Trial protocol and statistical analysis plan

Version 1.0：Effects of Tai chi Chuan on cognitive function in adults 60 years old or older with type 2 diabetes and mild cognitive impairment: A randomized clinical trial

Reference to original RCT protocol and statistical analysis plan (SAP): Published protocol and SAP for the 24-week intervention and 36-week follow-up (referenced in the original RCT publication: https://doi.org/10.1001/jamanetworkopen.2023.7004).

Protocol and SAP：Two-year follow-up assessment of a randomized controlled trial evaluating the durability of Tai Chi on cognitive function in older adults with type 2 diabetes and mild cognitive impairment

The follow-up study protocol (Protocol Version 2.0) was finalized immediately following completion of the original RCT (24-week intervention + 36-week follow-up), prior to initiating any longitudinal follow-up procedures.

The Statistical Analysis Plan for the 2-year endpoint follow-up (SAP Version 2.0) was approved after protocol finalization but before data collection commencement. Database lock occurred following completion of 2-year data collection, with SAP approval preceding unblinding.

Trial protocol

Introduction

Older adults with type 2 diabetes (T2D) exhibit high rates of cognitive impairment, with ~45% having concurrent mild cognitive impairment (MCI) [1]. T2D accelerates progression from MCI

to dementia [2,3], conferring 1.5–3-fold greater dementia risk than non-diabetic peers [4]. This imposes substantial functional and societal burdens [5]. International guidelines prioritize T2D-MCI as a critical window for dementia prevention via non-pharmacological interventions [6,7].

Exercise is fundamental to T2D management [8] and MCI care [9], yet optimal modalities for sustained cognitive preservation are unestablished. Tai Chi—a multimodal mind-body therapy—shows dual benefits for glycemic control [10] and short-term cognition in T2D-MCI [11]. Our prior RCT demonstrated 24-week Tai Chi's efficacy on global cognition and specific cognitive domains [12].

Two critical unresolved questions limit clinical translation. Sustainability: Whether cognitive benefits persist beyond 24 months—a timeframe pivotal for dementia prevention. Clinical relevance: Whether Tai Chi achieves minimal clinically important differences (MCID) in global cognition, a prerequisite for health policy recommendations.

The absence of robust evidence regarding Tai Chi's long-term efficacy against comparators (e.g., fitness walking or health education) and clinically meaningful outcomes impedes its integration into preventive geriatric care. This protocol addresses these gaps through a prespecified 2-year follow-up assessment of our completed RCT cohort.

Methods

Study design

Prospective, assessor-blinded, 2-year follow-up assessment of participants originally enrolled in the 3-arm, parallel-group, randomized controlled trial (RCT: Tai Chi vs. Fitness Walking vs.

Health Education Control) [12]. No further formal supervised intervention is provided during the follow-up period beyond the initial 24 weeks.

Participants

Source population

Participants of T2D-MCI who completed the baseline assessment and were randomized in the original RCT (n=328).

Inclusion criteria for follow-up

All participants originally randomized, regardless of adherence to the initial 24-week intervention or completion of the 36-week assessment (Intention-to-Treat principle). Participants providing informed consent for the extended follow-up.

Exclusion criteria for follow-up

Withdrawal of consent for participation in the 2-year assessment. (Participants lost to follow-up will be included in ITT analyses using appropriate statistical methods).

Sample size

The sample size was determined for the original RCT's primary outcome (MoCA at 36 weeks). The original RCT enrolled 328 participants, providing sufficient power (≥80%) to detect clinically relevant differences in MoCA between groups at 36 weeks, $\alpha=0.05$, and accounting for attrition.

Randomization and concealment

Randomization: Performed during the original RCT using block randomization (1:1:1 ratio, block size of 6), via a central computer-generated sequence.

Allocation Concealment: Implemented during the original RCT using sequentially numbered, opaque, sealed envelopes.

Blinding (Masking): Outcome assessors and data analysts remain blinded to group allocation throughout the 2-year follow-up period. Participants and intervention providers were necessarily unblinded during the active intervention phase.

