Study protocol

Project name: diet intervention in obesity-related complication
Solution Version Number/Date: 1.0/2024-6-23

Research background

Obesity has become a World epidemic disease. According to the latest "World Obesity Map" [1] released by the World Obesity Federation in 2023, it is expected that by 2035, more than 4 billion people in the world will be overweight or obese, accounting for more than half of the total population. Obesity-related complications are responsible for 4 million deaths per year worldwide, accounting for 7.1% of all-cause deaths [2]. Obesity-related complications, including diabetes, Metabolic associated Fatty Liver Disease (MAFLD), hyperlipidemia and other metabolic diseases listed in the Clinical Practice Guidelines for the Integrated Management of Obese Patients published by the American Association of Clinical Endocrinologists, And stress osteoarthritis, stress urinary incontinence, gastroesophageal reflux disease and other volume-load-related diseases [3]. Obesity is also closely associated with gastric mucosal injury diseases, such as gastric ulcer and gastric cancer.

A series of previously published studies suggest that obesity, especially centripetal obesity, is an independent risk factor for gastric ulcer [4]. A large retrospective cohort study with 226,953 participants found that there was a statistically significant difference in the incidence of gastric ulcer among people with different Body Mass Index (BMI) cut-off points, and BMI>25 kg/m2 was an independent risk factor for gastric ulcer [5]. Another prospective cohort study suggested that obese patients with BMI≥30.0 kg/m2 had an 83% increased risk of gastric ulcer compared with normal BMI. Compared with men with a Waist to Hip Ratio (WHR) of 0.85-0.89, men with a WHR≥1.00 also had an 88% increased risk of gastric ulcer [6]. In addition, many studies have revealed the relationship between obesity, precancerous lesions of gastric mucosa and gastric cancer [7]. A retrospective study [8] found that the risk of Intestinal Metaplasia (IM) in overweight and obese patients increased by 72% and 141%, respectively. A large epidemiological investigation study on global lifestyle and risk of gastrointestinal tumors published in 2021 showed that BMI was positively correlated with the prevalence of gastric cancer [9]. In addition, a meta-analysis of 3,097,794 subjects (including 9,492 patients with stomach cancer) found that obese patients had a 36% increased risk of stomach cancer compared to people with normal BMI [10]. Another meta-analysis of 10 prospective studies and 12 case-control studies confirmed that each 2.5 kg/m2 increase in BMI was associated with an 11% increased risk of gastric cancer. The risk of gastric cancer in overweight and obese patients increased by 71% and 134%, respectively [11]. Therefore, obesity-related gastric mucosal injury, as an important complication of obesity, needs urgent attention in clinical and basic research fields. It is of great significance to study the mechanism of obesity-related gastric mucosa injury for reducing the risk of obesity-related gastric cancer.

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Research purpose

Main Purpose

To investigate the effect of 12-week dietary intervention on gastric mucosal damage in adult obese patients

Secondary Purposes

To evaluate the effects of 12-week dietary intervention on glucose metabolism, lipid metabolism and thyroid metabolism in obese patients

To evaluate the effects of a 12-week dietary intervention on the liver of obese patients

Study endpoint

Primary end points

Changes in body weight (Kg) and gastric injury from baseline 0 to 12 weeks

Secondary endpoints

glycolipid metabolism and thyroid function

Fatty liver 【 Liver function indicators, liver tumor indicators, liver fat content and hardness values, improvement of liver enlargement or fatty liver 】

Research design

This clinical study was a 3-month prospective, single-center trial. Obese adults aged 18-65 years were recruited. After signing the informed consent, patients meeting the inclusion/exclusion criteria who completed baseline clinical visits and data collection were randomly assigned 1:1 to the intervention group (n=30). The intervention group was treated with low LA diet pattern for 12 weeks.

