Title

The efficacy and safety of metformin intervention in elderly overweight or obesity with mild cognitive impairment

study protocol

Scheme Design Institute: Huadong Hospital, Fudan University
Main Research Institution: Huadong Hospital, Fudan University

Main Investigator: Tao Xiaoming
Clinical Research Department: Endocrine Department
Sponsor: none
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一、 Programme summary

Protocol name	The efficacy and safety of metformin intervention in elderly overweight or obesity with mild cognitive impairment
Version number/ Version date	V1.0 7 August, 2025
Research objects	Elderly overweight or obesity patients with mild cognitive impairment
Research purposes	To explore the efficacy and safety of metformin in sufficient amount in elderly overweight or obese patients with MCI. To provide reliable evidence for the elderly overweight or obese patients with MCI to delay and reverse the cognitive dysfunction.
Research design	This study was a prospective, randomized controlled, single-center clinical cohort study. Elderly subjects who were overweight or obese with MCI and met the inclusion criteria and did not meet the exclusion criteria were included in this study with informed consent. The subjects were randomly divided into two groups using random number method. In the first group, subjects received metformin sustained-release tablets (1500mg once a day or reduced to the maximum tolerated dose if intolerable) and lifestyle intervention guidance. Subjects in the second group received only lifestyle intervention guidance. All subjects were followed for a period of 26 weeks. History collection, body measurement index evaluation, glycolipid metabolism biochemical test, medication record, and MoCA scale evaluation were conducted during the enrollment phase and the end of follow-up. The insulin sensitivity in central nervous system of subjects in the two groups before and after treatment was evaluated by nasal insulin inhalation combined with fMRI.
Inclusion/ exclusion	**Inclusion criteria**

criteria	A. age: 60 -75 years; B. Body mass index: ≧24kg/m^2; C. glycated hemoglobin < 8.5%; D. The scores of the MoCA scale are 20–26 (education > 10 years) or 20–25 (education ≦10 years); E. sign informed consent forms; **Exclusion criteria** A. Anyone who takes any hypoglycemic drug within 8 weeks prior to enrollment; B. type 1 diabetes; C. Central nervous system magnetic resonance imaging cannot be completed; D. A history of alcohol or drug addiction; E. Severe gastrointestinal diseases, history of gastrointestinal surgery, severe cardiopulmonary dysfunction, malignant tumor and other diseases; F. Abnormal liver function (liver enzyme index is more than 2.5 times the upper limit of the reference range) or renal function (estimated glomerular filtration rate < 45 mL/min); G. There were relevant situations that the investigator judged were not suitable for inclusion in the study.
Study drug	Drug name: metformin hydrochloride sustained-release tablets (Gehuazhi) Drug specification: 500mg
Sample size	The change in blood flow volume of cerebral regions shown by fMRI after nasal insulin inhalation was used as the research endpoint. According to the results of previous literature, the difference between groups after drug intervention was 20%, and the standard deviation was 20%. When the accuracy was 0.05 and the test efficiency 1-β was 0.8, the required sample size for each group was obtained from the sample size calculation formula: n1=n2=23. Considering the existence of a certain shedding ratio, and calculated based on the shedding rate of 15%, 54 patients, 27 in each group, were to be included in the study.

Statistical method	SPSS 22.0 statistical analysis software was used for calculation. All statistical tests were performed using a two-sided test, and P≦0.05 was considered statistically significant for the difference tested. Enumeration data were described by constituent ratio, and measurement data were described by mean, standard deviation, median, and interquartile. Statistical analysis: Appropriate methods were used for analysis according to the types of indicators. T test or Wilcoxon rank sum test was used for inter-group comparison of quantitative data, and chi-square test was used for classified data.

二、Contents

三、 Version of the study protocol

Scenario history

version number	Version date	Summary of revisions
1.0	7 August, 2025	first edition

四、 Research topic

The efficacy and safety of metformin intervention in elderly overweight or obesity with mild cognitive impairment

五、 Research purposes

To explore the efficacy and safety of metformin in sufficient amount in elderly overweight or obese patients with MCI. To provide reliable evidence for the elderly overweight or obese patients with MCI to delay and reverse the cognitive dysfunction.

