Statistical Analysis Plan & Protocol

for

Liver fat as a dietary target by Chinese Medical Nutrition Therapy (CMNT) diet for treating type 2 diabetes with nonalcoholic fatty liver disease

Protocol Approval
CMNT Targeting Liver Fat in T2D with NAFLD

Principal Investigator:

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Sponsor Name: State Key Laboratory of Subhealth Intervention Technology (Affiliated Institution: Hunan Agricultural University)

Date: February 12, 2025

Signature Page

Your signature on this page indicates that you confirm and agree to the statistical analysis of this trial in accordance with this plan.

Project Leader's Approval Signature: _____

Date: _____

Sponsor Name: State Key Laboratory of Subhealth Intervention Technology (Affiliated Institution: Hunan Agricultural University)

Institutional Review Board (Signature, Seal):

Contents

1. Original Statistical Analysis Plan for Version 2.0

1.1 Research Purpose

This study aims to explore the efficacy of Chinese Medicine Nutrition Intervention (CMNT), an intermittent energy restriction (iER) intervention, in managing Chinese adults with Type 2 diabetes (T2D) and non-alcoholic fatty liver disease (NAFLD). It further explores whether the glucose-lowering effect of CMNT is related to the reduction of liver fat and its mechanism in hepatic glucose and lipid metabolism.

1.2 Sample Size Justification

Approximately 120 participants are expected to be enrolled across participating centers during the study period. The sample size calculation is based on the reduction in Glycated hemoglobin (HbA1c). According to preliminary studies, the mean and SD values of HbA1c after intervention were 7.173±1.919% in the CMNT group and 8.124 ±1.144% in the control group. Forty-four participants in each group will be recruited, and the following formula will be used to calculate the required sample size. In consideration of stratification analysis on T2D and NAFLD comorbidity, we presume a dropout rate of 20%. In addition, as this study adds a 12-month follow-up compared to the control group's standard care, the sample size per group is increased to 60 cases.

1.3 Outcome Analysis

All statistical analyses will be conducted using IBM SPSS V.23. We will use a t-test or χ^2 test to determine the differences for normal data and Mann-Whitney U test for non-normal data between the CMNT group and usual care group. Data will be analyzed on an intention-to-treat basis, comparing the CMNT group with the usual care group. The controlled attenuation parameter (CAP) values and HbA1c values at baseline and after treatment will be evaluated by repeated measure analysis of variance. To control for confounders, the primary and secondary outcomes will be compared via analysis of covariance with age, sex, international physical activity questionnaire (IPAQ) score and adherence to the diet as covariates. Multivariate linear regression analysis will be performed to assess the association between changes in CAP values and changes in HbA1c values between two groups. P values < 0.05 are considered statistically significant and the data are analyzed using SPSS software

A statistical analysis will be performed on all the enrolled patients who meet the protocol selection criteria and for whom physical examination indicators and fecal samples are available. Data will be summarized using standard descriptive statistics:

-The continuous variables will be described in terms of data quantity, number of missing data, mean, SD, median, quartiles (Q1, Q3), minimum and maximum values. Means and standard deviations will be presented with one more decimal place than the collected data.

-The categorical variables will be described in terms of missing data frequency, frequency and percentage of each recorded category.

2. The Statistical Plan-Amendments for Version 3.0

2.1 Research Purpose

The main objective of this study is to investigate the effectiveness of CMNT (an intermittent fasting intermittent energy restriction intervention) in managing Chinese adults with T2D and NAFLD. It further explores whether the glucose-lowering effect of CMNT is related to reduced liver fat and its mechanism in hepatic glucose and lipid metabolism. This study is based on a small clinical pilot trial of 39 patients with type 2 diabetes and non-alcoholic fatty liver disease and is a part of the CMNT study (NCT05439226).

2.2 Sample Size Justification

Approximately 120 participants are expected to be enrolled in the study. The sample size calculation is based on the reduction in HbA1c. According to preliminary studies, the mean ± SD values of HbA1c after intervention were 7.173%±1.919 in the CMNT group and 8.124% ±1.144 in the control group.29 Forty-four participants in each group will be recruited, and the following formula will be used to calculate the required sample size, with consideration of stratification analysis on T2D and NAFLD comorbidity, we presume a dropout rate of 20%. In addition, as this study adds a 12-month follow-up compared to the control group's standard care, the sample size per group is increased to 60 cases. **Reference for sample size calculation：** Luo W, Zhou J, Yang X, et al. A Chinese medical nutrition therapy diet accompanied by intermittent energy restriction alleviates type 2 diabetes by enhancing pancreatic islet function and regulating gut microbiota composition. *Food Res Int* 2022;161:111744.

An interim analysis will use sample data from the first 39 enrolled patients. The interim analysis sample size design is similar to that of the previous study (CMNT study: NCT05439226). The sample size estimation is based on the reduction in HbA1c. The estimated sample size of this trial is 24 per group, allowing a 20% dropout rate. However, due to the COVID-19 pandemic in China, we are only able to include 19-20 subjects per group, which nevertheless proves sufficient to achieve > 80% statistical power. A subsequent analysis indicates the therapeutic potential of iER intervention in this study population. **Reference for re-estimating the sample size calculation:** Yang X*, et al.* Effect of an Intermittent Calorie-restricted Diet on Type 2 Diabetes Remission: A Randomized Controlled Trial. *The Journal of Clinical Endocrinology & Metabolism* **108**, 1415-1424 (2022).