Ethics

This study will adhere to the principles of the Declaration of Helsinki. Ethical approval has been obtained from the Ethics Committee of Fujian Provincial Hospital of Rehabilitation (2020KY-004-002). The trial is registered at ClinicalTrials.gov (NCT04416841). Written informed consent will be re-obtained from all participants specifically for participation in the 2-year follow-up assessments, detailing the procedures, time commitment, and continued blinding of assessors.

Recruitment and retention strategies

Recruitment: Completed during the original RCT.

Retention Strategies for Follow-Up: ① Establishment of WeChat-based participant groups for peer support and study updates (non-intervention content); ② Assignment of a dedicated study coordinator for participant liaison; ③ Quarterly contact via WeChat messages to maintain engagement and update contact details; ④ Pre-assessment visit reminder calls; ⑤ Travel expense reimbursement for assessment visits.

Interventions (recap from original RCT - completed prior to follow-up)

Tai Chi group (n= 107): received 24 weeks of 24-form Tai Chi training (1 hour/session, 3 sessions/week) in groups led by certified instructors, plus standard health education. Fitness walking group (n=110): received 24 weeks of supervised brisk walking (1 hour/session, 3 sessions/week, 50-70% max HR) in groups led by exercise instructors, plus standard health education. Health education group (n=111): Received standard health education sessions (30 min/session, every 4 weeks for 24 weeks) on diabetes management, diet, and complication prevention. Follow-up period (36 Weeks - 2 year): no structured, supervised intervention provided by the study team. Natural maintenance of activity levels will be monitored.

Outcomes

Outcome indicators and assessment time points are provided in Table 1.

Table 1. Outcomes and assessment time points.

Outcome Domain	Specific Measure(s)	Baseline	24 weeks (Post-Intervention)	36 weeks (Follow-up)	2 years (Follow-up)	MCID Definition
Global Cognition	Montreal Cognitive Assessment (MoCA) [0-30]	✓	✓	✓	✓	≥4 point increase
Executive Function	Stroop Test (Dot/Word/Color-Word Reaction Time, Errors)	✓	✓	✓	✓	N/A
	Trail Making Test Part B (Completion time, seconds)	✓	✓	✓	✓	N/A
Attention	Digit Symbol Substitution Test (DSST) [Number Correct]	✓	✓	✓	✓	N/A
Visuospatial Ability	Rey-Osterrieth Complex Figure [0-36]	✓	✓	✓	✓	N/A
Language Fluency	Boston Naming Test (BNT) [Spontaneous Correct Naming]	✓	✓	✓	✓	N/A
Memory	Wechsler Memory Scale (Memory Quotient)	✓	✓	✓	✓	N/A
Clinical/Metabolic	Glycated Hemoglobin (HbA1c)	✓	✓	✓	✓	N/A
	Fasting Blood Glucose	✓	✓	✓	✓	N/A
	Fasting Insulin / HOMA-IR (Calculated)	✓	✓	✓	✓	N/A
Physical Activity	International Physical	✓	✓	✓	✓	N/A

Outcome Domain	Specific Measure(s)	Baseline	24 weeks (Post-Intervention)	36 weeks (Follow-up)	2 years (Follow-up)	MCID Definition
	Activity Questionnaire (IPAQ-SF)					
Medication Use	Diabetes Medications	✓	✓	✓	✓	N/A
Safety	Adverse Events (AEs)		✓	✓	N/A	N/A

Data management

Standardized case report forms (CRFs), consistent with those utilized in the original RCT, will be employed for data collection. Electronic data capture (EDC) systems will serve as the primary method, with paper-based contingency protocols. Cognitive assessments will be administered by trained research assistants (blinded to group allocation), adhering to standardized administration/scoring protocols; Clinical staff (similarly blinded) will collect biological samples under standardized operating procedures, with subsequent analyses conducted at certified central laboratories using validated methodologies. Data validation will incorporate: ① double-data entry with discrepancy resolution protocols for paper CRFs, or ② automated range/consistency checks with audit trails in EDC systems; source data verification against original records will be performed for ≥20% randomly selected cases. All analysis conducted exclusively on deidentified data.

