviations: T2DM, type 2 diabetes mellitus; MCI, mild cognitive impairment; AGEs, advanced glycation end products; CNS, central nervous system; AD, Alzheimer's disease; BDNF, brain-derived neurotrophic factor; TNF-a, tumor necrosis alpha; IL-1b, interleukin 1beta; IL-6, interleukin 6; SOD, superoxide dismutase; RAGE, receptor of advanced glycation end products; BBB, blood–brain barrier; IP6, myo-inositol hexaphosphate; MRI, magnetic resonance imaging; MoCA, Montreal Cognitive Assessment Test; SAGE, Self-Administered Gerocognitive Test; NPT, neuropsychological test; DSST, digit and symbol substitution test; (TMT-A and TMT-B), line test in version A and B; RAVLT, Rey Auditory-Verbal Learning Test; ROFC, Rey–Osterrieth Complex Figure Copy and Recall; BNT, Boston Naming Test; DCN, Data Collection Notebook; APOE, apolipoprotein E; BIA, Bio-impedance analysis; NMR, nuclear magnetic resonance; QSM, quantitative susceptibility mapping; BCVA, best-corrected visual acuity; OCT-SD, optical coherence tomography; RNFL, retinal nerve fiber layer; GCL, ganglion cell layer; VD, vessel density; PD, perfusion density; FAZ, foveal avascular zone; AE, adverse event; CEI-IB, Ethics and Clinical Research Committee of the Balearic Islands; ICH, International Conference on Harmonization.

disease, and Huntington's disease, among others, are related to an increase in iron levels in the region of the brain affected. The level of iron accumulation also correlates with the severity of the disease (18, 19). In addition, iron overload is associated with age-related macular degeneration and a subset of psychiatric diseases (20, 21).

Ferroptosis plays a role in neuronal loss during acute or chronic brain injury, and blocking ferroptosis could decrease cell death and improve neurological function in animal models (22). Conservative iron chelation has been proposed as a new therapeutic concept for the treatment of neurodegenerative diseases associated with increased iron storage (21). Unlike the chelators used for the treatment of systemic diseases, these chelators need to cross the blood–brain barrier (BBB). Deferoxamine, a hexadentate ligand with high affinity for Fe(III), is capable of inhibiting amyloid formation *in vitro* (23). However, it has difficulty in crossing the BBB when administered orally and also has side effects (24, 25). Clioquinol, a lipophilic binder, has been used successfully in phase II trials in moderate AD, but it is not iron selective and has significant side effects (26). Bearing this in mind, developing a chelator for treating neurodegenerative diseases, especially in individuals with MCI and T2DM, which is effective, easy to administer, and free of side effects, presents a considerable challenge.

Phytate (myo-inositol hexaphosphate, IP6) is a natural compound present in seeds (e.g., cereals, legumes, and nuts) as a calcium–magnesium salt (phytin) (27, 28). It possesses the ability to chelate various divalent and trivalent cations, including iron (27, 29–31) with a higher affinity than commonly used chelating agents like EDTA, deferoxamine, or deferiprone (30). This suggests that the consumption of IP6 could reduce iron bioavailability, and IP6 is able to cross de BBB (28, 32, 33). Moreover, in a study where we evaluated the relationship between dietary phytate (34), mineral status, and phytate levels in the body, it was observed that the amount of iron found in the brains of rats fed phytate diets was significantly lower than that found in rats fed non-phytate diets. Recently, an observational study has reflected that phytate intake was positively associated with cognitive function (35). Our group has shown that IP6 prevents the formation of pathological calcifications *in vivo*, such as calcium kidney stones (36, 37), sialolithiasis (38), and vascular calcification (39, 40), and protects against osteoporosis (41). In animals and cell models, phytate could also provide protection against cancer (33) and Parkinson disease (42). In a recent clinical trial with high safety levels, we have shown that daily phytate intake for 3 months is capable of reducing the levels of advanced glycation end products in T2DM patients (40). To our knowledge, no randomized clinical trial with IP6 supplementation and cognitive impairment have been carried out so far.

Our hypothesis is that phytate, due to its ability to inhibit the formation of AGEs and to chelate transition metals, will improve cognitive ability and delay the progression of mild cognitive impairment to dementia in patients with T2DM and MCI. It will also slow/prevent/mitigate the accumulation of iron in the CNS and the changes associated with neurodegeneration both in the CNS and in the retina. Likewise, changes in the biochemical markers associated with neurodegeneration are to be expected.

2 Objectives

2.1 Main aims

The main aims of this study are as follows:

1. To evaluate the effect of daily phytate intake on cognitive ability and compare it with the placebo group.
2. To determine the effect of daily phytate intake on morphological changes and iron storage associated with cognitive impairment in diabetes by magnetic resonance imaging (MRI).

2.2 Secondary aims

The secondary aims of this study are as follows:

1. To evaluate the effect of daily phytate intake on the evolution of retinal neurodegeneration parameters and diabetic retinopathy.
2. To determine the effect of daily phytate intake on the evolution of plasmatic biomarkers associated with cognitive decline and aging.
3. To assess the effect of daily phytate intake on body composition (using bioimpedance vector and muscle ultrasound) and grip strength (by dynamometry).

3 Experimental design

3.1 Design

The PHYND trial is a two-arm, double-blind, placebo-controlled study designed to evaluate the effect of daily phytate supplementation on the progression of mild cognitive impairment, brain iron storage, and retinal involvement in patients with type 2 diabetes. Patients diagnosed with MCI, and whose consumption of phytates is in the low/moderate range, will be recruited by the Endocrinology and Nutrition Service of the Son Llátzer University Hospital. The treatment will be diet modification: Arm 1 will take capsules of phytate dietary supplement three times a day (in the form of Lit-Control pH Balance) compared to Arm 2, which will take placebo capsules three times a day.

The present protocol was elaborated following the Standard Protocol Items: Recommendations for Intervention Trials (SPIRIT) 2013 Statement (43). Reporting of the study will follow the CONSORT statement recommendations on RCTs (44). Table 1 summarizes all items included in the trial registry, as suggested by the World Health Organization (WHO, 2018) (45).

TABLE 1 Data collection by visits.

	Initial evaluation	Initial evaluation	Start	Continuation visit	Continuation visit	Continuation visit	Continuation visit	Last visit	Follow-up
Weeks number	−8	−4	0	4	14	28	42	56	65
Informed consent	✓								
Inclusion & Exclusion criteria	✓								
Questions about health and disease	✓	✓	✓	✓	✓	✓	✓	✓	✓
Physical examination	✓		✓	✓		✓		✓	✓
Blood pressure and heart rate measurement	✓	✓	✓	✓	✓	✓	✓	✓	✓
ApoE genotype		✓							
LUES serology		✓							
Food frequency questionnaire	✓			✓	✓	✓	✓	✓	
Medical history evaluation	✓								
Grip Strength	✓					✓		✓	
MOCA questionnaire	✓							✓	
SAGE questionnaire	✓							✓	
Magnetic Resonance Imaging		✓					✓		
Ophthalmologic evaluation		✓						✓	
Beck questionnaire		✓						✓	
Rey figure		✓						✓	
Pittsburg Sleep Quality Index			✓					✓	

(Continued)

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TABLE 1 Continued

	Initial evaluation	Initial evaluation	Start	Continuation visit	Continuation visit	Continuation visit	Continuation visit	Last visit	Follow-up
2-h urinary phytate determination		✓				✓		✓	
Body composition evaluation			✓			✓		✓	
Lab test		✓			✓	✓	✓	✓	✓
AD biomarkers determination (TNF-alpha, IL-1B, IL-6, NF, t-tau, p-tau 181, Ab-42, Ab-40)		✓						✓	
AGE and RAGE determination			✓					✓	
Adverse effect evaluation				✓	✓	✓	✓	✓	✓
Pill counting				✓	✓	✓	✓	✓	

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3.2 Baseline variables

Variables to be measured to evaluate inclusion criteria are the following:

1. Diagnosis of MCI: cognitive screening for MCI will be performed using the Montreal Cognitive Assessment Test (MoCA) and the Self-Administered Gerocognitive Test (SAGE). We stratified MoCA results by race/ethnicity and education level before applying a cutoff value for the MoCA score to achieve more accurate cutoffs as suggested by Milani et al. (2018). "Mild cognitive impairment" will be considered when SAGE<17 (46) and MoCA-adjusted score (47) corresponds to an MCI.
2. Neuropsychological test (NPT): in patients who score MoCA corresponds to a MCI considering race and years of education, a cognitive assessment will be carried out using the following neuropsychological tests (NPT): SAGE, digit and symbol substitution test (DSST), line test in version A and B (TMT-A and TMT-B), Rey Auditory-Verbal Learning Test (RAVLT), Rey–Osterrieth Complex Figure Copy and Recall (ROCF), Boston Naming Test (BNT), and phonetic and semantic verbal fluency will also be tested. Through these tests, we will examine the following domains: visual scanning and processing speed (DSST, TMT-A), divided attention and executive function (TMT-B and verbal fluency), learning, retention memory and verbal recall (RAVLT), constructive praxia (including motor skills), visual memory (ROCF), and language (BNT). All these tests are validated in Spanish language. NPTs will not be performed unless the diagnosis of MCI with MoCA is established.
3. Phytate dietary questionnaire: a score ≤5 will be considered low/moderate phytate consumption (48).
4. Apolipoprotein E (APOE) genotype in serum samples.