2.3 Outcome Analysis

All statistical analyses will be conducted using IBM SPSS V.23. We will use a t-test or $\chi 2$ test to determine the differences for normal data and Mann-Whitney U test for non-normal data between the CMNT group and usual care group. Data will be analyzed on an intention-to-treat basis, comparing the CMNT group with the usual care group. CAP values and HbA1C values at baseline and after treatment will be evaluated

by repeated measure analysis of variance. To control the confounders, the primary and secondary outcomes will be compared via analysis of covariance with age, sex, IPAQ score and adherence to the diet as covariates. The multivariate linear regression analysis will be performed to assess the association between changes in CAP values and changes in HbA1c values between two groups. P values <0.05 are considered statistically significant and the data are analyzed using SPSS software.

At baseline, the normality of continuous parameters will be tested. The test will be conducted in the modified Intention-to-Treat Analysis (ITT) population. If two or more of the Shapiro-Wilk, Cramer-von Mises, and Anderson-Darling tests are statistically significant at the 0.05 level, the normality of the distribution will be visually inspected. If necessary, simple transformations (e.g., taking the natural logarithm) can be applied to parameters to normalize the distribution.

Unless otherwise specified, the two groups will be made as follows:

- Continuous parameters: compared using t tests; if the normality of the distribution cannot be assumed, the non-parametric Wilcoxon test will be used.
- Categorical parameters: compared using the chi-square test; if the theoretical frequency is less than 5, Fisher's exact test or an equivalent method will be used.

Statistical analysis will be performed on all enrolled patients who meet the protocol selection criteria and for whom physical examination indicators and fecal samples are available. Data will be summarized using standard descriptive statistics:

- Continuous variables will be described by data quantity, number of missing data, mean ± standard deviation (SD), median, quartiles (25%, 75%), minimum and maximum values.
- Categorical (ordinal or nominal) variables will be described by missing data frequency, frequency and percentage of each recorded category.

3. Statistical Analysis Plan Amendments.

The main revisions and their justifications are as follows:

3.1 Clarification of Data Analysis Set Definition

In the final statistical plan, the modified ITT population is defined as all enrolled participants with CAP data at baseline, replacing the Full Analysis Set (FAS) in the original plan.

Rationale: To more precisely identify assessable participants directly related to the core research objectives, enhancing the clinical significance and reliability of the results.

3.2 Statistical Parameters

In the final statistical plan, tests for the normality of continuous parameters have been added, along with the specific methods for comparing the two groups.

Rationale: Adding normality tests for continuous parameters and specifying group-comparison methods in the final plan ensures the applicability of the statistical methods and the scientific rigor of the results.

3.3 Addition of Interim Analysis

Rationale: To evaluate the effectiveness of the CMNT dietary intervention and the safety of the energy-restricted diet during clinical trials, we conducted a mid-term analysis when 40% of the intended treatment population was reached. The study subjects were individuals with type 2 diabetes who had non-alcoholic fatty liver disease (NAFLD). It remains unknown whether their outcomes and risks differ from those of conventional care. Notably, due to differences in age, biochemical profiles, and the severity of underlying conditions, each participant's tolerance to the energy restricted diet varied. The purpose of the interim analysis was to assess the effectiveness of the CMNT dietary intervention, as well as to evaluate participants' adherence and the safety of the energy-restricted diet. Based on previous research, a smaller sample size of 24 participants per group was deemed statistically significant. Therefore, the first 40% of the participants were selected for the interim analysis.

References: Yang X, et al. Effect of intermittent calorie-restricted diet on the remission of type 2 diabetes: a randomized controlled trial. Journal of Clinical Endocrinology & Metabolism 108,1415-1424 (2022).

4. Original study Protocol (Version 2.0)

4.1 Summary of Original Protocol：

Principal Sponsor: State Key Laboratory of Subhealth Intervention Technology (Affiliated Institution: Hunan Agricultural University)
Title: Chinese Medicine Nutrition Therapy Targeting Liver Fat for the Treatment of Type 2 Diabetes with Non-Alcoholic Fatty Liver Disease
Abbreviation: CMNT Targeting Liver Fat in T2D with NAFLD
Principal Investigator (PI):
Dongbo Liu (Professor)
College of Horticulture, Hunan Agricultural University
State Key Laboratory of Subhealth Intervention Technology
Number of Research Centers:
Hunan Shanshui physical examination center；
Physical Examination Center of Gezhouba central hospital of Sinopharm
Jinheyuan Outpatient Department, National Sub-health Intervention Technology Achievement Application Center
Timeline: Recruitment Phase: June 5, 2022 to May 5, 2024 (anticipated)
Follow-up Phase: 1 years
Maximum Study Duration: 5 years

4.2 Research Population

-Patients officially diagnosed with T2D and NAFLD.
-Patients included in those using antidiabetic medications and newly-diagnosed patients not using such medications.

-All patients have undergone a risk assessment for iER intervention in multi-disciplinary team meetings (including endocrinologists, nutritionists, and researchers) and have been evaluated by physicians for the need for appropriate clinical medications.
-Patients included in the study undergo diagnostic examinations (imaging or biological) in conjunction with diet and drug therapy (if any), in accordance with the recommendations of the Chinese health authorities.