Intervention measure

Low LA nutritional diet description: The fat intake is mainly unsaturated fatty acids (such as linseed oil, olive oil, etc.), and the diet structure includes a diet containing fruits, vegetables

and grains, fish, shrimp and seafood, poultry and eggs. Cooking dishes using olive oil, reduce the intake of LA, can eat a moderate amount of nuts; Eat a small or moderate amount of yogurt or cheese every day; Appropriate intake of fish, shrimp and seafood; Eat eggs; During the intervention period, try not to consume red meat (cattle, sheep, pork); Drink the right amount of water every day.

Intervention process

1. For 12 weeks, the intervention group used olive oil for cooking, caloric restriction, 1300Kcal for women and 1600Kcal for men. A trained professional dietitian evaluates the menu weekly to limit LA intake.
2.At baseline visits, trained physicians conducted face-to-face interviews using standard questionnaires to obtain information on demographic attributes, lifestyle factors, health status and drug use.
3. Study participants were asked not to change their physical activity and to continue their chronic medications unless modified by their personal physician.
4. To help improve adherence to allowable caloric intake, all participants received dietary counseling throughout the duration of the trial. Dietary counseling is conducted under the guidance of a trained nutritionist. Participants received a written diet information brochure with some suggestions and sample menus.
5. At the beginning of the intervention, each participant was trained. The patients self-measured their fasting weight every day and recorded their sleep, step count and dietary adaptation.
6. Participants received weekly online healthy eating promotion training to guide them on how to firmly specify their diet for a long time, ensure the quality of their diet, and avoid overeating when they eat freely, to assess participants' adherence to the program and help participants achieve their caloric goal of weight loss within 3 months.
7. The participants measured their neck circumference, waist circumference and hip circumference at the end of each week and gave feedback on their eating situation.
8. in week 4 and week 8, the subjects were followed up in the outpatient department for biochemical examination, in body test, face-to-face communication with doctors to evaluate the effect of weight loss.
9. At baseline and at week 12, gastric tissue specimens were collected for gastric injury assessment.
10. Compliance is assessed by dividing the number of meals served by the number of meals served.
11. Physical examination, biochemical and imaging examinations were performed on all participants at the 12th week.

Subject population

The inclusion criteria were:
(1) Men and premenopausal women, aged 18-65 years at the time of signing the informed consent
(2) Obese patients were diagnosed based on body mass index (BMI) 28-40kg/m2
2. Exclusion criteria are:

1） Patients who do not have or lose the ability to make autonomous judgments, do not understand the purpose of the study, or are unwilling to cooperate;
2) Heart, liver, kidney dysfunction and systemic diseases, such as various malignant tumors, systemic allergic diseases, autoimmune diseases;
3） Drug or alcohol dependence, intellectual disability, mental disorders;
4） Pregnant and lactating women;
5) Confirmed Helicobacter pylori infection (PPI, bismuth and antibiotics were stopped for at least 4 weeks, and any of the following tests were positive: non-invasive Hp test such as urea breath test, monoclonal stool Helicobacter pylori antigen test, serum soluble Helicobacter pylori antigen test; Invasive Hp test included rapid urease test and tissue section staining);
6） diagnosis of acute gastric mucosal lesions;
7） According to the Consensus Opinion on Chronic Gastritis in China in 2017, the patient was diagnosed with chronic gastritis according to the new Sydney system visual simulation scoring method and the pathological diagnostic criteria of chronic gastritis in China;
8） Patients with gastroscopy and histological pathology who meet the diagnostic criteria for gastric ulcer
9） the Diagnosis and Treatment of Gastric Cancer, combined with endoscopic, histological and imaging examinations, adenocarcinoma was diagnosed

Termination criteria

Discontinue use of the test product in any of the following cases:

The subject requests withdrawal of informed consent

Inclusion in trials that violated inclusion and/or exclusion criteria and/or randomization criteria

Safety concerns are at the discretion of the investigators

Subjects will not tolerate serious adverse events

Excessive alcohol consumption (>70 g/wk) and smoking habits

Diagnosed with cancer, kidney disease or diabetes, lactose intolerance

Pregnant or trying to get pregnant

To participate in a clinical trial of another approved or unapproved investigational drug product

Research process

Screening period

Potential participants were recruited through advertising

All subjects are required to complete relevant screening tests before enrollment and be screened according to the admission criteria

Sc/Rz (Screening period) related content is as follows:

sign informed consent;

Demographic characteristics: sex, age

Medical history: including past medical history and drug use history

Physical examination: height and weight

Baseline Period

V0 (baseline period) : Subjects who meet the inclusion criteria and do not have exclusion

criteria can be directly enrolled.