3.3 Participants

3.3.1 *Inclusion criteria*

Those aged over 60 years, with a diagnosis of mild cognitive impairment, according to the MoCA and SAGE, low/moderate phytate intake, T2DM evolution of over 5 years, no history of cerebrovascular accident or neurodegenerative disease, no acute myocardial infarction or heart failure during the previous 6 months, no severe pre-proliferative retinopathy or proliferative retinopathy, and those who have signed the informed consent will be included.

3.3.2 *Exclusion criteria*

Those with illnesses or disorders that could affect deterioration, safety or adherence, immuno-suppressants, epilepsy, neoplasia in the previous 5 years, alcohol or drug abuse, anti-Parkinsonian or anticonvulsant medication, iron metabolism pathologies such as hemochromatosis and blood disorders requiring transfusions, skin neoplasia (history of cancer during the 5 years prior to the day of screening) with the exceptions of basal cell and squamous cell carcinoma of the skin and any carcinoma *in situ*, and those who are participating in any another clinical trial will be excluded.

3.3.3 Patient withdrawal criteria

The investigator must withdraw patients from the study when at least one of the following circumstances occurs: death, withdrawal of informed consent, and loss to follow-up. The reason and date of withdrawal from the study must be included in the Data Collection Notebook (DCN).

3.4 Sample calculation and randomization

There are no data on the effects of phytate supplementation on mild cognitive impairment. The sample size has been calculated based on other dietary intervention studies on MCI considering an alpha risk of 0.05 and a statistical power of 0.8 (49). Bearing in mind that we anticipate that there will be a difference of 30% in the progression of cognitive deterioration between those assigned diet 1 and those assigned diet 2, for a bilateral hypothesis test, a power of 80%, an alpha of 0.05, and a percentage of losses of 15%, we would obtain a minimum sample of 45 T2DM patients. Randomization will be 1:1 using fixed size stratified blocks. The stratification factors will be APOE genotype, age (75 years), gender, duration of diabetes (10 years), and previous cardiovascular disease (50).

4 Procedure

4.1 Description of interventions

Patients will have nine visits between week −8 (−8) and week 56 (w56), with week 0 (w0) being the point of randomization and start of treatment. These visits will take place in the endocrinology service of the Son Llàtzer University Hospital. Figure 1 sums up the different interventions that will take place in each visit. Furthermore, a follow-up visit will be performed at week 62 (w62).

4.1.1 Visit 1 (week −8)

At the patients' first visit (v1, w-8), inclusion criteria will be reviewed to ensure that they are eligible. To verify that the patient meets all the criteria deemed necessary to participate, the following procedures will be carried out:

- They will be informed about the different aspects of the study and given the information sheet so that they can read it carefully.
- They will be asked to read and sign the informed consent document.
- Questions about health and disease will be carried out.
- They will be asked questions about their medical history, and the researcher will check with them if they meet the requirements to participate in the project, including

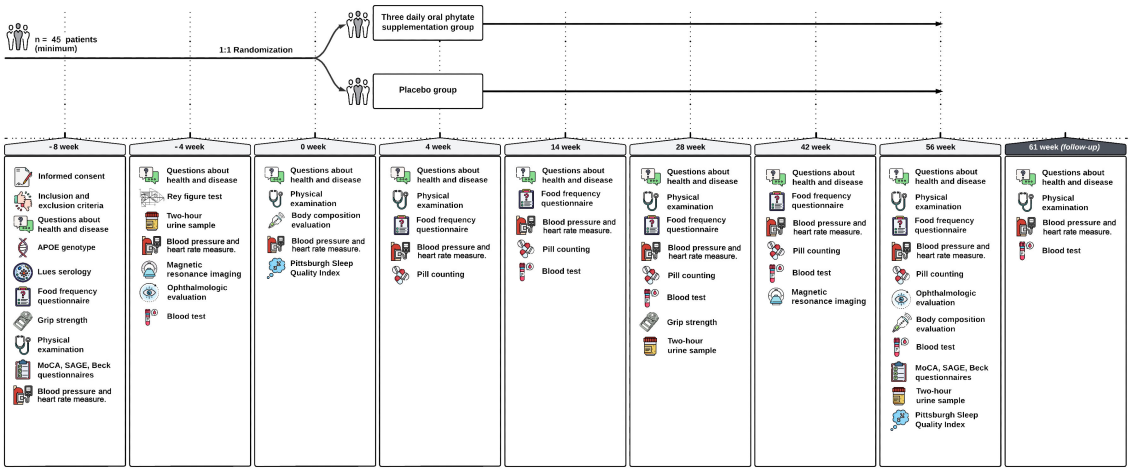

FIGURE 1
Outlines the actions to be conducted in each visit.

asking questions about their eating habits, consumption of tobacco, alcohol, and other substances.

- They will undergo screening tests for cognitive impairment and do the phytate dietary survey.
- A hand grip strength dynamometry will be performed to measure the grip strength in the dominant and contralateral hand.
- They will be asked if they are taking any other medication at the time of the project.
- The doctor will perform a complete physical examination, including taking height and weight measurements, to check their general state of health.
- Determination of APOE genotype and LUES serology will be requested.
- Their vital signs will be measured, including heart rate, blood pressure, and body temperature.
- They will be given a 2-h urine collection container to use for the next visit, and they will be scheduled for a baseline test at week −4.

4.1.2 Visit 2 (week −4)

On this visit:

- Questions about health and disease will be carried out.
- They will be asked if they are taking any other medication at the time of the project.
- Their vital signs will be measured, including heart rate, blood pressure, and body temperature.
- The relevant neuropsychological tests will be completed.
- They will be scheduled for a non-contrast MRI and ophthalmological tests to take place between week −4 and week 0.

4.1.3 Visit 3 (week 0)

In the case of patients who meet all the inclusion criteria and none of the exclusion criteria, the corresponding variables of the clinical history will be recorded in the DCN. On this visit:

- Questions about health and disease will be carried out.
- They will be asked if they are taking any other medication at the time of the project.
- The doctor will perform a complete physical examination, including taking height and weight measurements, to check their general state of health.
- Their vital signs will be measured, including heart rate, blood pressure, and body temperature.
- Body composition assessment will be carried out using vectorial bio-impedance analysis (BIA) and Nutritional Ultrasound to measure the diameter of the rectus femoris of the quadriceps and preperitoneal visceral adipose tissue (51).
- Non-contrast MRI and ophthalmological tests will take place between week −4 and week 0.
- Pittsburg Sleep Quality Index will be administered (52).

These patients will be randomly assigned to one of two treatment arms:

- Arm 1: 56 weeks with phytate dietary supplement
- Arm 2: 56 weeks with placebo

Diets will consist of:

- Treatment with phytate: patients will continue with their usual diets (low/moderate in phytate), and in addition, they will ingest three capsules a day (breakfast, lunch, and dinner) of a dietary supplement of 300 mg/sachet of

phytate (in the form of phytin) over the course of 56 weeks. The doctor will give them the correct number of capsules to last until the next visit. At the next visit, a blood test will be requested (in line with usual clinical practice), and a 2-h urine sample will be collected again to determine the urinary phytate concentration at weeks 0, 28, and 56.

- Placebo treatment: patients will continue with their usual diets (low/moderate in phytate) and, in addition, will ingest three placebo capsules a day (breakfast, lunch, and dinner) over the course of 56 weeks. The doctor will give them the correct number of capsules to last until the next visit. At the next visit, a blood test will be requested (in line with usual clinical practice), and a 2-h urine will be collected again to determine the urinary phytate concentration at weeks 0, 28, and 56.

4.1.4 Visits 4 (week 4), 5 (week 14), 6 (week 28), and 7 (week 42)

On these visits:

- Questions about health and disease will be carried out.
- They will be asked if they are taking any other medication at the time of the project.
- Safety will be reviewed. Any of the following outcomes will be recorded as serious adverse events: death, hospitalization of more than 24 h, persistent disability, and any other results that endanger the life of the subject. Episodes of clinically significant iron deficiency or anemia and episodes of hypoglycemia <54 mg/dl and level III hypoglycemia (that is, requiring the assistance of a third party) will be collected with particular interest.
- The doctor will perform a complete physical examination, including taking height and weight measurements, to check their general state of health at weeks 0 and 28.
- Their vital signs will be measured, including heart rate, blood pressure, and body temperature.
- At week 28, a urine sample will be collected.
- At weeks 14, 28, and 42, blood tests will be performed.
- Patients will be given a dietary survey to assess their adherence to the low phytate diet.
- Remaining supplement capsules will be collected and counted, and the correct number of capsules will be given to last until the next visit.
- Patients will be given a 2-h urine collection container that must be brought to their next visit at week 28.
- The final visit will be scheduled, where a blood test will be performed in line with usual clinical practice.