六、 Research background

1. **The prevalence of cognitive impairment in elderly obese patients is high, and early intervention is of great value.**

Cognitive impairment refers to one or more impairments in learning and memory, language, executive function, sensorimotor function, complex attention and social cognition, which is often accompanied by mental, behavioral and personality abnormalities, including mild cognitive impairment (MCI) and dementia [1]. Epidemiological studies have shown that the prevalence of MCI in people over 60 years old in China is 15.5%, and the number of people with MCI is 38 million [2]. More studies have confirmed the existence of a clear correlation between obesity and cognitive impairment [3]. Patients with MCI have less interference with cognitive deficits in daily life, but the proportion of patients progressing to dementia every year can reach 5-10%, which is much higher than the incidence rate of 1-2% of the general population every year [4]. 50% of patients with MCI can develop to dementia within five years [5], and 20-30% of patients with MCI can recover to normal cognition [6].

Therefore, early detection of MCI and timely intervention for elderly obese patients have important clinical significance and value.

2. **Central nervous insulin resistance plays a role in the pathogenesis of cognitive dysfunction. Functional magnetic resonance imaging (fMRI) can evaluate cognitive dysfunction by observing central insulin resistance.**

At present, the mechanisms of cognitive dysfunction in elderly obese patients include the pathophysiological role of microvascular mechanisms and fat-derived factors [3]. Insulin not only regulates peripheral metabolism, but also acts on the central nervous system to regulate cognitive function. All cell types in the brain have insulin signaling pathways, and some studies have shown that insulin resistance in the brain can damage the plasticity and memory function of hippocampal synapses, leading to cognitive decline [7]. The Montreal Cognitive Assessment Scale (MoCA) is usually used for the evaluation of cognitive function, which includes multiple dimensions such as memory, attention, executive function and language function, with a full score of 30. The score considering the diagnosis of MCI is 20-26 (education > 10 years) or 20-25 (education ≦10 years) [8]. However, in actual work, scale collection is affected by many factors. fMRI can display the changes of local cerebral blood flow and metabolic activities before the changes of brain structure, so as to understand the changes of brain function in depth. It is an important means for early diagnosis of cognitive dysfunction, efficacy monitoring and mechanism research [9]. The degree of central insulin resistance was assessed by fMRI combined with nasal insulin inhalation to observe the response of different brain regions to insulin. The absence of neuroimaging or neurophysiological reactions after nasal insulin inhalation is suggestive of central nervous system insulin resistance [7]. Previous studies have shown that improving central insulin resistance can delay or even reverse cognitive decline [10]. Therefore, the central insulin resistance observed by fMRI can be used as an objective basis for the assessment of cognitive function.

3. **Metformin may improve cognitive dysfunction by improving central insulin resistance in elderly obese patients.**

Metformin is widely used as the first-line medication for type 2 diabetes. Basic research showed that metformin had the potential to activate the 5'AMP-activated protein kinase (AMPK) pathway, which played a vital role in the pathogenesis of dementia [11]. Metformin can improve insulin resistance [12], reduce neuronal apoptosis [13], and reduce cerebral oxidative stress and inflammatory response [14]. Recent studies have shown that the use of metformin is associated with a significant

reduction in the risk of dementia in elderly patients with type 2 diabetes [15][16], but these studies are all diabetes patients in a large database. Comparing the use of metformin to reach the conclusion is subject to more interference factors. Other early studies have shown that long-term use of metformin even increases the risk of dementia [17], which may be related to vitamin B12 deficiency, the duration of metformin use, and the course of diabetes. Short-term controlled studies with small samples have shown that metformin can penetrate the blood-brain barrier and improve learning, memory and attention in non-diabetic MCI patients [18].

In summary, as a widely used traditional hypoglycemic drug, metformin has been shown to improve central insulin resistance in basic studies and has been proved to be feasible in clinical studies of diabetic patients. However, there are few studies investigating the efficacy of metformin in improving cognitive function in elderly obese patients with MCI.

七、 Research method

1. overall design

This study was a prospective, randomized controlled, single-center clinical cohort study. Those subjects who met the inclusion criteria were included in the study after they signed the informed consent. The study was conducted in the Drug Clinical Laboratory of Endocrinology Department, Huadong Hospital. The subjects were randomly divided into two groups using random number method. In the first group, subjects received metformin sustained-release tablets (1500mg once a day or reduced to the maximum tolerated dose if intolerable) and lifestyle intervention guidance. Subjects in the second group received only lifestyle intervention guidance. All subjects were followed up for 26 weeks with a diary card for dosing and adverse events and a telephone visit every 4 weeks 3 days after randomization.