Statistical methods

All outcome measures (including MoCA, WMS memory quotient, DSST, TMT-B, BNT, and ROCF scores) will undergo comparative analysis across the three intervention groups. the influence of different intervention on achieving the MCID of global cognitive function （an increase of ≥4 points on the MoCA score compared to before treatment is considered to have reached the MCID [13]）will also explored. Detailed analytical procedures are prespecified in the

statistical analysis plan. The threshold for statistical significance will be maintained at $\alpha=0.05$. effect sizes with 95% confidence intervals will supplement significance testing.

Safety

Given the the non-interventional nature of this 2-year follow-up phase where no supervised exercise is administered, formal safety assessments are not protocolized.

Funding

This work was supported by the Ministry of Science and Technology of the People's Republic of China [grant number 2019YFC1710301].

Statistical analysis plan

1 Introduction

Approximately 45% of older adults with type 2 diabetes (T2D) have concurrent mild cognitive impairment (MCI). T2D accelerates progression to dementia (1.5 – 3-fold increased risk), imposing substantial burdens; international guidelines prioritize T2D-MCI as a critical window for non-pharmacological dementia prevention.

While Tai Chi demonstrates short-term benefits for glycemic control and cognition in T2D-MCI, two key questions remain unresolved: the sustainability of cognitive gains beyond 24 months – a pivotal timeframe for dementia prevention, and whether it achieves the minimal clinically important difference (MCID) in global cognition necessary for policy recommendations.

This study aims to address these critical gaps by conducting a prespecified 2-year follow-up assessment of our completed RCT cohort, evaluating Tai Chi's long-term efficacy against fitness walking and health education, and determining its clinical relevance for sustainable cognitive preservation to inform its integration into preventive geriatric care.

2 Methods

2.1 Ethical approval

This study will adhere to the principles of the Declaration of Helsinki. Ethical approval has been obtained from the Ethics Committee of Fujian Provincial Hospital of Rehabilitation (2020KY-004-002). The trial is registered at ClinicalTrials.gov (NCT04416841). Written informed consent will be re-obtained from all participants specifically for participation in the 2-year

follow-up assessments, detailing the procedures, time commitment, and continued blinding of assessors.

2.2 Study design

Prospective, assessor-blinded, 2-year follow-up assessment of participants originally enrolled in the 3-arm, parallel-group, randomized controlled trial (RCT: Tai Chi vs. Fitness Walking vs. Health Education Control). No further formal supervised intervention is provided during the follow-up period beyond the initial 24 weeks.

3 Study outcome variables

Outcome indicators and assessment time points are provided below.

Table 1. **Outcomes and assessment time points.**

Outcome Domain	Specific Measure(s)	Baseline [a]	24 weeks (Post-Intervention) [a]	36 weeks (Follow-up) [a]	2 years (Follow-up)	MCID Definition
Global Cognition	Montreal Cognitive Assessment (MoCA) [0-30]	✓	✓	✓	✓	≥4 point increase
Executive Function	Stroop Test (Dot/Word/Color-Word Reaction Time, Errors)	✓	✓	✓	✓	N/A
	Trail Making Test Part B (Completion time, seconds)	✓	✓	✓	✓	N/A
Attention	Digit Symbol Substitution Test (DSST) [Number Correct]	✓	✓	✓	✓	N/A
Visuospatial Ability	Rey-Osterrieth Complex Figure [0-36]	✓	✓	✓	✓	N/A
Language Fluency	Boston Naming Test (BNT) [Spontaneous Correct Naming]	✓	✓	✓	✓	N/A
Memory	Wechsler Memory Scale (Memory Quotient)	✓	✓	✓	✓	N/A
Clinical/Metabolic	Glycated Hemoglobin (HbA1c)	✓	✓	✓	✓	N/A
	Fasting Blood Glucose	✓	✓	✓	✓	N/A
	Fasting Insulin / HOMA-IR (Calculated)	✓	✓	✓	✓	N/A

Outcome Domain	Specific Measure(s)	Baseline [a]	24 weeks (Post-Intervention) [a]	36 weeks (Follow-up) [a]	2 years (Follow-up)	MCID Definition
Physical Activity	International Physical Activity Questionnaire (IPAQ-SF)	✓	✓	✓	✓	N/A
Medication Use	Diabetes Medications	✓	✓	✓	✓	N/A
Safety	Adverse Events (AEs)		✓	✓	N/A	N/A

[a] Completed prior to 2-year follow-up.