4.1.5 Visit 8 (week 56)

At this end-of-treatment visit:

- Safety will be reviewed, as above.
- Neuropsychological and cognitive deterioration tests will be completed again.
- Urine samples will be collected, and a blood test will be performed.
- A dietary survey to assess patient adherence to the low phytate diet will be completed.
- Any surplus supplement capsules will be collected, counted, and accounted for.
- The doctor will perform a complete physical examination, including taking height and weight measurements, to check the patient's general state of health.
- Vital signs will be measured, including heart rate, blood pressure, and body temperature.
- A non-contrast MRI and ophthalmological tests will be performed this week.
- Body composition assessment will be carried out using vectorial BIA and Nutritional Ultrasound to measure the diameter of the rectus femoris of the quadriceps and preperitoneal visceral adipose tissue (51).
- Pittsburg Sleep Quality Index will be administered.

4.1.6 Visit 9 (week 62)

In the follow-up visit:

- Questions about health and disease will be carried out.
- Their vital signs will be measured, including heart rate, blood pressure, and body temperature.
- Complete physical examination will be performed.
- A routine blood test will be carried out.

4.2 Manufacture of phytate and placebo

The phytate supplement and the placebo were purchased from the company Devicare S.L. The phytate will be administered in the same pharmaceutical form as the Lit-Control® pH Balance dietary supplement with national code (CN) 183677.9. The placebo treatment will be manufactured at ELABORADOS DIETÉTICOS S.AU., as a sub-contracted manufacturing plant with Good Manufacturing Practice certification.

5 Results

5.1 The main variables (according to the main objectives) will be the following:

1. Progression of cognitive impairment as a dichotomous variable defined as a score decrease >1 SD in MoCA or in any of the cognitive domains studied [visual scanning and processing speed (DSST, TMT-A), divided attention and

executive function (TMT-B and verbal fluency), learning, retention memory and verbal recall (RAVLT), praxia, visual memory (ROCF) and language (BNT)] at week 0 and week 56.

2. Differences in the quantification of the iron deposit assessed by nuclear magnetic resonance (NMR) (T2W (R2* and R'2; QSM) (General Electrics, Signa Explorer 1.5T) at week 0 and week 56. We quantify iron content in specific brain areas using magnetic resonance imaging (MRI) techniques such as quantitative susceptibility mapping (QSM) or R2* relaxometry (53). QSM describes the magnetizability of a material to an applied magnetic field and is a substance-specific value (54). Iron causes MRI images to darken at a rate proportional to the iron load, with the half-life of this darkening defined as T2* (55). The rate of darkening, designated as R2*, is the reciprocal of T2* and is proportional to the iron content of the tissues (55). MRI scanning estimates tissue iron concentration both by gradient echo imaging, which provides T2*, and spin echo imaging, which provides T2, the reciprocal of R2 (55). Novel NMR techniques allowed an improvement of tissue metal mapping methods and also the understanding of the ferroptosis mechanisms (53, 55).

5.2 The secondary variables (depending on the secondary objectives) will be the following:

1. Quantitative differences detected by the different cognitive tests (W0 and W56).
2. Morphological differences in the MRI without contrast (W0 and W56): degree of atrophy of the medial temporal lobe (Scheltens score) (56), periventricular intensity and hyperintense lesions in deep white matter (Fazekas scale) (57), and number of lacunar infarcts detected.
3. Differences in retinal neurodegeneration parameters and evolution of diabetic retinopathy (W0 and W56).
4. Retinal function: best-corrected visual acuity (BCVA) and macular perimetry.
5. Structural evaluation of the retina:
    a. Seven-field retinography and staging of diabetic retinopathy according to the Early Treatment Diabetic Retinopathy Study (58). Image of the disk-centered fundus, used to study the caliber and morphology of the vessels.
    b. Wide-field images with and without fluorescein to examine the peripheral retina and the degree of hypoperfusion (59) (60).
    c. Optical coherence tomography (OCT-SD) to assess neurodegeneration and macular edema parameters such as retinal nerve fiber layer (RNFL) thickness, ganglion cell layer (GCL) thickness, and choroidal thickness will be collected (61).
    d. OCT-angiography: to evaluate in detail the capillary plexuses of the macula, both the vessel density (VD), perfusion density (PD) in both superficial capillary plexus, and the foveal avascular zone (FAZ) will be collected (60).
6. Effect on AD biomarkers (W0 and W56):
    a. Inflammation mediators: TNF-a, IL-1b, and IL-6 (Multiplex)
    b. Neuronal injury markers: neurofilament (NF) (SimoaTM)
    c. AD biomarkers: t-tau, p-tau181, Ab42 and Ab40 (ELISA)
7. AGEs and RAGEs (ELISA)
8. Differences in sleep quality reported by Pittsburg Sleep Quality Index.
9. Differences body composition assessed with vectorial bio-impedance analysis and Nutritional Ultrasound to measure the diameter of the rectus femoris of the quadriceps and preperitoneal visceral adipose tissue.
10. Effect on hand grip strength dynamometry in the dominant and contralateral hand.

5.3 Other variables

Other variables include the following:

1. As the cognitive status of participants can be influenced by depression and educational level, this information will be collected, and a Beck Depression Inventory test will be performed to assess depressive symptoms (W0 and W56).
2. Laboratory parameters (W0, W14, W28, W42, W56, and W62). Glucose, HbA1c, liver profile, iron, ferritin, transferrin, transferrin saturation, calcium, phosphate, 25-(OH)-vitamin D (W0, W28, W56, and W62), PTH (W0, W28, and W56), creatinine, profile lipid, complete blood count, microalbuminuria/creatinine (W0, W28, and W56), and cystatin C, insulin, and hsPCR (W0 and W56). FIB-4 will be calculated.
3. Urinary phytate in 2 h urine (W-4, W28, and W56) (62).
4. Clinical history and results of physical examination: age, sex, race, educational level, date of diagnosis of T2DM, height (W-8, W0, and W56), weight, BMI, blood pressure, heart rate, waist circumference (W0, W14, W28, W42, W56, and W62), hypertension, cardiovascular disease, diabetic retinopathy, alcohol and tobacco consumption, history of polyneuropathy (W-8, W0, and W56), treatment of T2DM, and intake of other medications (W0, W14, W28, W42, W56, and W62).

5.4 Statistical analysis

For the analysis of primary and secondary objectives, differences within groups will be assessed using ANOVA for repeated

measures, and difference between groups will be assessed using ANCOVA for repeated measures. Qualitative variables will be analyzed using the chi-square test or the McNemar's test. The analysis will be adjusted for confounding variables. With regard gender dimension, women have a higher risk of dementia; therefore, an additional analysis will be carried out, stratifying results by sex. A value of $p<0.05$ will be considered an indicator of a significant difference. The analysis will be performed using SPSS software (IBM Corp., version 24.0).

5.5 Ethical considerations

The clinical researchers involved in the trial have a valid certificate of Good Clinical Practice. The study will adhere to law 14/2007 on biomedical research, the principles of the Declaration of Helsinki, and the Council of Europe Convention on Human rights and Biomedicine. Our protocol has been approved by the Ethics and Clinical Research Committee of the Balearic Islands (CEI-IB) with number "IB4719/21". The study will be carried out according to the standards of Good Clinical Practice and Guidelines of the International Conference on Harmonization (ICH).

6 Safety

The treatment that is used, Lit-Control® pH Balance, has been developed with ingredients that have been internationally recognized as safe. No incidents have been reported by its consumers since the beginning of its commercialization on said date. However, in case that a possible serious adverse event was to be detected and recorded, the treatment would be suspended and the investigator would proceed as follows:

- The sponsor would be immediately informed of all adverse events. Immediate notification must be followed by timely detailed written reports. The immediate and follow-up report must identify subjects by a unique code assigned to the trial subject and not by the subject's name, personal identification numbers, or subject's address. The investigator must also comply with the legal regulations regarding the notification of serious and unexpected adverse events to the relevant authorities and to the CEI-IB.
- When the researcher notifies a death, he must provide the promoter and the CEI-IB with all the complementary information that they request (e.g., the autopsy report and the most recent medical reports). An adverse event (AE) will be considered any adverse health incident that occurs in a patient who has received a treatment, even where there is not necessarily a causal relationship with said treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of treatment, whether or not related to it.

According to current regulations, "serious adverse events" will be considered as "any adverse reaction that causes death, may be life-threatening, requires hospitalization of the patient or prolongation of existing hospitalization, causes significant or persistent disability or invalidity, or constitutes a congenital anomaly or birth defect. Those suspected adverse reactions that are considered important from a medical point of view, even if they do not meet the above criteria, such as those that put the patient at risk, or require intervention to prevent any of the above outcomes, will also be treated as serious, as will all suspicions of transmission of an infectious agent through a medication (RD 1090/2015, art. 22.1.d "Serious adverse reaction")".