4.3 Selection Criteria

4.3.1 Inclusion Criteria：

-Patients with T2DM diagnosed according to the 1999 WHO diagnostic criteria for diabetes and with fatty liver, diagnosed according to Chinese standards.
- BMI at screening between 18.0 and 35.0 kg/m^2 (inclusive); age at screening between 18 and 75 years (inclusive), both males and females;
- Willing and able to accurately self-monitor blood glucose using a home glucose meter;
-Able to understand and comply with the trial procedures, voluntarily participate in the trial, and provide informed consent.

4.3.2 Exclusion Criteria:

-Patients with type 1 diabetes, pancreatic - related diabetes, or secondary diabetes (e.g., diabetes caused by Cushing's syndrome or acromegaly).
-Use of any of the following medications or treatments:
-Use of other medications that may affect glucose metabolism within the past 2 months, including systemic glucocorticoids (excluding inhaled or topical use), growth hormones, etc.;
-Use of antihypertensive or lipid-lowering medications that have not reached a stable dosage before screening;
-Presence of any of the following medical histories or conditions:
-History or presence of any cardiac disease within the past 6 months;
-Decompensated heart failure (NYHA Class III or IV);
-Unstable angina, myocardial infarction, coronary artery bypass grafting, or coronary stenting;
-Uncontrolled or severe arrhythmias (e.g., long QT syndrome), as assessed by the investigator as unsuitable for trial participation;
-History of hemorrhagic or ischemic stroke within the past 6 months, as assessed by the investigator as unsuitable for trial participation;
-Medical history of cerebral thrombosis, cerebral vascular blockage, encephalic angioma, transient ischemic attack, cerebral hemorrhage, stroke, hydrocephalus, or malignant brain tumor;
-History of carotid artery stenting;
-Urinary system conditions such as nephrotic syndrome, uremia, polycystic kidney disease, kidney transplantation, unilateral nephrectomy/congenital solitary kidney, renal atrophy, renal tumor;
-Digestive system conditions such as ascites, liver cirrhosis, liver fluke infection, severe

hepatitis, gastric varices;
-Nervous system conditions such as cerebellar atrophy, demyelinating diseases, cerebral palsy, Parkinson's disease, mania, schizophrenia;
-Respiratory system conditions such as pulmonary embolism, cor pulmonale;
-Musculoskeletal system conditions such as arterial rupture, myeloma;
-Immune system conditions such as Behçet's disease, lupus erythematosus;
-Conditions such as chondrosarcoma, liposarcoma, brucellosis, leukemia;
-History of malignant tumor within the past 5 years, or currently under evaluation for potential malignancy;
-History of unstable or treatment-requiring proliferative retinopathy or macular edema within the past 6 months;
-History of diabetic ketoacidosis or hyperosmolar nonketotic diabetic coma within the past 6 months;
-Currently suffering from lower extremity arteriosclerotic occlusive disease;
-History of severe infection or severe trauma within the past 1 month;
-History of ≥2 severe hypoglycemic episodes within the past year;
-Currently suffering from clinically significant urinary tract/genital infection, or a history of complicated urinary tract infections, or recurrent urinary tract infections within the past 6 months;
-Currently suffering from uncontrolled hypertension, defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥100 mmHg at screening/baseline, or systolic blood pressure ≤90 mmHg and/or diastolic blood pressure ≤60 mmHg;
-Currently suffering from uncontrolled thyroid dysfunction;
-History of other severe endocrine diseases, such as multiple endocrine neoplasia;
-History of severe hepatic or renal disease;
-Suspected or confirmed history of alcohol or drug abuse;
-Blood donation or blood loss ≥400 mL within the past 3 months;
-Presence of severe psychiatric disorders or language barriers, unwillingness or inability to fully understand and cooperate;
-Pregnant or breastfeeding women;
-Any other conditions deemed unsuitable for trial participation by the investigator.

4.4 Research Objectives

4.4.1 Primary Objective

The effect of CMNT intervention on liver fat and blood glucose in patients with T2D and NAFLD

4.4.2 Secondary objective

- Analyze whether blood glucose improvement is related to liver fat reduction.
- Changes in liver function.
- Changes in blood lipid levels.
- Changes in systemic inflammatory levels.
- Improvement in insulin resistance for this comorbidity.

- Changes in liver fibrosis.
- Changes in gut microbiota.

4.5 Outcome Measures

4.5.1 Primary Endpoints

Liver fat content measured by transient elastography
Glycated hemoglobin (HbA1c)