V0 (baseline period) related content is as follows:

Physical examination: height and weight

Laboratory tests: glucose metabolism, lipid metabolism, thyroid function, liver and kidney function

Laboratory examination: liver and kidney function, glucose metabolism, lipid metabolism

Imaging examination: hardness data ultrasound

24 hour Dietary Review Scale

Gastric tissue assessment of gastric injury

Participants were provided with a written dietary information brochure containing some of the recommendations and sample menus

Intervention Period

Record the daily diet, collect and analyze the diet according to the weekly diet recall table.

in week 4 and week 8, the subjects were followed up in the outpatient department for biochemical examination, in body examination, face-to-face communication and evaluation with doctors

Participants' fasting weight was measured every morning, and the number of meals, eating time, eating feelings, sleep and step count were recorded.

The adverse reactions and severity during the intervention were recorded.

Healthy diet publicity and education: Online healthy diet training will be conducted once a week to promote regular and quantitative eating, avoid overeating, and ensure diet quality.

Endpoint Period (Week 12)

measurement same as base line

Statistical methods

Sample size estimation

It has always been a very important link in clinical trials to select research objects through random sampling technology to determine what sample size is representative. Sample size estimation is related to the reliability, repeatability and efficiency of the research results, which reflects the balance between clinical significance and statistics. In any study, it is impractical to study the population as a whole. Sample size refers to the minimum number of test units (study individuals) determined by corresponding statistical methods under the premise of ensuring the reliability of research conclusions. In the actual research process, a group of participants is selected from the population. This group of participants can not only weigh all aspects of the trial to ensure the efficiency of the test, but also fully represent the overall population, and draw a conclusion for the overall population according to the final test results. Therefore, sample size estimation is one of the key links of clinical trials.

Statistically speaking, the main parameters that affect the sample size are: Class I error α, Class II error β or certainty of the experiment (1 to β), tolerance error γ, and population correlation information. Three factors are often considered in optimal parallel randomized controlled trials, namely, the size of the difference between the experimental intervention and the control intervention, the requirement for the accuracy of the trial, and the compliance of the test subjects. The sample size is calculated as follows:

$$n = \frac{2\sigma^2 \times f(\alpha, \beta)}{(\mu_1 - \mu_2)^2} \#$$

Where, n is the required sample size for each group, u1 is the basic measurement value, u2 is the measurement value to be reduced, σ is the standard deviation, f(α, β) is a constant, which can be obtained by looking up the table according to the difference between α and β. In test reports with explicit formulas, researchers often set Class I error α to 0.05 and Class II error β to 0.2. These parameters were originally set to avoid the problem of false positives and false negatives, and are not set in stone, but there are few reports to adjust the size of the parameters according to the actual situation.

Relevant parameters: In general, sample size estimates are closely related to the target disease. Combined with the target disease of the test, the size of class I error α and class II error β are appropriately adjusted to ensure that the β is as small as possible when the α is sufficiently small, so as to maintain the rejection of H0 and not make big mistakes. At the same time, it is necessary to clarify the relevant values of target diseases and target drugs. Such as: standard deviation σ, tolerance error γ, etc.

Compliance: For clinical trials, the shedding rate refers to the proportion of cases that failed to complete the trial according to the trial design after enrollment. For RCT, the shedding rate refers to the number of people who fail to complete the test. The total sample size required for the trial was 2n/(1-10%) with 10% dropout or loss of follow-up considered.