7 Validity and reliability

The current study will provide important information on the effect of oral phytate supplementation on cognitive impairment progression, cerebral iron deposition, and diabetic retinopathy on patients living with T2DM. The PHYND study will include a control sample of participants that will receive placebo. The free-living conditions and the study design will allow us to obtain robust results. Internal validity will be warranted by a randomization process based on allocation sequence generation, blinded to the PI and staff involved in the intervention. Moreover, the data analyst and the PI will be blinded to patient allocation to reduce biases in the evaluation of the intervention. Lastly, the interventions are based on the latest scientific evidence.

8 Discussion

The present study will provide information on the efficacy of an oral phytate supplementation intervention (≈1g/day) to reduce cognitive impairment progression, cerebral iron deposition, and diabetic retinopathy on patients living with T2DM. There is no specific treatment available to reduce cognitive impairment progression on patients with T2DM. Diets known to be high in phytate are associated with lower cognitive decline (33). So far, there is no randomized clinical trial assessing the effect of phytate intake in cognitive decline and neurodegenerative disease. Our work will be the first one in assessing the effects of oral phytate supplementation in amounts corresponding to a healthy and balanced diet rich in nuts and legumes (≈1g/day) on cognitive impairment progression in outpatients conditions.

The PHYND study presents some limitations. First, it focuses on patients with T2DM and MCI, potentially restricting the generalizability of results to broader populations. Second, ensuring participant compliance with the daily phytate supplementation regimen is crucial, yet despite monitoring mechanisms like pill counts and urinary phytate level assessments, inconsistent adherence may impact result interpretation. Third, despite rigorous blinding procedures and the use of validated outcome measures to minimize placebo

effects, improvements related to the placebo effect within the placebo group are still possible, which could influence the interpretation of study outcomes. The most important limitation will be patient adherence to the treatment and the study, given its length, i.e., 56 weeks of intervention. However, this issue will be mitigated by frequent follow-up, frequently telephonic reminders of the appointments, and motivational interview. Another issue is the small sample size. We anticipate a 15% drop off based on other dietary intervention studies on MCI considering an alpha risk of 0.05 and a statistical power of 0.8(49); a minimum sample of 45 T2DM patients is required. Keeping this in mind, we will recruit 60 patients per arm. Thus, reducing the probability of small sample is a limitation of our work. In addition, as the study is single center (the sample will be taken at the Endocrinology and Nutrition service of the Son Llàtzer Hospital), we are aware that it could not be possible to generalize the results obtained.

However the strengths of our work are a multidisciplinary approach, where the effect of phytate supplementation on the brain and the retina will be objectively examined using validated ophthalmic tests, magnetic resonance imaging iron measurement, evaluation of cognitive impairment through validated psychological tests, extensive laboratory tests (using the accepted laboratory parameters, inflammatory mediators, markers of neuronal injury, AD biomarkers, and advanced glycation products), and measurement of body composition and strength using nutritional ultrasound and bio-impedance vector analysis and dynamometry. In addition, as the cognitive status of participants can be influenced by depression, educational level and quality, and quantity of sleep, this information will be collected with validated tests. Phytate levels will be monitored by measuring adherence to diet supplementation and serial measurement of urinary phytate levels.

Finally, the results of our study could contribute to better nutritional strategies for the management of cognitive impairment and diabetic-related complications. Given the increasing prevalence of T2DM and cognitive impairment, determining interventions that could prevent or reduce diabetes-related complications and reduce the progression of cognitive impairment could have a great impact on the lives of our population.

Ethics statement

The studies involving humans were approved by Ethics and Clinical Research Committee of the Balearic Islands (CEI-IB) with number "IB4719/21". The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

AP: Conceptualization, Data curation, Investigation, Validation, Writing – original draft, Writing – review & editing. PS: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Visualization, Writing – original draft, Writing – review & editing. MT: Conceptualization, Investigation, Methodology, Project administration, Validation, Writing – review & editing. JN: Conceptualization, Investigation, Methodology, Writing – review & editing. FG: Funding acquisition, Investigation, Methodology, Resources, Validation, Visualization, Writing – review & editing. AEsp: Investigation, Methodology, Validation, Visualization, Writing – review & editing. AEst: Conceptualization, Methodology, Validation, Writing – review & editing. ER: Investigation, Methodology, Writing – review & editing. GA: Investigation, Methodology, Writing – review & editing. MR: Investigation, Methodology, Writing – review & editing. JR: Investigation, Methodology, Writing – review & editing. IG: Investigation, Methodology, Writing – review & editing. OS-S: Investigation, Methodology, Writing – review & editing. LM: Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing, Conceptualization, Funding acquisition, Investigation, Methodology, Project administration.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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EDITED BY
Joana Nicolau,
Son Llatzer Hospital, Spain

REVIEWED BY
Inha Jung,
Korea University Ansan Hospital, Republic of Korea
Dorota Formanowicz,
Poznan University of Medical Sciences, Poland

*CORRESPONDENCE
Worku Chekol Tassew
workukid16@gmail.com

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Cognitive impairment and associated factors among patients with diabetes mellitus in Africa: a systematic review and meta-analysis

Worku Chekol Tassew[1*], Yeshiwas Ayal Ferede[2] and Agerie Mengistie Zeleke[3]

[1]Department of Medical Nursing, Teda Health Science College, Gondar, Ethiopia, [2]Department of Reproductive Health, Teda Health Science College, Gondar, Ethiopia, [3]Department of Clinical Midwifery, Teda Health Science College, Gondar, Ethiopia

Background: Inappropriate management of blood sugar in patients with diabetes mellitus leads to micro-vascular and macro-vascular complications, subsequently leading to high morbidity and mortality rates. In addition, diabetes independently increases the occurrence of cognitive impairment complicated by dementia. Scientific evidence on the magnitude of cognitive impairment will provide a sound basis for the determination of healthcare needs and the planning of effective healthcare services. Despite this, there are no comprehensive data on the prevalence and associated factors of cognitive impairment among patients with diabetes in Africa.

Methods: To identify relevant articles for this review, we searched PubMed, Cochrane Library, Science Direct, African Journals Online, and Google Scholar. After extraction, the data were imported into Stata software version 11 (Stata Corp., TX, USA) for further analysis. The random-effects model, specifically the DerSimonian and Laird (D+L) pooled estimation method, was used due to the high heterogeneity between the included articles. Begg's and Egger's regression tests were used to determine the evidence of publication bias. Sub-group analyses and sensitivity analyses were also conducted to handle heterogeneity.

Results: The pooled prevalence of cognitive impairment among patients with diabetes in Africa is found to be 43.99% (95% CI: 30.15–57.83, $p < 0.001$). According to our analysis, primary level of education [pooled odds ratio (POR) = 6.08, 95% CI: 3.57–10.36, I^2 = 40.7%], poorly controlled diabetes mellitus (POR = 5.85, 95% CI: 1.64–20.92, I^2 = 87.8%), age above 60 years old (POR = 3.83, 95% 95% CI: 1.36–10.79, I^2 = 63.7%), and diabetes duration greater than 10 years (POR = 1.13; 95% CI: 1.07–1.19, I^2 = 0.0%) were factors associated with cognitive impairment among patients with diabetes.

Conclusion: Based on our systematic review, individuals with diabetes mellitus exhibit a substantial prevalence rate (43.99%) of cognitive impairment. Cognitive impairment was found to be associated with factors such as primary level of

education, poorly controlled diabetes mellitus, age above 60 years, and diabetes duration greater than 10 years. Developing suitable risk assessment tools is crucial to address uncontrolled hyperglycemia effectively.

Systematic review registration: https://www.crd.york.ac.uk/prospero, identifier CRD42024561484.

KEYWORDS

cognitive impairment, diabetes mellitus, systematic review, meta-analysis, Africa

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders characterized by elevated levels of glucose in the blood resulting from defects in insulin secretion, insulin action, or both (1). The burden of DM is increasing worldwide, especially in developing countries (2). In 2014, the global prevalence of diabetes was 422 million, and by 2040, this number is expected to rise to more than 642 million. The healthcare costs for DM reached 162 billion dollars in 2019 and will be 185 billion dollars in 2045 (3, 4). Inappropriate management of blood sugar in patients with DM leads to micro-vascular and macro-vascular complications, subsequently leading to high morbidity and mortality rates. In addition, diabetes independently increases the occurrence of cognitive impairment (CI), which is complicated by dementia (5–7).