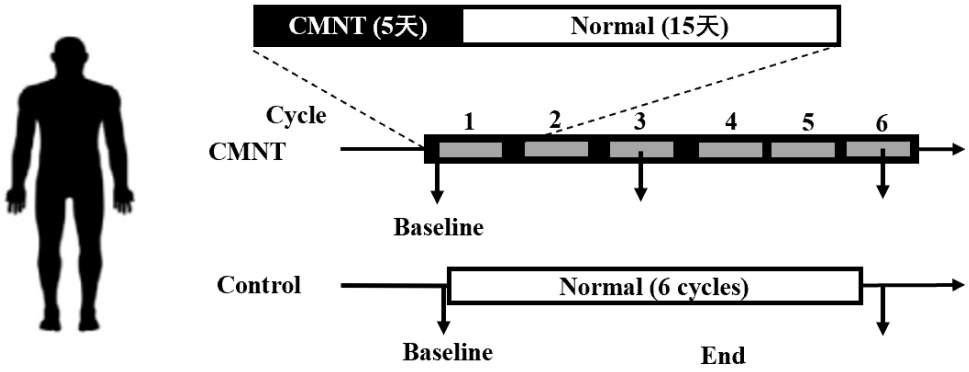
4.5.2 Secondary Endpoints

Anthropometric measures: height, weight, waist circumference, hip circumference, chest circumference, pulse, blood pressure, heart rate, body temperature, respiratory rate;
Body fat composition analysis;
Complete blood count;
Fasting blood glucose; Fasting insulin;
Fasting C-peptide;
12-item liver function tests: total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, direct bilirubin, indirect bilirubin, ALT, AST, GGT, ALP, AST/ALT; 7-item lipid profile: triglycerides, total cholesterol, HDL, LDL, apolipoprotein A, apolipoprotein B, apolipoprotein A/apolipoprotein B ratio;
High-sensitivity C-reactive protein (hsCRP);
β-hydroxybutyrate;
Insulin - like growth factor -1 (IGF-1);
Free fatty acids;
Food frequency questionnaire (FFQ);
Gut microbiota.

4.6 Research Methods

This is a multi-center, prospective, longitudinal study to be jointly conducted at three clinical recruitment centers in China: Hunan Shanshui Health Check-up Center; Sinopharm Gezhouba Central Hospital; and the National Sub - health Intervention Technology Application Center (Jinhe Garden Clinic).

4.7 Dietary Intervention Pattern



CMNT Intervention Group: The CMNT diet adopts a "5 + 10" intermittent energy restriction pattern, with a 15 - day CMNT cycle consisting of 5 consecutive days of CMNT diet followed by 10 days of normal diet for participants in the trial group. During the 5-day CMNT diet period, participants in the CMNT group receive a pre-prepared CMNT diet totaling 917 Kcal daily. Specifically, breakfast includes a packet (50g) of fruit and vegetable porridge; lunch consists of a packet (60g) of nutritional rice and a cup (25g) of solid beverage; dinner comprises two blocks (30g) of meal-replacement biscuits and a cup of solid beverage. The 10-day normal diet follows the standards of *the China Type 2 Diabetes Prevention and Control Guidelines*. **The control group** adheres to the conventional diet as per *China Type 2 Diabetes Prevention and Control Guidelines*.

4.8 Patient Recruitment

During dietary intervention and routine diabetes management counseling, researchers will inform patients of the possibility to participate in the study. Patients will receive both verbal and written information on the study protocol, primary objectives, implementation, follow - up, and sample collection (as part of the study protocol). All sample collections will coincide with routine clinical examinations during conventional treatment. Researchers will distribute information leaflets to patients and collect signed informed consent forms.

4.9 Research Procedures

All procedures outlined in the study protocol will be conducted concurrently with routine clinical examinations in conventional treatment. Procedures specific to this study include:

Collection of one blood and one fecal sample at three time points:

-At recruitment (Baseline).

-Post-treatment (90 days).

-During follow-up (6, 12, and 24 months).

- High-frequency blood glucose monitoring using a glucose meter, with data uploaded to the CMNT mobile app for timely access by nutritionists and endocrinologists.
- Electronic collection of patient - completed responses to quality-of-life and food questionnaires. These questionnaires will be distributed in paper form during the

baseline physical examination.
- Electronic collection of patient-completed responses to the ethnic background questionnaire. This questionnaire will only be requested after an additional informed consent form is signed.

In accordance with the "Administrative Measures for Ethical Review of Life Sciences and Medical Research Involving Human Beings" jointly issued by the National Health Commission, Ministry of Education, Ministry of Science and Technology, and the National Administration of Traditional Chinese Medicine, this study is considered research involving human subjects.

4.10 Patient Follow-up

Each patient will be followed up according to the conventional diabetes management pathway, including:
- Follow-ups at 6, 12, and 24 months post - intervention.
- Systematic follow-up or telephone contact within one year to assess disease status.
- The data collection methods for this study refer to the six major data collection methods summarized in "Research Methods, Design, and Analysis" by Larry Christensen and Burke Johnson. The collected data are health-related and are deemed appropriate, relevant, and not excessive for the research purposes.
The following data will be collected for each patient participating in the study:
- Identification information:
- The identification number consists of the center number in the study, a sequential number increasing according to the order of recruitment (with the first number in the study being 1), and the first two letters of the surname and the initial of the given name.
- The aforementioned inclusion and exclusion criteria.
- Data strictly necessary for conducting the research:
  -Age.
  -Clinical examination.
  -Laboratory tests.
  -Results of specific biomarker analyses in blood samples related to liver function.
  -Medical imaging (transient elastography).
  -Results of gut microbiota detection in fecal samples.
  -Data on any adverse events and/or side effects occurring during the study period.
  -Personal or family medical history.
  -Dates related to the study (especially the recruitment date and visit dates).
  -Family status.
  -Socio - occupational category/occupation.
  -Participation in other studies or research to ensure compliance with inclusion criteria.
  -Tobacco, alcohol, and drug use.
-The quality-of-life and dietary questionnaires.