2. Study Population

Any elderly subject who is overweight or obesity with mild cognitive impairment and is admitted to the study center may be considered for this study. The primary investigator bore the primary responsibility for subject enrollment. You can expect to limit the speed of grouping for the following reasons:

- The number of outpatient visits and the number of subjects who met the inclusion and exclusion criteria;
- The willingness and informed consent of the subjects were evaluated at baseline.

八、 Inclusion criteria, exclusion criteria and exit criteria of subjects

1. Inclusion criteria

A. age: 60 -75 years;

B. Body mass index: ≧24kg/m^2；

C. glycated hemoglobin < 8.5%;

D. The scores of the MoCA scale are 20–26 (education > 10 years) or 20–25 (education ≦10 years);

E. sign informed consent forms;

2. Exclusion criteria

A. Anyone who takes any hypoglycemic drug within 8 weeks prior to enrollment;

B. type 1 diabetes;

C. Central nervous system magnetic resonance imaging cannot be completed;

D. A history of alcohol or drug addiction;

E. Severe gastrointestinal diseases, history of gastrointestinal surgery, severe cardiopulmonary dysfunction, malignant tumor and other diseases;

F. Abnormal liver function (liver enzyme index is more than 2.5 times the upper limit of the reference range) or renal function (estimated glomerular filtration rate < 45 mL/min);

G. There were relevant situations that the investigator judged were not suitable for inclusion in the study.

3. Exit criteria

All subjects had the right to withdraw from the study at any stage of the study and the investigator had the right to discontinue observation of subjects who:

1) Continuing to participate in the seminar would cause unnecessary damages to the subjects;

2) The subjects had poor compliance and did not cooperate with the researchers in

relevant examinations and interventions;

3) Subjects who were found not to meet the inclusion criteria after participating in the study;

4) Subjects who were found to meet the exclusion criteria after participation in the study.

九、 Sample size assessment

The change in blood flow volume of cerebral regions shown by fMRI after nasal insulin inhalation was used as the research endpoint. According to the results of previous literature, the difference between groups after drug intervention was 20%, and the standard deviation was 20%. When the accuracy was 0.05 and the test efficiency 1-β was 0.8, the required sample size for each group was obtained from the sample size calculation formula: n1=n2=23. Considering the existence of a certain shedding ratio, and calculated based on the shedding rate of 15%, 54 patients, 27 in each group, were to be included in the study.

十、 Study Endpoints

1. Effectiveness

The main evaluation indexes were the changes of cerebral blood flow shown by fMRI after nasal insulin inhalation. The secondary evaluation index was MoCA scale score. To explore the influence of metformin intervention on cognitive dysfunction under different glycosylated hemoglobin baselines.

2. Safety

Intestinal reaction, such as abdominal pain, diarrhea, abdominal distension; other uncomfortable symptoms or events affecting living activities.

十一、 Research process

1. **Screening period**: whether the screening met the inclusion criteria, and there was no exclusion criterion.

2. **V0**: A total of 54 elderly subjects who were expected to be enrolled as overweight or obesity with MCI, taking medical history (previous illness, medication history and concomitant medication, smoking history, alcohol consumption history, and educational level), demographic data (gender, date of birth), comprehensive physical examination (height, weight, body temperature, heart rate, respiration, blood pressure, waist circumference, hip circumference, body composition, nervous system, and cardiopulmonary abdomen), Laboratory tests (fasting blood glucose, fasting insulin, fasting C-peptide, glycated hemoglobin, blood lipids, routine blood tests, liver and kidney function, and routine 12-lead electrocardiogram), MoCA scale evaluation, and nasal insulin inhalation combined with fMRI imaging were performed.

54 patients were randomly divided into two groups. In the first group, subjects received metformin sustained-release tablets (1500mg once a day or reduced to the maximum tolerated dose if intolerable) and lifestyle intervention. Subjects in the second group received only lifestyle intervention guidance. All subjects were followed for a 26-week follow-up period and diary cards were issued to record dosing and adverse events. A 24-hour meal was recorded during the first week of intervention; Exercise instruction.

3. **V1** (intervention 4 weeks): The patient was interviewed by phone for any adverse events.