CI is defined as a disturbance in memory, acquiring knowledge, focusing, or making decisions that have a negative impact on activities of daily life (8). Patients with DM are more likely to develop cognitive problems and dementia than patients without DM (9). DM can lead to the accumulation of waxy protein in the neuron by decreasing its excretion through cerebrospinal fluid, ultimately resulting in cognitive decline (10). Extended exposure of nerve cells to high levels of glucose weakens the connections between neurons, leading to their distortion, a condition directly linked to cognitive dysfunction (7).

Different studies have suggested that the exact pathophysiology of CI in diabetic patients may be due to blood vessel abnormality, insulin transmission disturbance in the cerebrum, recurrent attack of nerve cells with hyperglycemia and hypoglycemia, and accumulation of waxy protein in the neuron (11, 12). The global prevalence of CI is 45% (13) with the lowest and highest prevalence being 21.8% and 67.5%, respectively (14, 15). The global prevalence of complications of CI (dementia) in 2010 was 35.6 million, which is expected to rise to 65.7 million by 2030 (16). The overall prevalence of CI in sub-Saharan African countries among the general population has been estimated to be between 6.3% and 25% (17). The comorbidity of DM and CI is the major challenge for the long-term management of DM. DM not only causes CI but also induces the complications of CI (18). The global healthcare cost of CI complicated by dementia is 1.5 times higher than that of patients without dementia (19).

An increase in the magnitude of CI with the high cost of healthcare will impose a serious social, medical, and economic burden, causing a major challenge to the already strained healthcare system of African countries. CI is a major problem that affects the effective long-term management of diabetes. Early diagnosis of CI in patients with diabetes is important for the recovery of cognitive function and the delay of cognitive decline, as well as improving medication adherence in people with diabetes. Scientific evidence on the magnitude of CI will provide a sound basis for the determination of healthcare needs and the planning of effective healthcare services.

Despite this, there are no comprehensive data on the prevalence and associated factors of CI among patients with diabetes in Africa. We therefore designed this review to assess the prevalence and associated factors of CI among patients with diabetes in Africa.

Abbreviations: DM, diabetes mellitus; CI, cognitive impairment; IBCS, institutional-based cross-sectional study; POR, pooled odds ratio; PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Materials and methods

The international Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines were used (20) to report the findings of this systematic review and meta-analysis (Supplementary Material 1).

Publication search strategy

To identify pertinent articles for this review, we conducted searches in several databases, including PubMed, Cochrane Library, Science Direct, African Journals Online, and Google Scholar. These searches were performed by two authors (WCT and AMZ) between 10 November 2023 and 10 January 2024. We used the following MeSH terms while searching from the above electronic databases: "cognitive dysfunction" OR "cognitive impairment" OR "neurocognitive disorder" OR "cognitive decline" AND "diabetes"

OR "diabetes mellitus" OR "diabetes mellitus type 2" OR "diabetes mellitus type II" OR "type II diabetes mellitus" OR "type 2 diabetes mellitus" OR "type 2 diabetes" OR "diabetes mellitus type 1" OR "diabetes mellitus type I" OR "type I diabetes mellitus" OR "type 1 diabetes mellitus" OR "type 1 diabetes". The snowball technique from the searched articles was also used to obtain additional articles.

Eligibility criteria

Inclusion criteria

We included the following types of primary studies: cross-sectional, case–control, and cohort studies that reported the prevalence of CI among patients with diabetes; peer-reviewed studies published in English; studies conducted inside Africa among patients with diabetes; moderately and highly qualified studies; and freely accessible studies.

Exclusion criteria

The review excluded studies that did not involve patients with diabetes, those that did not provide data on CI prevalence, case reports, low-quality studies, or studies that were published in languages other than English.

Outcome of interest

The outcome of this review is the prevalence of CI and associated factors among patients with diabetes. CI was assessed using the Mini-Mental State Examination (MMSE) in the primary studies.

Article selection and data extraction

Duplicate articles were deleted after importing all articles into the EndNote version X7 software. Then, two authors (YAF and AMZ) screened the articles critically for eligibility criteria. The corresponding author, publication year, study setting, study design, study population, sampling procedure, total sample size, response rate (participant), associated factors, and prevalence of CI among patients with diabetes were extracted by two authors (YAF and AMZ) using a standardized Microsoft Excel data extraction format. Associated factors were extracted based on the following eligibility criteria: having a similar categorization, having a similar operational definition, having been reported with a similar statistical measure (odds ratio), having a similar direction of association, and having been associated with two or more articles.

Quality assessment

Two authors (WCT and YAF) assessed the quality of articles using tools assessing the risk of bias in prevalence studies: modification of an existing tool and evidence of interrater agreement developed by Hoy and Brooks (21). The tool consists of 10 items addressing four domains of bias plus a summary risk of bias assessment. The 10 items are representativeness, sampling frame/procedure, random selection, non-response bias, direct data collection from patients, acceptability of case definition, study tool reliability and validity, same mode of data collection, appropriate length of prevalence period, and appropriateness of numerator and denominator. Uncertain or unclear items were considered to have a high risk of bias. After summarizing the high risk of bias, the overall risk of bias was evaluated as low (≤2), moderate (3–5), and high (≥5) (Supplementary Material 2).

Statistical analysis

After extraction, the data were imported to Stata software version 11 (Stata Corp LLC., TX, USA) for further analysis. Heterogeneity between the included articles was assessed using Cochran's Q chi-square test at a significance level of less than 0.05 and inverse variance (I^2 index). Values of 0%–40%, 40%–60%, 60%–90%, and 90%–100% indicated low, medium, substantial, and high heterogeneity, respectively (22). The random-effects model, specifically the DerSimonian and Laird (D+L) pooled estimation method, was used due to the high heterogeneity among the included articles (23). Sub-group analyses and sensitivity analyses were also conducted to handle heterogeneity. Egger's and Begg's regression tests and visual inspection of the funnel plot were utilized to assess the evidence of publication bias.

Results

Study selection

Our systematic search found a total of 613,277 articles from five databases: [Google Scholar (18,200), PubMed (588,989), Cochrane Library (402), Science Direct (5352), and African Journal Online (334)]. After importing all articles into Endnote, 433,547 articles were removed due to duplication. Of the remaining articles, 178,868 were excluded after title screening. The abstract text of 862 articles was assessed for eligibility criteria; finally, 13 articles ultimately met the inclusion criteria and were included in the review. A summary of the steps involved in the screening process and the reasons for the exclusion of articles is provided (Figure 1).

Baseline characteristics of the included publications

The review analyzed the results of 13 articles (11 studies are institutional-based cross-sectional, and the remaining 2 are cohort and case–control studies). The included articles were conducted in different countries in Africa; three from Ethiopia (24–26), four from Nigeria (27–30), three from Egypt (31–33), and the remaining from Cameroon, Congo, and Morocco (34–36). Detailed baseline characteristics of the included articles are presented in Table 1.

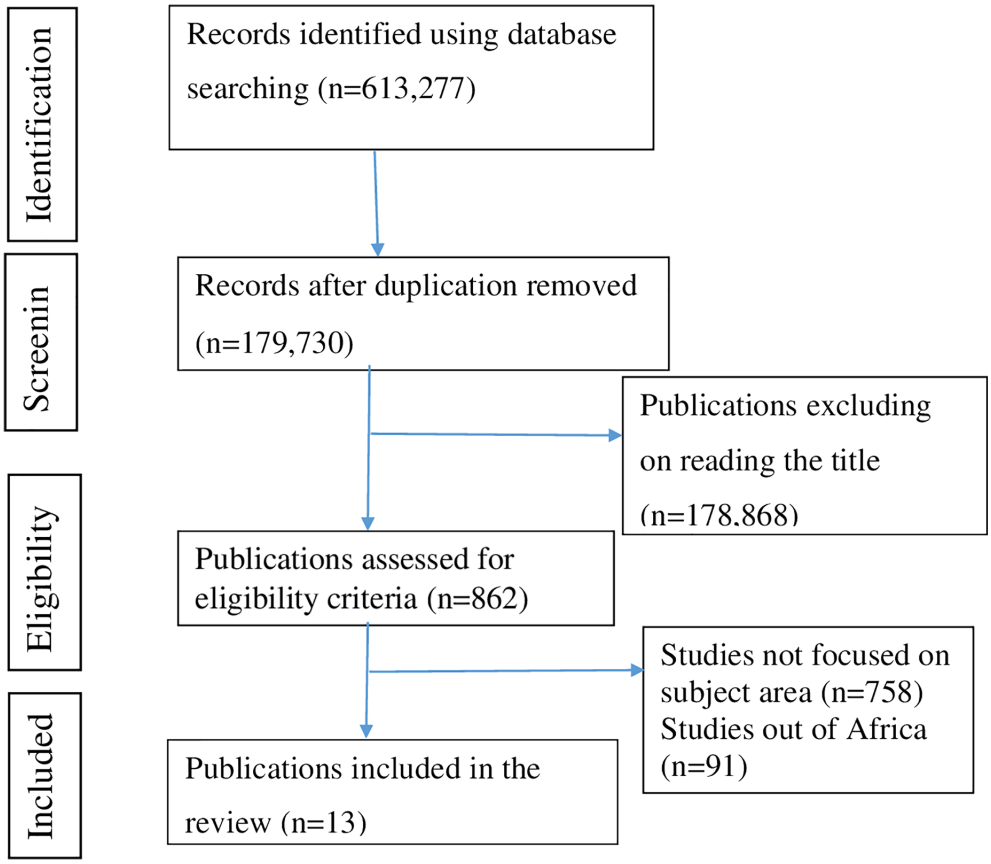

FIGURE 1
PRISMA flow diagram of the selection of publications for a systematic review and meta-analysis on the prevalence and associated factors of cognitive impairment among patients with diabetes in Africa (*N* = 13).