4.11 Efficacy and Safety Evaluation Monitored Adverse Effects/Toxicity

All risks and discomforts associated with calorie-restricted diets include hunger,

anxiety, somnolence, dizziness, headache, muscle pain, fatigue, low blood pressure, and in rare cases, fainting. These dietary interventions may also lead to arrhythmias, short-term nutritional deficiencies, and weakened immune responses. For individuals already malnourished, long-term low-calorie diets are particularly hazardous. Participants may withdraw from the dietary intervention and resume their normal diet at any time during the study. If significant discomfort occurs during the study, participants should seek immediate medical attention. During the dietary intervention, participants should drink sufficient water to prevent dehydration and avoid intense activities or exercises. They should refrain from operating motor vehicles and heavy machinery, avoid high-temperature environments such as hot showers or baths, and abstain from alcohol. If participants have any questions about the dietary intervention or feel unwell, they should contact the researcher, consult their personal physician, or seek immediate medical care. After the dietary intervention, participants should avoid overeating and gradually return to a normal diet, starting with liquid foods like soups and juices, then gradually transitioning to light meals. Participants may feel dizzy during blood collection. In rare cases, participants may experience bruising, excessive bleeding, infection (a minor risk when the skin is punctured), dizziness, and fainting. Participants may stop the blood collection procedure at any time. If bleeding or infection occurs during blood collection, participants should contact their personal physician or seek immediate medical care.

Participants may withdraw from the dietary intervention and resume their normal diet at any time during the study. If participants experience significant discomfort during the study, they should seek immediate medical attention.

Adverse Event Reporting: Procedures for Reporting Unexpected and Fatal Toxicities within 10 working days of becoming aware of the event, the following serious adverse events (SAEs) or serious suspected adverse reactions will be reported to the IRB:
- Death;
- Hospitalization;
- Persistent or significant disability;
- Severe impact on the ability to perform normal life activities;
- Events endangering the participant; or
- Events potentially requiring medical or surgical intervention to prevent any of the above - listed outcomes.

4.12 Sources of Data for Research Subjects

-Responses to the quality of life and food questionnaires provided by research participants. In this research, these questionnaires will be distributed in paper form.
-Data related to diabetes management and the study, provided by researchers: results of clinical examinations, laboratory tests, medical imaging (transient elastography), data on adverse events and side effects occurring during the study period, personal or family medical history.
Researchers will input all this data into an electronic case report form from the patient's medical records.

-Results of gut microbiota detection in fecal samples analyzed by a sequencing company.

All collected data will be anonymized and stored securely.

4.13 Review of Patient Diabetes Management Data

All anonymized diabetes management data included in the study (mainly routine physical examination and imaging data) will be reviewed by medical experts to ensure correct classification. Data reviewers will remain blinded to the results of fecal sample sequencing. If the medical reviewer identifies any inconsistencies between the data reported by the centers in the study and the review results, clarification will be requested.

5. Study Protocol- Amendments (Version 3.0)

5.1 The Summary of Study Protocol

Principal Sponsor: State Key Laboratory of Subhealth Intervention Technology (Affiliated Institution: Hunan Agricultural University)
Title: Chinese Medicine Nutrition Therapy Targeting Liver Fat for the Treatment of Type 2 Diabetes with Non-Alcoholic Fatty Liver Disease
Abbreviation: CMNT Targeting Liver Fat in T2D with NAFLD
Principal Investigator (PI):
Dongbo Liu (Professor)
College of Horticulture, Hunan Agricultural University
State Key Laboratory of Subhealth Intervention Technology
Number of Research Centers:
Hunan Shanshui physical examination center；
Physical Examination Center of Gezhouba central hospital of Sinopharm;
Jinheyuan Outpatient Department, National Sub-health Intervention Technology Achievement Application Center;
Yiyang Central Hospital (Hunan Province, China);
Shaoyang Central Hospital (Hunan Province, China);
Zhuzhou People's Hospital (Hunan Province, China);
Timeline: Recruitment Phase: June 5, 2022 to May 5, 2025 (anticipated)
Follow-up Phase: 1 years
Maximum Study Duration: 5 years

5.2 Research population

-Patients officially diagnosed with T2D and NAFLD.
-The study includes patients using antidiabetic medications and newly-diagnosed patients not using such medications.
-All patients have undergone a risk assessment for intermittent energy restriction diet intervention in multidisciplinary team meetings (including endocrinologists, nutritionists, and researchers) and have been evaluated by physicians for the need for appropriate clinical medications.

-Patents included in the study undergo diagnostic examinations (imaging or biological) in conjunction with diet and drug therapy (if any), in accordance with the recommendations of the Chinese health authorities.

5.3 Selection Criteria

5.3.1 Inclusion Criteria:

- Patients with type 2 diabetes mellitus diagnosed according to the 1999 WHO diagnostic criteria for diabetes and with fatty liver, diagnosed according to Chinese standards.
- BMI at screening greater than 18.0 kg/m^2; age at screening between 18 and 75 years (inclusive), both males and females;
- Willing and able to accurately self - monitor blood glucose using a home glucose meter;
- Be able to understand and comply with the trial procedures, voluntarily participate in the trial, and provide informed consent.