4. **V2** (eight weeks of intervention): The patient was interviewed by phone for any adverse events.

5. **V3** (intervention 12 weeks): The patient was interviewed by phone for any adverse events.

6. **V4** (intervention for 16 weeks): The patient was interviewed by phone for any adverse events.

7. **V5** (intervention of 20 weeks): The patient was interviewed by phone for any adverse events.

8. **V6** (24 weeks of intervention): The patient was interviewed by phone for any adverse events.

9. **V7** (intervention for 26 weeks): Medical history (previous disease history, medication history and concomitant medication history, smoking history, drinking history, and educational level) demographic data (gender, date of birth), comprehensive physical examination (height, weight, temperature, heart rate, respiration, blood pressure, waist circumference, hip circumference, body composition, nervous system, and cardiopulmonary abdomen), laboratory tests (fasting blood glucose, fasting insulin, fasting C-peptide, glycated hemoglobin, blood lipid, blood routine, liver and kidney function, routine 12-lead electrocardiogram), MoCA scale evaluation, and nasal insulin inhalation combined with fMRI imaging were collected again.

10. **V8**: A safety visit 4 weeks after the end of the intervention to ask the patient over the phone for any adverse events. The report was analyzed and recommendations were made for treatment protocol adjustments based on the report.

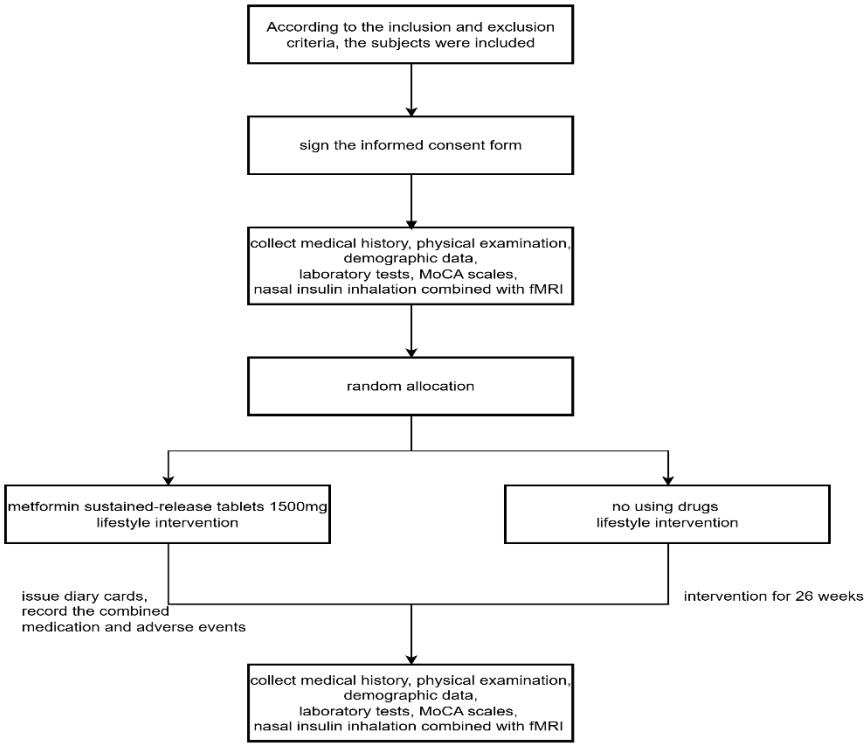


Figure 1 research flow chart

visit	V-1	VO	V1	V2	V3	V4	V5	V6	V7	V8
stage	screening period	baseline visit	telephone follow-up	telephone follow-up	telephone follow-up	telephone follow-up	telephone follow-up	telephone follow-up	follow-up at the end of the treatment period	follow-up after the treatment period
time(weeks)	2	0	4	8	12	16	20	24	26	30
window period (days)			±3d	±3d	±3d	±3d	±3d	±3d		±7d
sign the informed consent form		√								
check the inclusion and exclusion criteria	√	√								
formulate or adjust the medication plan for lowering blood glucose		√								
collect medical history (previous illness, medication history and concomitant medication, smoking history, alcohol consumption history, and educational level)		√							√	
demographic data (gender, date of birth)		√							√	
comprehensive physical examination (height, weight, body temperature, heart rate, respiration, blood pressure, waist circumference, hip circumference, nervous system, and cardiopulmonary abdomen)		√							√	
body composition		√							√	
routine 12-lead electrocardiogram		√							√	
routine blood tests		√							√	
liver and kidney function, eGFR(based on renal function assessment)		√							√	
blood lipids(CHO, TG, HDL-C, and LDL-C)		√							√	
fasting blood glucose, fasting insulin, fasting C-peptide		√							√	
HbA1c		√							√	
MoCA scale evaluation		√							√	
nasal insulin inhalation combined with fMRI imaging		√							√	
diet and exercise guidance		√								
record adverse events		√	√	√	√	√	√	√	√	