TABLE 1 Baseline characteristics of the included articles on the prevalence and associated factors of cognitive impairment among patients with diabetes in Africa (*N* = 13).

Author	Publication year	Country	Study design	Study population	Sample size	Prevalence (%)	Sampling method
Ashebir et al. (24)	2024	Ethiopia	IBCS	Patients with DM	421	56.3	Systematic random
Mulugeta et al. (25)	2013	Ethiopia	IBCS	Patients with DM	384	45	Simple random
Abba et al. (34)	2017	Cameroon	IBCS	Patients with DM	223	14.8	Consecutive
Eze et al. (27)	2015	Nigeria	IBCS	Patients with DM	113	40	Systematic random
Abdellatif et al. (31)	2020	Egypt	IBCS	Patients with DM	200	34	Consecutive
Williams et al. (28)	2020	Nigeria	IBCS	Patients with DM	485	33.4	Consecutive
Dagnew et al. (26)	2017	Ethiopia	IBCS	Patients with DM	210	53.3	Consecutive
Mohamed et al. (32)	2023	Egypt	IBCS	Patients with DM	400	50	Simple random
Anwar et al. (33)	2018	Egypt	IBCS	Patients with DM	100	18	Consecutive
Bashir and Yarube (29)	2022	Nigeria	IBCS	Patients with DM	93	88.5	Systematic random
Ossou et al. (35)	2019	Congo	Case control	Patients with DM	200	57	Consecutive
Tlemcani et al. (36)	2022	Morocco	Cohort	Patients with DM	100	47.5	Consecutive
Adebayo et al. (30)	2022	Nigeria	IBCS	Patients with DM	274	27	Systematic random

*IBCS, institutional-based cross-sectional study.

Quality of the included studies

Based on the quality assessment results, 11 studies (84.61%) of the included articles have a low risk of bias, and 2 have a moderate risk of bias. The detailed results of the quality assessment of the articles are provided in the Supplementary File (Supplementary File 2).

Publication bias

Begg's and Egger's regression tests were used to determine the evidence of publication bias. Based on our results, there is no significant publication bias indicated with Egger's regression test p-value >0.05 ($p = 0.532$) and symmetrical inspection of the funnel plot (Figure 2).

Sub-group analysis

Sub-group analysis was done based on sampling techniques. The results showed that the highest prevalence of CI was reported in articles that used systematic random sampling [53.52% (CI: 23.41, 83.62)] and there was no heterogeneity in articles that used simple random sampling (Figure 3).

Meta-analysis

The pooled prevalence of CI among patients with diabetes in Africa is found to be 43.99% (95% CI: 30.15–57.83, $p < 0.001$). The analysis showed a high heterogeneity between the included articles ($I^2 = 92.7\%$, $p < 0.001$). As a result, a random-effects model, specifically the DerSimonian and Laird (D+L) pooled estimation method, was used to estimate the pooled prevalence of CI (Figure 4).

Sensitivity analysis

Sensitivity analysis was also conducted using the random-effects model, and the results showed that no single study influenced the pooled prevalence of CI among patients with diabetes (Figure 5).

Associated factors of cognitive impairment

Four factors are associated with CI among patients with diabetes, based on extracted factors from the primary articles. They are primary educational level, uncontrolled DM, age greater than 60 years old, and duration of DM greater than 10 years. According to our analysis, the random pooled odds ratio of developing CI was 6.08 times (POR = 5.85, 95% CI: 1.64–20.92, $I^2 = 87.8\%$) higher among patients with diabetes who completed primary education compared to college and post-college education(Figure 6). The random pooled odds ratio of developing CI was 5.85 times higher (POR = 3.83, 95% CI: 1.36–10.79, $I^2 = 63.7\%$) among patients with DM whose blood glucose was uncontrolled as compared to patients with DM whose blood sugar was controlled (Figure 7). Patients with diabetes whose age was above 60 years old were 3.83 times more likely to develop CI (POR: 1.13; 95% CI: 1.07–1.19, $I^2 = 0.0\%$) than patients whose age was lower than or equal to 60 years old (Figure 8). The review also found that patients with DM who had diabetes for more than 10 years were 1.13 times more likely to develop CI (POR: 1.13; 95% CI: 1.07–1.19, $I^2 = 0.0\%$) than patients with DM for less than 10 years (Figure 9).

Discussion

According to the review, the pooled prevalence of CI among patients with diabetes in Africa is found to be 43.99% (95% CI: 30.15–57.82, $p < 0.001$). This result is in line with the findings of

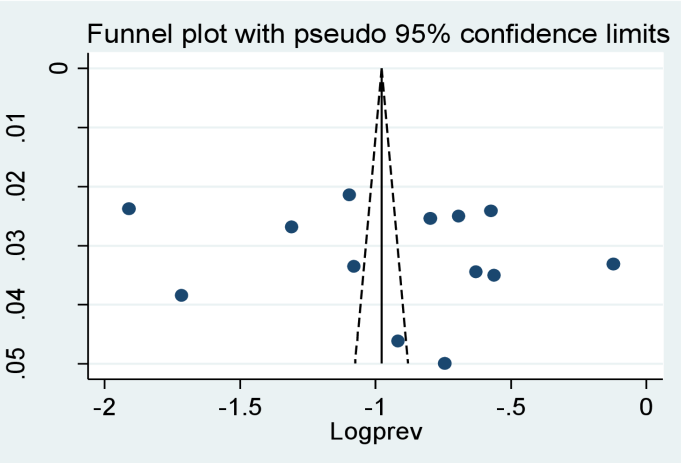

FIGURE 2
Funnel plot showing the absence of publication bias in the systematic review and meta-analysis of prevalence and associated factors of cognitive impairment among patients with diabetes in Africa ($N = 13$).

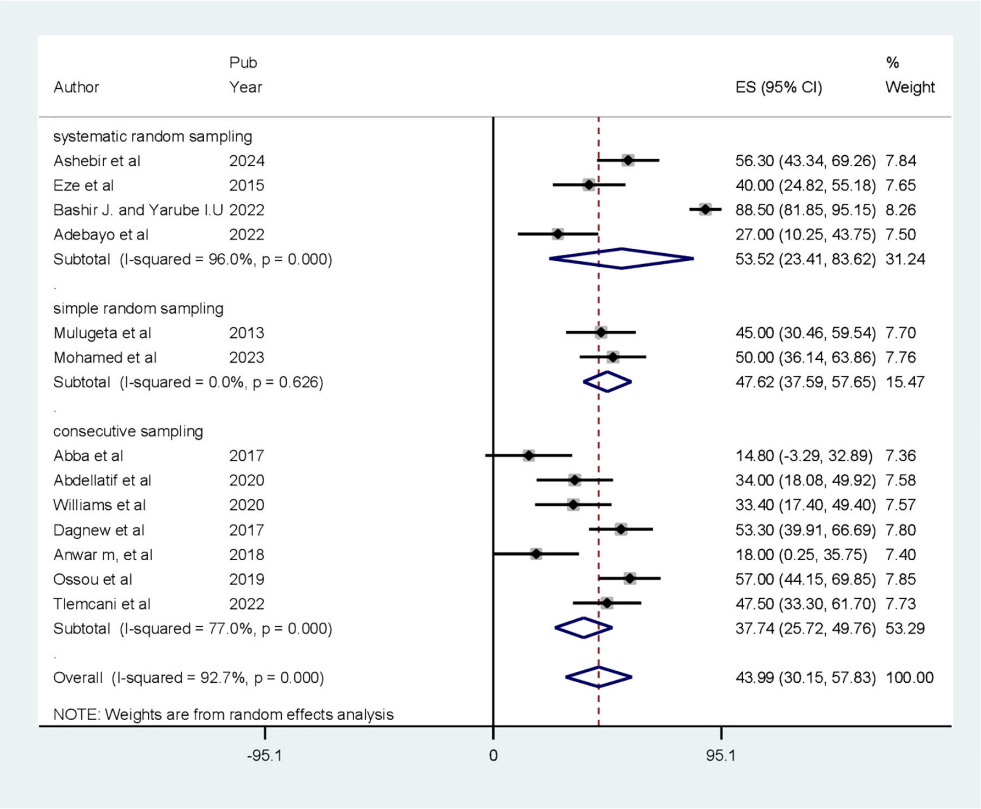

FIGURE 3
Forest plot of the sub-group analysis showing the prevalence of cognitive impairment among patients with diabetes based on sampling technique in Africa (*N* = 13).

studies conducted in China, 45.0% (95% CI: 36.0–54.0) (13); India, 33.73% (37); Korea, 31.5% (38); Philippines, 45% (39); and Poland, 32.7% (40).