4 General considerations

4.1 Data set

The intention-to-treat (ITT) population includes all randomized eligible participants, regardless of treatment adherence or dropout status. For the analysis of global cognition (evaluating group, time, and group-by-time interaction effects on MoCA scores), missing data were handled using multiple imputation. Other outcomes [Minimal Clinically Important Difference (MCID) in global cognition, executive function, attention, visuospatial ability, and language fluency] were analyzed using available-case analysis without imputation.

The per-protocol population comprises participants who: (1) completed the original 24-week RCT intervention with ⩾75% attendance in assigned sessions (Tai Chi/fitness walking/health education), (2) attended 2-year follow-up including endpoint MoCA assessment, (3) exhibited no major protocol deviations during the 2-year extension phase — specifically no initiation of prohibited cognition-altering medications (e.g., cholinesterase inhibitors), no participation in structured cognitive training programs (>20 hours total), and no cross-over between intervention arms — and (4) provided primary outcome data (MoCA score) at baseline and 24-month assessments. This PP analysis serves as a sensitivity analysis to assess robustness against non-adherence and missing data.

4.2 Covariates

For the linear mixed models analyzing cognitive outcomes (global and domain-specific), self-reported physical activity levels measured at all four assessment timepoints (baseline, 24 weeks, 36 weeks, and 2 years) will be included as a random effect covariate to account for its potential confounding influence. For the time-dependent Cox regression model analyzing the attainment of the Minimal Clinically Important Difference (MCID) in global cognition (MoCA improvement ≥4 points), the following baseline covariates will be adjusted for: sex, age, Body Mass Index (BMI), duration of diabetes (years), years of education, medical history, smoking history, physical activity level, fasting blood glucose, glycated hemoglobin (HbA1c), and fasting insulin. These covariates were selected based on their established or potential relevance to cognitive function in older adults with type 2 diabetes and mild cognitive impairment from the literature and clinical rationale.

4.3 Missing data

Given the follow-up duration, substantial missing data is anticipated. Upon identifying missingness, we will: (1) quantify missing data proportions, (2) investigate missingness patterns using Little's MCAR test, and (3) use multiple imputation. The imputation model will incorporate baseline demographic characteristics with 50 imputations. Complete-case analyses will be conducted for sensitivity reporting in manuscripts.

4.4 Interim analysis

Interim analysis will not be conducted.

5 Statistical analysis

5.1 Data management and general analysis

The Research Electronic Data Capture (REDCap) electronic data capture system will be employed for data collection and management. An independent data management committee will oversee data validity and integrity.

All statistical analyses were performed using SPSS 26.0 software. Statistical analyses will include descriptive and inferential methods. Continuous variables will be summarized as mean (standard deviation) for normally distributed data or median (interquartile range) for non-normally distributed data. Categorical variables will be reported as frequencies (percentages). Differences of demographic characteristics of 3 Groups at baseline and 2-year follow-up were assessed by one-way analysis of variance (ANOVA) or Kruskal-Wallis H tests for continuous variables and chi-square tests for categorical variables. A bilateral test with α level of 0.05 was adopted, A p-value of < 0.05 was considered indicative of a statistically significant difference.

5.2 Analysis of group, time, and group-by-time interaction effects on MoCA score)

As for the change in global cognitive function measured by the Montreal Cognitive Assessment (MoCA) score from baseline to 2-year follow-up. Analysis will be performed using linear mixed models (LMM) with the following specifications: (1) dependent variable: MoCA score, (2) fixed effects: intervention group (Tai Chi, fitness walking, health education), time (baseline, 24 weeks, 36 weeks, 2 years), and group-by-time interaction, (3) random effect: self-reported physical activity levels (measured via IPAQ-SF) at the four assessment timepoints, and (4) covariance structure: unstructured covariance matrix.