5.3.2 Exclusion Criteria:

-Patients with type 1 diabetes, pancreatic - related diabetes, or secondary diabetes (e.g., diabetes caused by Cushing's syndrome or acromegaly).
-Use of any of the following medications or treatments:
-Use of other medications that may affect glucose metabolism within the past 2 months, including systemic glucocorticoids (excluding inhaled or topical use), growth hormones, etc.;
-Use of antihypertensive or lipid-lowering medications that have not reached a stable dosage before screening;
-Presence of any of the following medical histories or conditions:
-History or presence of any cardiac disease within the past 6 months;
-Decompensated heart failure (NYHA Class III or IV);
-Unstable angina, myocardial infarction, coronary artery bypass grafting, or coronary stenting;
-Uncontrolled or severe arrhythmias (e.g., long QT syndrome), as assessed by the investigator as unsuitable for trial participation;
-History of hemorrhagic or ischemic stroke within the past 6 months, as assessed by the investigator as unsuitable for trial participation;
-Medical history of cerebral thrombosis, cerebral vascular blockage, encephalic angioma, transient ischemic attack, cerebral hemorrhage, stroke, hydrocephalus, or malignant brain tumor;
-History of carotid artery stenting;
-Urinary system conditions such as nephrotic syndrome, uremia, polycystic kidney disease, kidney transplantation, unilateral nephrectomy/congenital solitary kidney, renal atrophy, renal tumor;
-Digestive system conditions such as ascites, liver cirrhosis, liver fluke infection, severe hepatitis, gastric varices;

-Nervous system conditions such as cerebellar atrophy, demyelinating diseases, cerebral palsy, Parkinson's disease, mania, schizophrenia;
-Respiratory system conditions such as pulmonary embolism, cor pulmonale;
-Musculoskeletal system conditions such as arterial rupture, myeloma;
-Immune system conditions such as Behçet's disease, lupus erythematosus;
-Conditions such as chondrosarcoma, liposarcoma, brucellosis, leukemia;
-History of malignant tumor within the past 5 years, or currently under evaluation for potential malignancy;
-History of unstable or treatment-requiring proliferative retinopathy or macular edema within the past 6 months;
-History of diabetic ketoacidosis or hyperosmolar nonketotic diabetic coma within the past 6 months;
-Currently suffering from lower extremity arteriosclerotic occlusive disease;
-History of severe infection or severe trauma within the past 1 month;
-History of ≥2 severe hypoglycemic episodes within the past year;
-Currently suffering from clinically significant urinary tract/genital infection, or a history of complicated urinary tract infections, or recurrent urinary tract infections within the past 6 months;
-Currently suffering from uncontrolled hypertension, defined as systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥100 mmHg at screening/baseline, or systolic blood pressure ≤90 mmHg and/or diastolic blood pressure ≤60 mmHg;
-Currently suffering from uncontrolled thyroid dysfunction;
-History of other severe endocrine diseases, such as multiple endocrine neoplasia;
-History of severe hepatic or renal disease;
-Suspected or confirmed history of alcohol or drug abuse;
-Blood donation or blood loss ≥400 mL within the past 3 months;
-Presence of severe psychiatric disorders or language barriers, unwillingness or inability to fully understand and cooperate;
-Pregnant or breastfeeding women;
-Any other conditions deemed unsuitable for trial participation by the investigator.

5.4 Research Objectives

5.4.1 Primary Objective

The effect of CMNT intervention on liver fat and blood glucose in patients with T2D and non-alcoholic fatty liver disease.

5.4.2 Secondary Objectives

- Analyze whether blood glucose improvement is strongly related to liver fat reduction.
- Changes in liver function.
- Changes in blood lipid levels.
- Changes in systemic inflammatory levels.
- Improvement in insulin resistance for this comorbidity.
- Changes in liver fibrosis.
- Changes in gut microbiota.

- Use metabolomics to identify key metabolites.
- Based on gut microbiota findings, screen for key microbial taxa post-CMNT intervention, and validate the causal relationship between diet and gut microbiota using fecal microbiota transplantation.

5.5 Outcome Measures

5.5.1 Primary Endpoints

-Liver fat content measured by transient elastography
-Glycated hemoglobin (HbA1c)

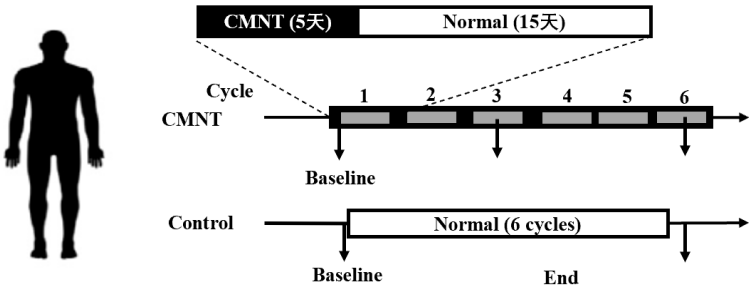
5.5.2 Secondary Endpoints

-Anthropometric measures: height, weight, waist circumference, hip circumference, chest circumference, pulse, blood pressure, heart rate, body temperature, respiratory rate;
-Body fat composition analysis;
-Complete blood count;
-Fasting blood glucose; Fasting insulin;
-Fasting C-peptide;
-Liver function tests: total protein, albumin, globulin, albumin/globulin ratio, total bilirubin, direct bilirubin, indirect bilirubin, ALT, AST, GGT, ALP, AST/ALT;
-Lipid profile: triglycerides, total cholesterol, HDL, LDL, apolipoprotein A, apolipoprotein B, apolipoprotein A/apolipoprotein B ratio;
-High - sensitivity C-reactive protein (hCRP);
-β-hydroxybutyrate;
-Insulin-like growth factor-1 (IGF-1);
-Free fatty acids;
-Food frequency questionnaire (FFQ);
-Gut microbiota;
-Untargeted metabolomics;
-Fecal and single-strain transplantation.