However, the finding is higher than the result of studies done in Japan, 26% (41); China, 21.8% (14) and China, 13.5% (42); USA, 25.6% (43); and New York, 28% (44). The elevated prevalence observed in our review could stem from several factors. First, in the setting of the aforementioned study, hospitalized patients may typically experience better plasma glucose management as physicians prioritize close monitoring of their levels, potentially leading to a lower prevalence in this group. Conversely, in our study setting, the study population tends to be of lower socioeconomic status, which is linked to poorer cognitive function due to limited resources and healthcare access. Additionally, variations in the study populations, such as the inclusion of individuals with advanced DM in the Nigerian study, may significantly contribute to the occurrence of complications and cognitive decline. Finally, the substandard quality of healthcare services in African contexts may also play a role in the higher prevalence observed. The other interesting reason for this discrepancy is that carriers of apolipoprotein E (*APOE*) ε4 are at high risk for cognitive decline and Alzheimer's disease. The ε4 allele is more common in Black than white individuals. The ε4 allele is associated with a risk of cognitive decline and dementia (45).

However, the pooled prevalence of CI in our review is lower than the findings of studies done in India, 58.29% (46) and 64.86% (47) and Pakistan, 67.3% (15). A potential explanation could be the variation in the age demographics of the subjects studied. The research conducted in Pakistan involved older patients with DM, who are more prone to age-related cognitive decline. Conversely, studies in India focused on patients with chronic DM, who are at higher risk for various complications, including CI.

According to our analysis, the random pooled odds ratio of CI among patients with diabetes who completed primary education was 6.08 times higher as compared to those whose educational level was college or above. The finding is similar to the results of studies conducted in China and Germany (48, 49). This may be due to the fact that individuals with higher education tend to have a larger "cognitive reserve," meaning that they have a greater capacity for mental processing and can better compensate for age-related declines in brain function. This reserve may be built through years of stimulating mental activity, including learning new information, problem solving, and engaging in complex tasks.

The random pooled odds ratio of developing CI among patients with DM whose blood glucose was uncontrolled was 5.85 times higher compared to patients with DM whose blood sugar was controlled. The finding is consistent with the results of studies done in New York and India (38, 50). This is explained by the fact that elevated blood sugar levels in diabetes can directly damage

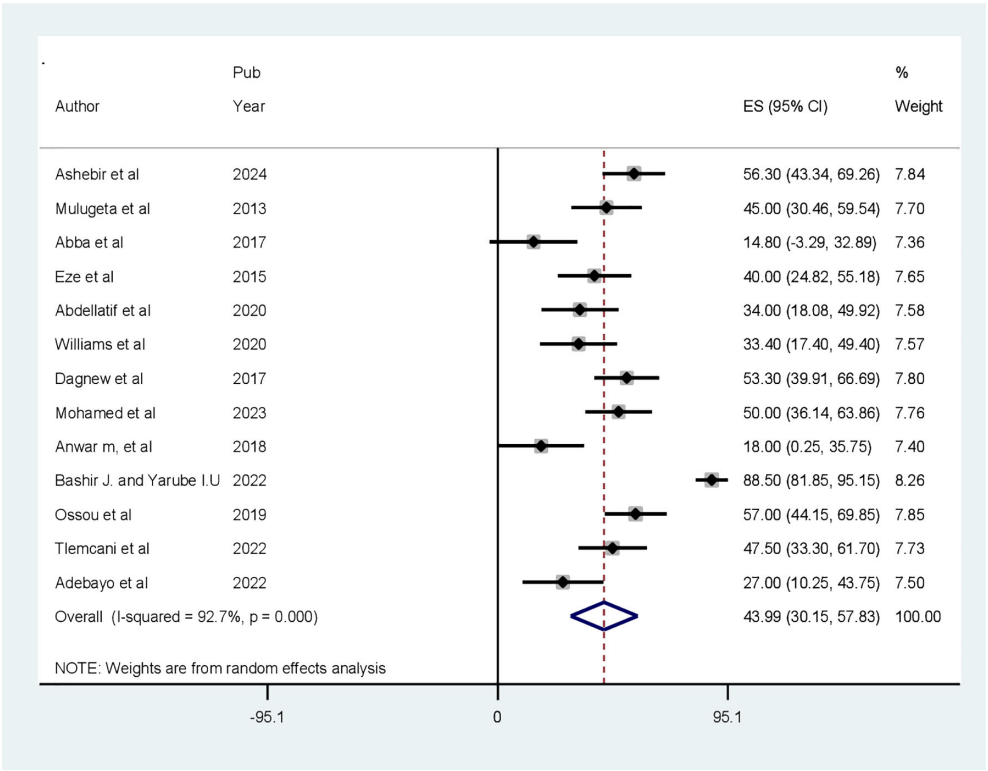

FIGURE 4
Forest plot showing the prevalence of cognitive impairment among patients with diabetes in Africa (*N* = 13).

neurons through an increased polyol pathway, advanced glycation end products, protein kinase C (PKC) activation, and increased production of free radicals (highly reactive molecules that damage cells and DNA) (51).

Patients with diabetes whose age was above 60 years old were 3.83 times more likely to develop CI than patients whose age was lower than or equal to 60 years old. This is in line with the findings of a study done in India (52). The possible justification for this

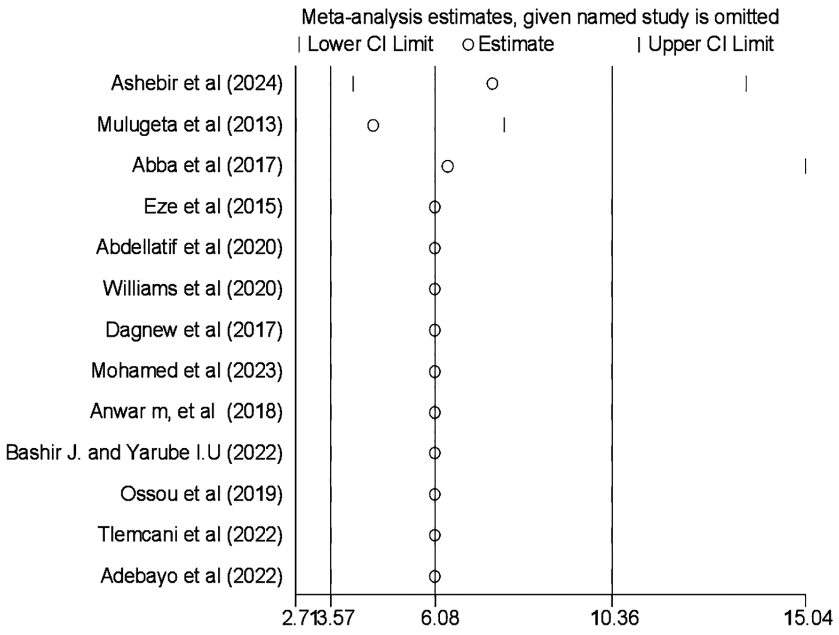

FIGURE 5
Result of the sensitivity analysis on the prevalence and associated factors of cognitive impairment among patients with diabetes in Africa (*N* = 13).

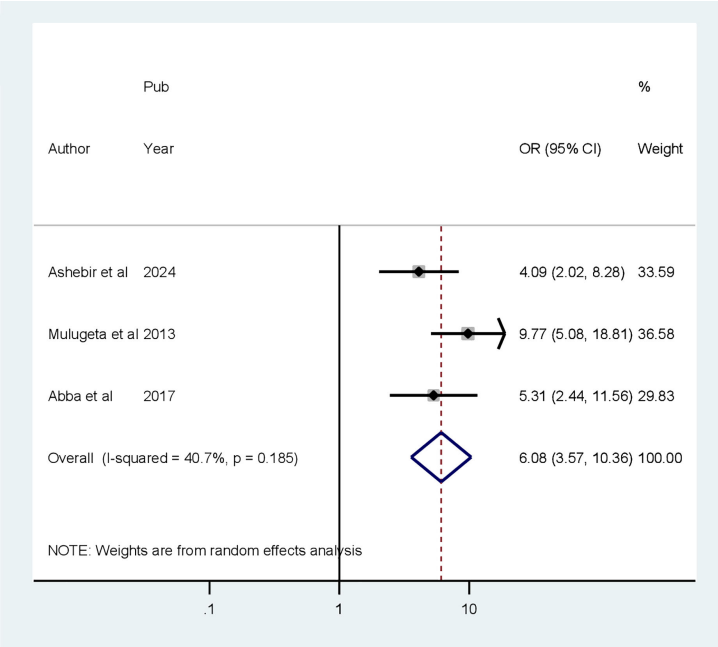

FIGURE 6
Forest plot showing the association between primary education level and cognitive impairment among patients with diabetes in Africa (*N* = 13).