Figure 2 Follow-up plan (duration and content of follow-up)

Note:

(1) After a 5-minute rest, systolic and diastolic blood pressure as well as pulse were measured by sitting quietly. Blood pressure, waist circumference, grip strength, and body function were assessed using the uniform method provided. The body composition was determined using Beijing Yueqi BIA-140.

(2) All tests were performed in the test center. There were two blood tests in total, and 5ml venous whole blood was drawn each time.

(3) ECG: 12 leads were used, and the same subject should be kept consistent.

(4) Early withdrawal from trial visit: Participants who withdrew from the trial early should return to the study center on an empty stomach for the early withdrawal visit within 7 days after the investigator was informed. A repeat examination for the same item may be waived if the visit is less than 7 days from the date of the previous visit.

(5) Dietary life guidance rules: during the formulation of nutrition treatment plan, attention should be paid to appropriately increase the protein and energy intake, to encourage the elderly subjects to choose regular exercise that can be insisted on for a long time, and to select the appropriate exercise mode (such as aerobic exercise and resistance training).

十二、 Serious adverse events

Definition of adverse event (AE): Any adverse medical event that occurred between the time the subject signed the informed consent and was enrolled in the study and the last visit, regardless of causality to study drug, was considered an adverse event. Serious adverse events (SAE) was a clinical event indicating a significant hazard, contraindication, side effect, or caution with respect to a test article

at any dose or at any time during observation. Adverse events are classified as serious adverse events if one or more of the following criteria are met: 1. Death 2. Life-threatening (meaning that the patient in whom the event occurred was at risk of dying immediately at the time of the event; Does not include those events which, if more serious, could result in the death of the patient) 3. Lead to hospitalization or prolonged hospitalization 4. Lead to permanent or significant loss of labor or disability 5. Congenital malformations. Medical events that have not yet resulted in death, life risk, or hospitalization that, after appropriate medical judgment, may be hazardous to the patient or subject or require medical or surgical treatment to avoid such occurrences should also be considered SAE.

If any adverse event occurs, the patient should report it to the researcher in time. The researcher should make a detailed record at the corresponding position on the case report form. The researcher should assist the patient to actively handle the adverse event and strive for the best prognosis while respecting the patient's will and choice. Meanwhile, possible adverse events are prevented and treated. As metformin was used in this study, it was expected that possible adverse events in this study would include: gastrointestinal reaction conditions, such as abdominal pain, diarrhea, and abdominal distension. In the case of serious adverse events, the Ethics Committee and relevant health authorities will be reported within 24 hours and the subject will be treated promptly.

十三、 Statistical Analysis Plan

SPSS 22.0 statistical analysis software was used for calculation. All statistical tests were performed using a two-sided test, and P≦0.05 was considered statistically significant for the difference tested. Enumeration data were described by constituent ratio, and measurement data were described by mean, standard deviation, median, and interquartile. Statistical analysis: Appropriate methods were used for analysis according to the types of indicators. T test or Wilcoxon rank sum test was used for inter-group comparison of quantitative data, and chi-square test was used for classified data.

十四、 Testing-related Ethics

1. compliance with the requirements of the declaration of helsinki and laws and regulations

2. Each subject should read the informed consent form before inclusion, and the patient should sign the informed consent form after the doctor answers all questions of the patient (see annex for the informed consent form).
3. This study is a randomized controlled trial. The test drug metformin has been used clinically and can be used together with diet and exercise to improve blood glucose control of adult patients with type 2 diabetes. Lifestyle intervention was conducted in the control group in accordance with the principle of fairness and justice. All trials will be conducted after approval by the Ethics Committee of Huadong Hospital and accept supervision by the Ethics Committee of Huadong Hospital.

