A Randomized Controlled Study of Fish Oil in Obese Patients with Mild Cognitive Impairment

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Research Program

I. Project Title

A randomized controlled study of fish oil in obese patients with mild cognitive impairment。

II. Recruitment Program

1、Recruitment method

Recruitment by community health service centers: Screening patients through community health service centers; family doctors help recruit obese patients who meet the requirements.

2、Execution process

Initial screening: Investigators carry questionnaires, tape measures and body composition meters to the investigation site (community health center) for on-site assessment.

Community hospitals provide obese people who meet the inclusion criteria and participate voluntarily (BMI ⩾ 28.0 kg/m2 or waist circumference ⩾ 90 cm for men and ⩾ 85 cm for women) based on past work records. Participants who met the criteria were offered a small gift.

On-site assessment: Investigators measured participants' height and weight using a body composition instrument, participants' waist circumference using a tape measure, and subjects who met the criteria for obesity were assessed for cognition using questionnaires such as the Montreal Cognitive Assessment (MoCA) scale. Participants who achieved obesity with mild cognitive impairment were included in the study.

Signed consent: Those who passed the assessment signed an informed consent form and left their contact information and address.

Baseline information collection: participants who were determined to participate had 8 ml of blood collected by a nurse from the community hospital and stool samples were collected (blood collection tubes and fecal cups were provided by the subject group), and the food frequency questionnaire was completed by the investigator.

Randomized grouping: participants were randomly divided into intervention and control groups.

Intervention and follow-up: Fish oil capsules and placebo were distributed regularly, and the remaining dosage was recorded to assess adherence; follow-up visits were made at home or at the community hospital at 6 months for blood and stool sample collection.

III. Specific research program

Based on a multicenter nutrition and chronic disease prospective cohort, patients with obesity and mild cognitive impairment (MCI) who met the inclusion and exclusion criteria of this study were selected through the Montreal Cognitive Assessment Scale (MCAS) and body mass index (BMI) for initial screening, and were matched 1:1 with gender, age, and education level. The patients were matched 1:1 according to gender, age, and education level, and fish oil capsules were used for 12 months of continuous intervention.

1. Inclusion Criteria

(1) Meet the diagnostic criteria of obesity and MCI; (2) Age between 35 and 80 years old;

(3) Those who did not diet or stop dieting in the last 3 months, did not take fish oil supplements, or were willing to stop self-administration of fish oil 3 months before the intervention; (4) Voluntarily participate in the program with informed consent, and sign the informed consent form.

2. Exclusion Criteria

(1) Neuropsychiatric diseases such as stroke, epilepsy or schizophrenia; (2) Cognitive impairment caused by depression, thyroid disease, traumatic brain injury, drug or alcohol poisoning; (3) History of cerebrovascular disease or cognitive impairment caused by severe cardiac, hepatic, pulmonary or renal impairment; (4) Complete vegetarians who are unable to comply with the balanced dietary pattern; (5) Those who suffer from severe angina pectoris, wheezing bronchitis, severe infections, systemic lupus erythematosus, or severe infections; or , systemic lupus erythematosus, malignant tumors, and other diseases that may affect one's dietary habits and life; (6) Celiac disease and lipid malabsorption.

3.Sample size estimation.

This study is a randomized controlled study, review of the relevant literature can be obtained, fish oil intervention and placebo group Addenbrooke's cognitive test scale scores were 92.5 ± 0.9, 91.8 ± 0.9, respectively, to obtain the sample size required for each group of 35 cases, taking into account the loss of visit and refusal of 20% of the case calculation, the final need for inclusion of at least 44 cases in each group, a total of 88 cases of the study subjects of the 2 groups. The sample size formula is as follows.

$$2\sigma^2 \left(Z_{\alpha/2} + Z_{\beta}\right)^2 \Big/ \delta^2$$

n tabulates the number of samples in each group, σ is the standard deviation 0.9, and δ is the difference between the means of the two groups 0.7; Z-values are based on a=0.05, 1-β=90% lookup table.

4. Ethical issues and informed consent

This project was reviewed by the Ethics Committee of Capital Medical University (Ethical Review No.:2023SY072). And it was registered with the China Clinical Trial Registry before the study started. The study followed the comprehensive standard reporting guidelines for reporting trials of randomized clinical trials.

① Before the start of the questionnaire survey, the purpose and risks of the trial and other details were truthfully described and explained to the subjects, the voluntary signed informed consent was obtained, the subjects were clearly informed that they had the right to withdraw from the project at any time, and effective measures were taken to protect the legitimate rights and interests of the subjects.

② When blood is drawn from a vein, there may be transient discomfort and/or bruising at the time of the needle prick. Infection, excessive bleeding, clotting or fainting may also occur. A medical team of specialized doctors and nurses will act as a monitoring team.

IV. Baseline survey

1. General Survey

Using a questionnaire designed by the research team and validated in the previous study, the survey covered the age, gender, ethnicity, marital status, occupation, education level, place of residence, household income, living status (living alone, with family), dietary

habits and application of nutritional supplements, lifestyle (smoking, alcohol consumption, and physical activity), family history, personal history of disease (dementia, history of MCI), and chronic disease (hypertension, diabetes, hyperlipidemia, etc.).), and chronic disease status (hypertension, diabetes, hyperlipidemia, etc.). The investigators mainly completed the collection of the above basic information during the inclusion and exclusion phases of the respondents, as well as during the later follow-up visits.