might be that as age increases, the brains naturally undergo changes that can contribute to cognitive decline, including neuronal loss (gradual death of neurons throughout the brain, particularly in areas critical for memory, learning, and reasoning), synaptic dysfunction (weakening of connections between neurons, hindering communication and information processing), and neuro-inflammation (chronic low-grade inflammation in the brain, which can damage neurons and impair cognitive function) (48). The other justification for this may be due to a decline in processing speed, working memory, and episodic memory (recalling specific events) with age (52). Another reason for this may be due to early-onset familial Alzheimer's disease (EOFAD),

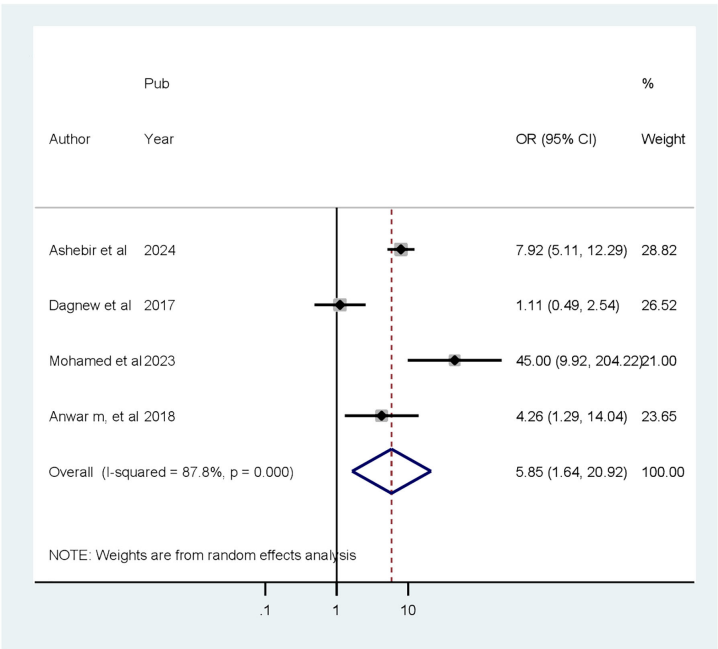

FIGURE 7
Forest plot showing the association between uncontrolled DM and cognitive impairment among patients with diabetes in Africa (*N* = 13).

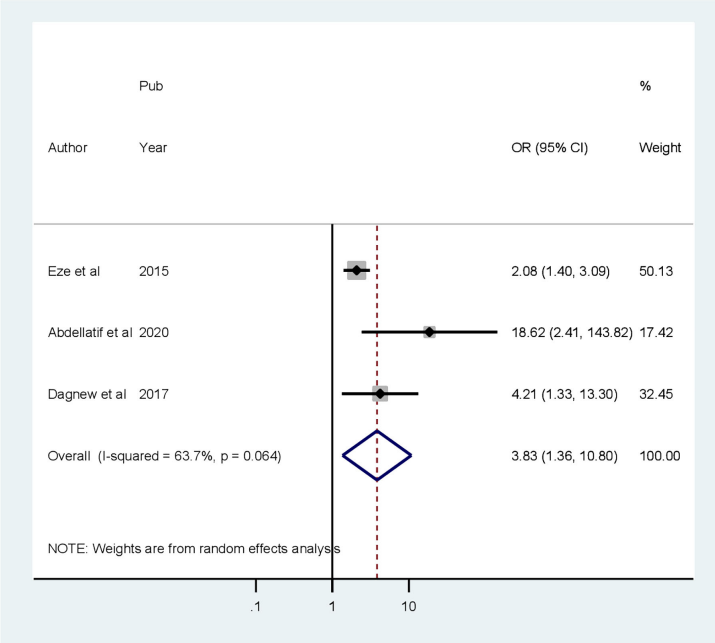

FIGURE 8
Forest plot showing the association between age greater than 60 years old and cognitive impairment among patients with diabetes in Africa (*N* = 13).

which has some distinctive features including early age at onset, positive family history, a variety of non-cognitive neurological symptoms and signs, and a more aggressive course. There is marked phenotypic heterogeneity among different mutations in EOFAD.

The review also identified that patients with DM who had diabetes for more than 10 years were more than 1.13 times more likely to develop CI than patients with diabetes duration of less than 10 years. The finding is in line with the studies done in China, Mexico, and London (42, 53, 54). This is justified by the long duration of DM as an atherogenic factor; it may increase the risk of cognitive dysfunction through well-recognized associations with stroke, causing cerebral disease and cerebral infarction (55).

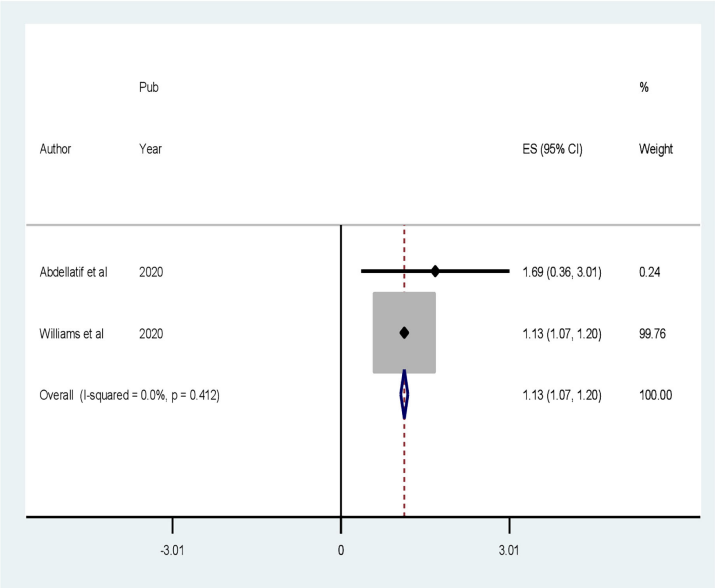

FIGURE 9
Forest plot showing the association between the duration of DM greater than 10 years and cognitive impairment among patients with diabetes in Africa (*N* = 13).

Limitations

This review has some limitations. The sub-group analysis did not indicate adequate factors to explain the observed high heterogeneity; important factors associated with CI were not analyzed in our review because of the lack of information in the primary studies and inconsistent categorization, and the number of included articles was not adequate (N = 13), which may affect the representativeness of the results.

Conclusion and recommendation

Based on our systematic review, individuals with DM exhibit a substantial prevalence rate (43.99%) of CI. CI was found to be associated with factors such as primary level of education, poorly controlled DM, age above 60 years, and diabetes duration greater than 10 years. Developing suitable risk assessment tools is crucial to address uncontrolled hyperglycemia effectively. Healthcare professionals in Africa ought to prioritize the monitoring of cognitive function in patients with DM. Early identification of CI among patients with DM is beneficial for both restoring cognitive function and slowing down cognitive decline. Healthcare institutions need to create diagnostic and treatment plans tailored to individuals with chronic diabetes to address cognitive issues, with a particular emphasis on the elderly population. Additional interventional research endeavors focused on mitigating cognitive decline in DM, particularly those targeting novel risk factors in primary care settings, are recommended.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

WC: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. YF: Data curation, Writing – original draft. AZ: Data curation, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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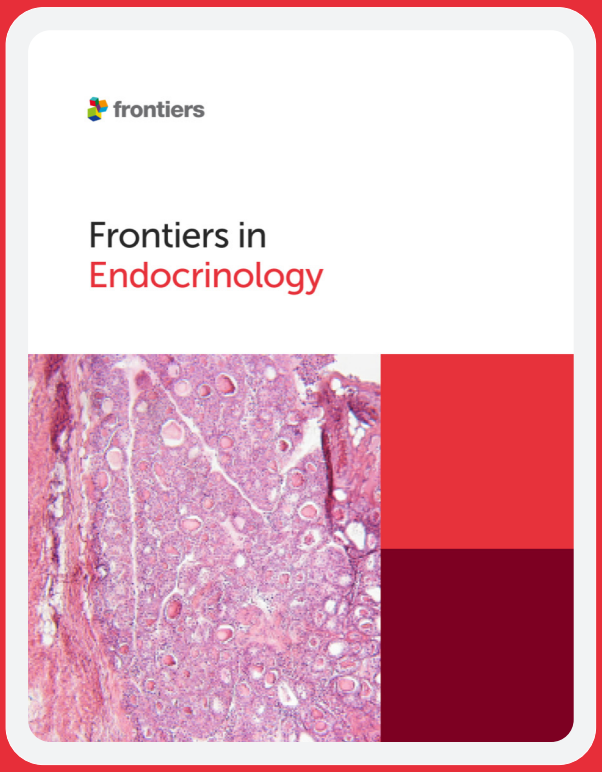

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