Pairwise comparisons between groups at 2-year will be adjusted using the Bonferroni method. Statistical significance is defined as two-sided $p < 0.05$.

5.3 Analysis of other endpoints

5.3.1 Minimal clinically important difference (MCID) of MoCA score attainment

A time-dependent Cox proportional hazards regression model will be employed to analyze time-to-event data for achieving MCID in global cognition, defined as an increase of ≥4 points [14] from baseline on the MoCA scale. The model will include intervention groups as the primary predictor and adjust for prespecified covariates: sex, age, body mass index, duration of diabetes, years of education, medical history, smoking status, physical activity level, fasting blood glucose, glycated hemoglobin, and fasting insulin. The proportionality assumption will be verified using Schoenfeld residuals. Results will be reported as hazard ratios (HR) with 95% confidence intervals. Participants without MCID attainment by the 2-year endpoint will be right-censored at their last assessment.

5.3.2 Domain-specific cognitive function analyses

Changes in cognitive subdomains (executive function, attention, visuospatial ability, language fluency, and memory) will be analyzed using linear mixed models with the same hierarchical structure as the MoCA scores analysis. Each subdomain score (e.g., Stroop test reaction times, Rey-Osterrieth copy scores) will serve as dependent variables in separate models, incorporating fixed effects for group, time, group-by-time interaction, and random intercepts for participants. Model covariates will include physical activity levels measured at four assessment points. Between-group differences at the 2-year follow-up will be estimated using Bonferroni-adjusted pairwise comparisons.

5.4 Sensitivity analysis

Sensitivity analyses will assess the robustness of key findings. For the group, time, and group-by-time interaction effects on MoCA score, Prespecified sensitivity analyses will include: per-protocol analysis limited to compliant participants who completed ≥75% intervention sessions and attended 2-year follow-up including endpoint MoCA assessment, without multiple imputation.

Reference

[1] You, Y., Liu, Z., Chen, Y., Xu, Y., Qin, J., Guo, S., Huang, J., & Tao, J. 2021. The prevalence of mild cognitive impairment in type 2 diabetes mellitus patients: a systematic review and meta-analysis. Acta Diabetol, 58(6), 671-685. https://doi.org/10.1007/s00592-020-01648-9

[2] Ciudin, A., Espinosa, A., Simó-Servat, O., Ruiz, A., Alegret, M., Hernández, C., Boada, M., & Simó, R. 2017. Type 2 diabetes is an independent risk factor for dementia conversion in patients with mild cognitive impairment. J Diabetes Complications, 31(8), 1272-1274. https://doi.org/10.1016/j.jdiacomp.2017.04.018

[3] Umegaki, H. 2015. Dementia: type 2 diabetes has a slow and insidious effect on cognition. Nat Rev Neurol, 11(3), 127-128. https://doi.org/10.1038/nrneurol.2015.17

[4] Koekkoek, P. S., Kappelle, L. J., van den Berg, E., Rutten, G. E., & Biessels, G. J. 2015. Cognitive function in patients with diabetes mellitus: guidance for daily care. Lancet Neurol, 14(3), 329-340. https://doi.org/10.1016/s1474-4422(14)70249-2

[5] Jia, L., Du, Y., Chu, L., Zhang, Z., Li, F., Lyu, D., Li, Y., Li, Y., Zhu, M., Jiao, H., Song, Y., Shi, Y., Zhang, H., Gong, M., Wei, C., Tang, Y., Fang, B., Guo, D., Wang, F., Zhou, A., Chu, C., Zuo, X., Yu, Y., Yuan, Q., Wang, W., Li, F., Shi, S., Yang, H., Zhou, C., Liao, Z., Lv, Y., Li, Y., Kan, M., Zhao, H., Wang, S., Yang, S., Li, H., Liu, Z., Wang, Q., Qin, W., & Jia, J. 2020. Prevalence, risk factors, and management of dementia and mild cognitive impairment in adults aged 60 years or older in China: a cross-sectional study. Lancet Public Health, 5(12), e661-e671. https://doi.org/10.1016/s2468-2667(20)30185-7