2. Physical and body composition examination

In addition to detecting the basic conditions of height, weight, waist circumference and hip circumference, the InBody Body Composition Analyzer was used to detect the total score, body composition analysis (total body water, protein, inorganic salts, and body fat), visceral fat area, fat in each section of the body (right upper limb, left upper limb, torso, right lower limb, and left lower limb), and the basal metabolic rate, the amount of body cells, and the index of the skeletal muscles of the limbs, and so on.

3. Assessment of overall cognitive function and diagnosis of MCI

The Montreal Cognitive Assessment (MoCA) scale was used to assess the overall cognitive functioning of the subjects, with a score of 30 out of 30, which included cognitive functioning tests in several dimensions, including visuospatial and executive functioning, naming, memory, attention, language, abstraction, delayed recall, and orientation.

MCI diagnostic criteria: <6 years of education, MoCA score ⩽19; >7 years of education but ⩽12 years, MoCA ⩽22; >12 years of education, MoCA ⩽24.

4. Dietary composition, nutrient intake and dietary inflammation index calculation

Food frequency questionnaire (FFQ) was used for the survey assessment. With reference to the FFQ questionnaire used in the Nutrition and Health Status Survey of Chinese Residents, a total of 72 food questionnaire items were included in 11 food categories, with a focus on n-3 PUFA-rich foods, as well as the intake of edible oils and condiments. Auxiliary survey tools such as the Retrospective Dietary Survey Auxiliary Atlas were used to help respondents recall. The Standardized Version of the Chinese Food Composition Table (6th Edition) was used to calculate the energy and nutrient intake of the population, including the intake of dietary fatty acids. Based on the mean and standard deviation of the per capita daily intake of each dietary component or nutrient and the corresponding dietary effect index of each component, the dietary inflammatory index (DI) of each dietary component/nutrient was calculated separately for each individual. The dietary inflammatory index (DI) was calculated separately for each individual for each dietary component/nutrient.

V. Intervention Methods

1. Grouping of study subjects

Eighty-eight study subjects who met the inclusion and exclusion criteria were randomly (gender, age, education level, BMI) divided into 2 groups, the placebo group and the fish oil intervention group, with 44 people in each group.

2. Intervention program

Placebo group: Diet based on the principle of balanced diet, taking placebo capsules with the same appearance and odor as fish oil capsules.

Fish oil intervention group: Except for the diet based on the principle of balanced diet, the

subjects in this group were additionally supplemented with n-3 PUFA (provided in 2g fish oil capsules), and the rest of the interventions were the same as those in the placebo group. The dosage was based on relevant studies and formulated according to the dosage of Fish Oil Softgel Capsules.

The study was double-blind, and neither the study implementer, nor the subjects were aware of the specific intervention allocation plan. After the follow-up and intervention were completed, the compliance of the subjects in the 2 groups was assessed, and questionnaires and biological samples were collected and tested for relevant indicators.

Ⅵ.Intervention Indicators and Testing Methods

1. Diet and exercise survey of the population

Dietary survey is the same as the baseline survey. In the baseline and intervention, Acti Graph was utilized to record the energy consumption, pedometer and activity intensity of the subjects for one week.

2. Cognitive function assessment and multidimensional cognitive domain function assessment of the population

Same as the baseline survey.

3. Detection of n-3 PUFA content in erythrocyte membranes of the population

Gas chromatography (GC) was used to detect C18:3n-3%, C20:3n-3%, C20:5n-3% (EPA), C22:6n-3% (DHA), and total n-3 PUFAs in the erythrocyte membranes, and to calculate the c-3 (Q3I) index.

4. Detection of the effects of intestinal anabolic bacteria and their metabolite levels in the population

① Anthelmintic detection method: design and amplification of anthelmintic specific sequences, and then use RT-qPCR to detect anthelmintic in feces.

② Anthrobacter metabolites (acetic acid and propionic acid) detection method: Targeted high performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) was used to detect the levels of short-chain fatty acids in feces.

5、Plasma Beige Fat Marker Levels in the Population

Plasma beige fat markers, including uncoupling protein1 (UCP1), Transmembrane protein 26 (TMEM26), TBox-1, CD40, CD81, CD137, are detected by double antibody sandwich ELISA.

6、Population plasma inflammation-related factors detection

The plasma levels of interleukin-3 (IL-3), IL-4, IL-13, IL-17A, monocyte chemoattractant protein-1 (MCP-1) and other inflammatory factors in the population were detected by using high-throughput liquid-phase protein microarray.

VII. Quality control

1、Assessment of MCI: Completed by professionals who have received unified training.

2、Questionnaire data: Collected by uniformly trained investigators of the subject group to ensure the homogeneity of data collection. The survey process was conducted by the questionnaire investigators one-on-one, face-to-face interviews. The content of each questionnaire is verified to ensure the quality of data.

3、Quality control :The questionnaire data take double entry and consistency test. For

inconsistent data, timely review and revision to ensure the accuracy of the questionnaire.

VIII. Statistical Methods

All data were organized using the software Epidata3.1 to establish a database, the implementation of double entry, checking and error correction. Statistical analysis was performed using the software SAS 9.4 as well as R3.5.1. Variables included in this study included basic information about the population, laboratory tests, and disease indicators, which were categorized into count data and measured data. For variables that met the normal distribution, t-test was used for intergroup comparisons; non-parametric tests were used for information whose distribution did not meet the normality. The pre and post-intervention comparative analyses were performed using an analytic factorial design; generalized estimating equations were used to compare the relevant indicators between the control group and the intervention group at baseline, after follow-up, and after the intervention, and mixed-effects models were used for the interaction analyses. Statistical tests were performed using two-sided tests, and differences were considered statistically significant when $P<0.05$.