5.6 Study Method

This is a multi-center, prospective, longitudinal study initially conducted at three clinical recruitment centers: Hunan Shanshui Health Check-up Center; Sinopharm Gezhouba Central Hospital; and the National Sub-health Intervention Technology Application Center (Jinhe Garden Clinic). The study will be expanded to six research centers, with new additions signing letters of intent with the undertaking organization.
The Interim Analysis: In the 120-participant CMNT intervention project, interim analyses will be added to compare intervention effects before and after CMNT diet optimization and with the conventional control group.

5.7 Dietary Intervention Pattern



CMNT Intervention Group: The CMNT diet adopts a "5 + 10" intermittent energy restriction pattern, with a 15-day CMNT cycle consisting of 5 consecutive days of CMNT diet followed by 10 days of normal diet for participants in the trial group. During the 5-day CMNT diet period, participants in the CMNT group receive a pre-prepared CMNT diet totaling 917 Kcal daily. Specifically, breakfast includes a packet (50g) of fruit and vegetable porridge; lunch consists of a packet (60g) of nutritional rice and a cup (25g) of solid beverage; dinner comprises two blocks (30g) of meal-replacement biscuits and a cup of solid beverage. The 10-day normal diet follows the standards of *the China Type 2 Diabetes Prevention and Control Guidelines*. **The control group** adheres to the conventional diet as per *China Type 2 Diabetes Prevention and Control Guidelines*.

5.8 Patient Recruitment

During dietary intervention and routine diabetes management counseling, researchers will inform patients of the possibility to participate in the study. Patients will receive both verbal and written information on the study protocol, primary objectives, implementation, follow-up, and sample collection (as part of the study protocol). All sample collections will coincide with routine clinical examinations during conventional treatment. Researchers will distribute information leaflets to patients and collect signed informed consent forms.

5.9 Research Procedures

All procedures outlined in the study protocol will be conducted concurrently with routine clinical examinations in conventional treatment. Procedures specific to this study include:

- Collection of one blood and one fecal sample at three time points:
  -At recruitment (baseline).
  -Post-treatment (90 days).
  -During follow - up (6, 12, and 24 months).
- High-frequency blood glucose monitoring using a glucose meter, with data uploaded to the CMNT mobile app for timely access by nutritionists and endocrinologists.
-Electronic collection of patient-completed responses to the quality-of-life and food questionnaires. These questionnaires will be distributed in paper form during the baseline physical examination.
- Electronic collection of patient-completed responses to the ethnic background

questionnaire. This questionnaire will only be requested after an additional informed consent form is signed.

In accordance with the "Administrative Measures for Ethical Review of Life Sciences and Medical Research Involving Human Beings" jointly issued by the National Health Commission, Ministry of Education, Ministry of Science and Technology, and the National Administration of Traditional Chinese Medicine, this study is considered research involving human subjects.

5.10 Patient Follow-up

Each patient will be followed up according to the conventional diabetes management pathway, including:

-Follow-ups at 6, 12, and 24 months post-intervention.

-Systematic follow - up or telephone contact within one year to assess disease status.

The data collection methods for this study refer to the six major data collection methods summarized in "Research Methods, Design, and Analysis" by Larry Christensen and Burke Johnson. The collected data are health-related and are deemed appropriate, relevant, and not excessive for the research purposes.

The following data will be collected for each patient participating in the study:

-Identification information:

-The identification number consists of the center number in the study, a sequential number increasing according to the order of recruitment (with the first number in the study being 1), and the first two letters of the surname and the initial of the given name.

-The aforementioned inclusion and exclusion criteria.

-Data strictly necessary for conducting the research:

-Age.

-Clinical examination.

-Laboratory tests.

-Results of specific biomarker analyses in blood samples related to liver function.

-Medical imaging (transient elastography).

-Results of gut microbiota detection in fecal samples.

-Data on any adverse events and/or side effects occurring during the study period.

-Personal or family medical history.

-Dates related to the study (especially the recruitment date and visit dates).

-Family status.

-Socio-occupational category/occupation.

-Participation in other studies or research to ensure compliance with inclusion criteria.

-Tobacco, alcohol, and drug use.

-The quality of life and dietary questionnaires.

5.11 Safety Risk Assessment

Risks and discomforts associated with calorie - restricted diets include hunger, anxiety, somnolence, dizziness, headache, muscle pain, fatigue, low blood pressure, and in rare cases, fainting. These dietary interventions may also lead to arrhythmias, short-

term nutritional deficiencies, and weakened immune responses. For individuals already malnourished, long-term low-calorie diets are particularly hazardous. Participants may withdraw from the dietary intervention and resume their normal diet at any time during the study. If significant discomfort occurs during the study, participants should seek immediate medical attention. During the dietary intervention, participants should drink sufficient water to prevent dehydration and avoid intense activities or exercises. They should refrain from operating motor vehicles and heavy machinery, avoid high-temperature environments such as hot showers or baths, and abstain from alcohol. If participants have any questions about the dietary intervention or feel unwell, they should contact the researcher, consult their personal physician, or seek immediate medical care. After the dietary intervention, participants should avoid overeating and gradually return to a normal diet, starting with liquid foods like soups and juices, then gradually transitioning to light meals. Participants may feel dizzy during blood collection. In rare cases, participants may experience bruising, excessive bleeding, infection (a minor risk when the skin is punctured), dizziness, and fainting. Participants may stop the blood collection procedure at any time. If bleeding or infection occurs during blood collection, participants should contact their personal physician or seek immediate medical care.