十五、 Informed consent

The optional informed consent procedures in this study are described in detail below and should be in compliance with local ethics committee regulations.

1. Subject informed consent

Subjects should provide informed written consent whenever possible. Subjects were briefed by clinicians familiar with the protocol to explain consent to participate in the study.

2. Informed consent of the agent

If the subject is unable to provide informed consent, the subject's representative may provide informed consent on behalf of the subject.

3. Withdrawal of informed consent

Informed consent briefings provided to subjects and/or their agents clearly indicated that subjects could withdraw from the study at any time without explanation and that subjects would not be prejudiced or impaired thereby. This condition should be documented in the subject file.

4. Confidentiality and privacy

Care should be taken to respect the privacy of subjects during the conduct of the study. To ensure subject privacy, submissions will be de-labeled. However, in monitoring the quality of the data and adherence to the study protocol, the auditor has access to the medical records at the hospitals participating in the study.

十六、 Research progress planning

① 2024.10 – 2025.3: study protocol was optimized and various preparations such as the investigator's manual and patient's diary were completed; Subjects were screened into the group; Subjects were instructed to take medications and were followed up

②2025.4 – 2025.9: subjects were screened into the group; Subjects were instructed to take medications and were followed up

③2025.10 – 2026.3: subjects were screened into the group; Subjects were instructed to take medications and were followed up

④ 2026.4 – 2026.9: All data were entered, statistically analyzed, articles were written, and the final questions were completed.

十七、 Safety Visit

A safety visit was conducted one week after the study and any adverse events were reported.

十八、 Handling of subject information and biological samples

To ensure evaluation and supervision by the Research Coordination Center of Endocrinology Department, Huadong Hospital, Fudan University, the researchers should agree to keep all research materials (including original records, informed consent network authorization of all patients, and all CRF), and assign a special counter to lock and store them. Shelf life 15 years. All materials for this clinical study were owned by the project collaboration group organized by Huadong Hospital, Fudan University.

The samples of the subjects were tested in the test center, and the biological samples were stored in the -80℃ refrigerator for a period of 5 years. After the expiration of the storage period, the biological samples were destroyed in accordance

with the Shanghai Measures for the Administration of Medical Waste and other laws and regulations, and were recorded and retained.

十九、 Data and copyright

1. Data management

1) fill in and transfer of case report form

The case report form was completed by the investigator and must be completed for each enrolled case. The completed case report forms were entered and managed by the data administrator.

2) data entry and modification

For questions existing in the case report form, the data administrator will generate a question answer form and send the questions to the investigator, who should answer and return the questions as soon as possible. The data administrator will modify, confirm and enter the data according to the investigator's answers, and then send the questions again if necessary.

3) data lock

After reviewing and confirming the correctness of the established database, the project principal, main researcher and statistical analyst locked the data. The locked data file is not changed. Problems found after data lock-in were corrected in the statistical analysis procedure after validation.

4) data processing

After all the study data were entered and locked, the database was submitted to the statistical analyst for statistical analysis according to the requirements of the statistical plan. After the statistical analysis was completed, the general and sub-reports for statistical analysis were written by the statistical analysts and submitted to the principal investigator of the trial for writing the study report.

5) Data confidentiality

All subject information used in this study must be kept strictly confidential, and information about subjects who participated in the study and who were enrolled in the study must be kept confidential. Subject information and study data will be identified by study number. Information that identifies him or her will not be disclosed to members outside the study group unless subject approval is obtained. Subject identity was to be kept confidential by all study members. The subject's profile will be

maintained in a locked cabinet for access by the investigator only. To ensure that the study was conducted as required, members of the ethics committee were provided with access to subject profiles at the study unit as necessary. No personal information will be disclosed for the subject when the results of this study are published.

All study members should keep the study data confidential. They should not inform the members other than the research group of the study data without the permission of the main investigator, and should not transfer the study data to external units without the permission of the hospital.

6) Quality control

The lead investigator organized training to ensure that the study was conducted in a standardized manner, that the case report forms were completed, and that other reports complied with GCP principles and the study protocol. All data and information must be verifiable, and all observations and findings should be verifiable in order to assure the reliability of the study data. Quality control will be applied at each stage of the study to assure reliability of all data and accuracy of study procedures. The lead investigator should ensure that the investigator followed the protocol, confirmed the accuracy of the data, and documented the completeness of the report, and obtained informed consent from all subjects prior to study initiation. Any deviations or deviations from the protocol should be reported to the ethics committee in a timely manner. When necessary, the research group will designate standard operating procedures and carry out quality control procedures at each link of the implementation of research and data processing to ensure the specification and reliability of research implementation and data operation.