[6] Dementia and Cognitive Impairment Group, Chinese Society of Neurology, Chinese Medical Association. Chinese Medical Association. 2022. Chinese expert consensus on the diagnosis and treatment of Alzheimer's disease-related mild cognitive impairment 2021. Chinese Journal of Neurology, 55(5), 421-440. https://doi.org/10.3760/cma.j.cn113694-20211004-00679

[7] Petersen, R. C., Lopez, O., Armstrong, M. J., Getchius, T. S. D., Ganguli, M., Gloss, D., Gronseth, G. S., Marson, D., Pringsheim, T., Day, G. S., Sager, M., Stevens, J., & Rae-Grant, A. 2018. Practice guideline update summary: Mild cognitive impairment: Report of the Guideline Development, Dissemination, and Implementation Subcommittee of the American Academy of Neurology. Neurology, 90(3), 126-135. https://doi.org/10.1212/wnl.0000000000004826

[8] American Diabetes Association Professional Practice Committee. 2025. Older Adults: Standards of Care in Diabetes-2025. Diabetes Care, 48(Supplement_1), S266-S282. https://doi.org/10.2337/dc25-S013

[9] Kanaley, J. A., Colberg, S. R., Corcoran, M. H., Malin, S. K., Rodriguez, N. R., Crespo, C. J., Kirwan, J. P., & Zierath, J. R. 2022. Exercise/Physical Activity in Individuals with Type 2 Diabetes: A Consensus Statement from the American College of Sports Medicine. Med Sci Sports Exerc, 54(2), 353-368. https://doi.org/10.1249/mss.0000000000002800

[10] Wang, X. Z., Jin, F. J., & Wang, X. D. 2022. The effects of Tai Chi on glucose and lipid metabolism in patients with diabetes mellitus: A meta-analysis. Complement Ther Med, 71, 102871. https://doi.org/10.1016/j.ctim.2022.102871

[11] Chen, H., Wang, Y., Zhang, M., Wang, N., Ge, S., & Liu, Y. 2024. Effectiveness of Tai Chi on cognitive function among older adults with mild cognitive impairment: a systematic review and meta-analysis of randomized controlled trials. Aging Ment Health, 28(2), 285-293. https://doi.org/10.1080/13607863.2023.2253183

[12] Chen, Y., Qin, J., Tao, L., Liu, Z., Huang, J., Liu, W., Xu, Y., Tang, Q., Liu, Y., Chen, Z., Chen, S., Liang, S., Chen, C., Xie, J., Liu, J., Chen, L., & Tao, J. 2023. Effects of Tai Chi Chuan on Cognitive Function in Adults 60 Years or Older With Type 2 Diabetes and Mild Cognitive Impairment in China: A Randomized Clinical Trial. JAMA Netw Open, 6(4), e237004. https://doi.org/10.1001/jamanetworkopen.2023.7004

[13] Feeney, J., Savva, G. M., O'Regan, C., King-Kallimanis, B., Cronin, H., & Kenny, R. A. 2016. Measurement Error, Reliability, and Minimum Detectable Change in the Mini-Mental State Examination, Montreal Cognitive Assessment, and Color Trails Test among Community Living Middle-Aged and Older Adults. J Alzheimers Dis, 53(3), 1107-1114. https://doi.org/10.3233/jad-160248

[14] Feeney J, Savva GM, O'Regan C, King-Kallimanis B, Cronin H, Kenny RA (2016) Measurement Error, Reliability, and Minimum Detectable Change in the Mini-Mental State Examination, Montreal Cognitive Assessment, and Color Trails Test among Community Living Middle-Aged and Older Adults. J Alzheimers Dis 53(3): 1107-1114. https://doi.org/103233/jad-160248