Participants may withdraw from the dietary intervention and resume their normal diet at any time during the study. If participants experience significant discomfort during the study, they should seek immediate medical attention.

Adverse Event Reporting: Procedures for Reporting Accidental and Fatal Toxicities

Within 10 working days of becoming aware of the event, the following serious adverse events (SAEs) or serious suspected adverse reactions will be reported to the IRB:

- Death;
- Hospitalization;
- Persistent or significant disability;
-Severe impact on the ability to perform normal life activities;
- Events endangering the participant; or
- Events potentially requiring medical or surgical intervention to prevent any of the above the listed outcomes.

5.12 Data Sources

-Responses to the quality-of-life and food questionnaires provided by study participants. In this study, these questionnaires will be distributed in paper form.

-Data related to diabetes management and the study, provided by researchers: results of clinical examinations, laboratory tests, medical imaging (transient elastography), data on adverse events and side effects occurring during the study period, personal or family medical history. Researchers will input all this data into an electronic case report form from the patient's medical records.

-Results of gut microbiota detection in fecal samples analyzed by a sequencing

company.
-Results of fecal and single-strain transplantation validation.
All collected data will be anonymized and stored securely.

5.13 Review of Patient Diabetes Management Data

All anonymized diabetes management data included in the study (mainly routine physical examination and imaging data) will be reviewed by medical experts to ensure correct classification. Data reviewers will remain blinded to the results of fecal sample sequencing.

If the medical reviewer identifies any inconsistencies between the data reported by the centers in the study and the review results, clarification will be requested.

6 Study Protocol Amendments.

The main revisions and their justifications are as follows:

6.1 Addition of Interim Analysis.

Rationale: The purpose of adding an interim analysis is to evaluate the effectiveness of the CMNT dietary intervention during this clinical trial, while also assessing participant compliance and the safety of the energy-restricted diet. The baseline CMNT intervention follows a "5+10" intermittent energy restriction structure (also referred to as iER, intermittent Energy Restriction), with the first 5 days using a fixed 917 kcal/day meal replacement model. Our prior research has shown that this baseline CMNT dietary structure is superior to conventional care in glycemic control for type 2 diabetes (T2D) management, with more participants achieving diabetes remission. However, this study focuses on T2D patients comorbid with non-alcoholic fatty liver disease (NAFLD), and whether its efficacy and risks differ from conventional care remains unknown. Notably, individual responses to energy restriction vary due to differences in age, metabolic characteristics, and baseline disease severity. For some participants, this fixed dietary model may not be the optimal intervention. Therefore, we have incorporated an interim analysis to preliminarily assess the efficacy and potential risks of the baseline CMNT diet in this comorbid population. Based on the unblinded interim data, the Data and Safety Monitoring Board (DSMB) will vote on whether to continue the original CMNT dietary protocol, using the following predefined criteria:
-Continue the original CMNT protocol if it demonstrates significantly better efficacy in glycemic and lipid control compared to conventional care, with no additional safety risks.
- Optimize the dietary protocol if efficacy is equivalent or inferior to conventional care, provided no safety concerns exist.
-Terminate the dietary protocol if safety risks are significantly higher than conventional care, even if efficacy is observed.

6.2 Expansion of BMI Inclusion Criteria.

The BMI inclusion criterion was revised from "18-35" to " > 18" (removing the upper limit) to include patients with higher BMI values.

Rationale: In clinical practice, some patients with a BMI over 35 have shown interest in participating in this study. After evaluation by physicians, these patients can be included.

6.3 Increase in the Number of Multicenter Sites

The number of centers increased from 3 to 6, including multiple municipal medical institutions (Yiyang Central Hospital, Shaoyang Central Hospital, Zhuzhou People's Hospital).

Rationale: Expanding the number of multicenter sites accelerates participant recruitment. Given the study's focus on patients with T2D and NAFLD, the recruitment of patients with comorbidities is relatively challenging. Additionally, expanding the number of centers enhances the generalizability of the study's conclusions, making them applicable to patients in more regions.

6.4 Clarification and Revision of Secondary Objectives and Corresponding Evaluation Criteria.

-Addition of metabolomics content to identify key metabolites in CMNT intervention.

-Screening for key microbial taxa post - CMNT intervention based on gut microbiota results.

-Validation of the causal relationship between diet and gut microbiota using fecal transplantation.

-These secondary objectives give rise to the following outcome measures: untargeted metabolomics, fecal and single - strain transplantation trials.

Rationale: Incorporating gut microbiota and metabolomics indicators helps better explore the mechanism of CMNT dietary intervention in this population.

6.5 The participant recruitment period has been extended to May 5, 2026.

Rationale: Due to the severe COVID-19 pandemic during 2022-2023, coupled with the fact that this study focuses on comorbidities, the number of eligible participants has been significantly lower compared to studies involving general T2D patients. Furthermore, as most individuals currently do not prioritize fatty liver screening, participant recruitment has progressed more slowly than anticipated. Therefore, an extension of the recruitment period is necessary.

All revisions have been approved by the Ethics Committee.