2. Copyright

Authors of published articles must conform to the following guidelines for authors issued by the editorial board of international medical journals:

1) The author must make a substantial contribution to the conception and design of the test, the acquisition of data, or the analysis of data, and the interpretation of the results;

2) The author must draft and publish the article in person or contribute to important revisions to the original (data analysis, interpretation, or other important intellectual content) that have been approved by other authors during the review process;

3) The author must provide an approval of the final draft of the article before it is submitted to the publication for publication.

All contributors who do not meet the above criteria for authorship will be listed in the acknowledgement column of the publication, if permitted by the journal, in accordance with the guidelines for acknowledgements issued by the editorial board of the International Journal of Medicine.

二十、 References

[1] Arvanitakis Z, Shah RC, Bennett DA. Diagnosis and Management of Dementia: Review. JAMA， 2019，322(16):1589-1599.

[2] Ren Rujing, Yin Peng, Wang Zhihui, etc.China Alzheimer's Disease Report 2021. Theory and practice of diagnostics, 2021, 20(4):317-337.

[3] Balasubramanian P, Kiss T, Tarantini S et al. Obesity-induced cognitive impairment in older adults: a microvascular perspective. Am J Physiol Heart Circ Physiol, 2021, 320(2):H740-H761.

[4] Sanford AM. Mild Cognitive Impairment. Clin Geriatr Med, 2017, 33(3):325-337.

[5] Morley JE. An Overview of Cognitive Impairment. Clin Geriatr Med, 2018, 34(4):505-513.

[6] Anderson ND. State of the science on mild cognitive impairment (MCI). CNS Spectr, 2019, 24(1):78-87.

[7] Kellar D, Craft S. Brain insulin resistance in Alzheimer's disease and related disorders: mechanisms and therapeutic approaches. Lancet Neurol, 2020,19(9):758-766.

[8] Tan SN, Tan C. Vortioxetine improves cognition in mild cognitive impairment. Int Clin Psychopharmacol, 2021, 36(6):279-287.

[9] Canu E, Sarasso E, Filippi M et al. Effects of pharmacological and nonpharmacological treatments on brain functional magnetic resonance imaging in Alzheimer's disease and mild cognitive impairment: a critical review. Alzheimers Res Ther, 2018, 10(1):21.

[10] Xu J, Ni B, Ma C et al. Docosahexaenoic acid enhances hippocampal insulin sensitivity to promote cognitive function of aged rats on a high-fat diet. J Adv Res, 2023, 45:31-42.

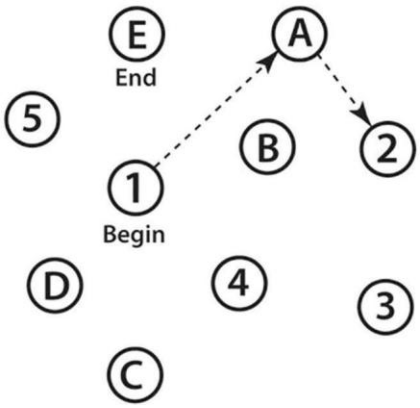
[11] Nikbakhtzadeh M, Shaerzadeh F, Ashabi G. Highlighting the Protective or Degenerative Role of AMPK Activators in Dementia Experimental Models. CNS Neurol Disord Drug Targets, 2021, 20(9):786-801.

[12] Ford RJ, Fullerton MD, Pinkosky SL et al. Metformin and salicylate synergistically activate liver AMPK, inhibit lipogenesis and improve insulin sensitivity. Biochem J, 2015, 468(1):125-32.
[13] Li LX, Liu MY, Jiang X et al. Metformin inhibits Aβ25-35 -induced apoptotic cell death in SH-SY5Y cells. Basic Clin Pharmacol Toxicol, 2019, 125(5):439-449.
[14] Obafemi TO, Olasehinde OR, Olaoye OA et al. Metformin/Donepezil combination modulates brain antioxidant status and hippocampal endoplasmic reticulum stress in type 2 diabetic rats. J Diabetes Metab Disord, 2020, 19(1):499-510.
[15] Sun M, Chen WM, Wu SY et al. Metformin in elderly type 2 diabetes mellitus: dose-dependent dementia risk reduction. Brain, 2024, 147(4):1474-1482.
[16] Tang H, Guo J, Shaaban CE et al. Heterogeneous treatment effects of metformin on risk of dementia in patients with type 2 diabetes: A longitudinal observational study.Alzheimers Dement, 2024,20(2):975-985.
[17] Imfeld P, Bodmer M, Jick SS et al. Metformin, other antidiabetic drugs, and risk of Alzheimer's disease: a population-based case-control study. J Am Geriatr Soc, 2012, 60(5):916-21.
[18] Koenig AM, Mechanic-Hamilton D, Xie SX, et al. Effects of the Insulin Sensitizer Metformin in Alzheimer Disease: Pilot Data From a Randomized Placebo-controlled Crossover Study. Alzheimer Dis Assoc Disord, 2017, 31(2):107-113.

二十一、 Appendix

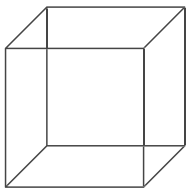
MoCA scale assessment (v0, v7)

VISUOSPATIAL / EXECUTIVE



[ ]

Copy cube



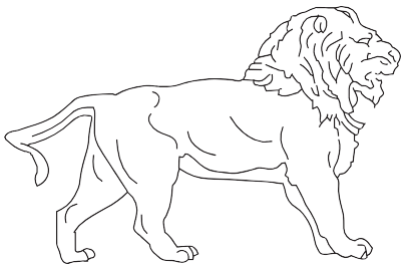
[ ]

Draw CLOCK (Ten past eleven)
(3 points)

POINTS

__/5

NAMING



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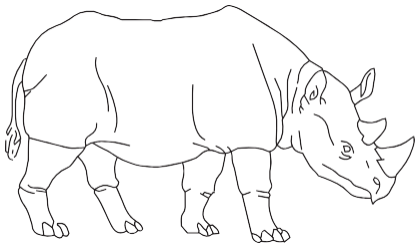

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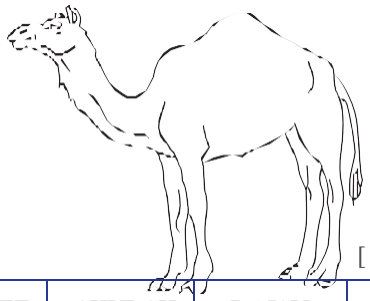

[ ]

__/3

MEMORY

Read list of words, subject must repeat them. Do 2 trials, even if 1st trial is successful. Do a recall after 5 minutes.

	FACE	VELVET	CHURCH	DAISY	RED
1ST TRIAL					
2ND TRIAL					

NO POINTS

ATTENTION

Read list of digits (1 digit/ sec). Subject has to repeat them in the forward order. [ ] 2 1 8 5 4

Subject has to repeat them in the backward order. [ ] 7 4 2

__/2

Read out the following numbers. Each time the number is 1, the patient knocks 1 on the table. No score is given if the error number is greater than or equal to 2.

[]5213941180621519451114190 5112

__/1

Serial 7 subtraction starting at 100. [ ] 93 [ ] 86 [ ] 79 [ ] 72 [ ] 65

4 or 5 correct subtractions: 3 pts, 2 or 3 correct: 2 pts, 0 or 1 correct: 0

__/3

LANGUAGE

Repeat: I only know that Zhangliang is the one to help today. [ ]

The cat always hid under the couch when dogs were in the room. [ ]

__/2

Fluency: Name maximum number of animal names in one minute. [ ] _____ (N≥11 words)

__/1

ABSTRACTION

Similarity between e.g. banana - orange = fruit [ ] train - bicycle [ ] watch - ruler

__/2

DELAYED RECALL

	FACE	VELVET	CHURCH	DAISY	RED	Points for UNCUED recall only
Has to recall words WITH NO CUE	[ ]	[ ]	[ ]	[ ]	[ ]	
Category cue						
Multiple choice cue						

__/5

ORIENTATION

[ ] Date [ ] Month [ ] Year [ ] Day [ ] Place [ ] City

__/6

TOTAL /30