· Calculation process:

A randomized controlled study was conducted to investigate plant-based diet intervention in obesity. Patients were divided into treatment group and control group (no intervention group) by completely randomized method. The main outcome indicator was the weight change of obesity after intervention.

The sample size was determined according to the weight change in the previous pre-experiment, and the weight index was a continuous variable. The average weight of the control group was expected to be 80.31±15.63 (before intervention), and the weight of the intervention group was expected to be reduced by 3.67±2.42 after the plant-based diet. For bilateral test, α was 0.05, the ratio of sample size between the two groups was 1:1, and the certainty (test efficiency) was 1-β=90%. PASS15 software was used to calculate the required sample size.

Safety monitoring, reporting and medical treatment

Definition of Adverse Events (AE)

An adverse event is any adverse medical event that occurs after a patient or subject receives a drug that is not necessarily causally related to the treatment. Therefore, an adverse event can be any adverse physical sign (including abnormal laboratory results), symptom, or disease that has a time correlation with the use of the investigational drug, regardless of whether a causal relationship with the investigational drug is considered. Adverse events include Serious adverse events (SAE) and non-serious adverse events.

SAE Definition

SAE refers to the occurrence of medical events during clinical trials that require hospitalization or prolonged hospitalization, disability, affect work ability, endanger life or death, and lead to

congenital malformations. Includes the following medical events:
1) events leading to death;
2) life-threatening events (defined as subjects in immediate danger of death at the time of the event);
3) Events requiring hospitalization or prolonged hospitalization;
4) Events that can cause permanent or severe disability/disability/affect the ability to work;
5) Congenital abnormalities or birth defects;
Other medically important events (defined as events that endanger subjects or require intervention to prevent the occurrence of any of the above).

Adverse event recording, collection, reporting and handling

Collection and recording of AE

All aes that occur between the signing of the informed consent and the provision of the plant-based diet should be recorded in the CRF table.

The record of the AE should include: description of the AE and all associated symptoms, time of occurrence, severity, duration, correlation with the test food, action taken, and final outcome and outcome. The recording of AE must use medical terminology, and if the subject's signs and symptoms can be summarized by a common cause, the diagnosis should be recorded as far as possible. In addition to indicators related to disease progression, all clinical events and clinically significant laboratory adverse events can be treated with reference to the Common Adverse Event Evaluation Criteria (CTCAE) version 5.0. Adverse reactions to treatment will be recorded by the investigator.

Pregnancy

Women who are fertile should use an effective contraceptive method for the period specified in the study. Before recruiting fertile women into the study, researchers must inform women of childbearing age about the importance of avoiding pregnancy during study participation, as well as potential risk factors for unwanted pregnancies. Subjects must sign informed consent forms to show that the issues have been discussed and fully understood.

Pregnancy test requirements: Urine pregnancy test results must be negative for all fertile women during screening, baseline, and treatment. In addition, all fertile women should be advised to report a possible pregnancy (such as menopause or delayed menstruation) to the investigator immediately at any time during the trial.

Pregnancy reporting: If pregnancy or suspected pregnancy is found during trial administration, the study drug should be discontinued.

Criteria for judging AE severity

Researchers will evaluate severity according to the five-level criteria developed by the NCI CTCA version 5.0:

Grade 1, mild; Asymptomatic or with mild signs; For clinical or diagnostic observation only, without medical intervention;

Grade 2, moderate; Age-appropriate limited functions of daily living (such as cooking, shopping, making phone calls, etc.);

Level 3, serious or medically important but not immediately life-threatening; Resulting in hospitalization or prolonged hospitalization; Disability; Limited daily self-care activities (daily self-care activities refer to bathing, dressing, undressing, eating, toilet use, medication, etc., but not bedridden);

Level 4, life-threatening, requiring urgent medical treatment;
Level 5, AE-related death;

Other responsibilities of investigators during follow-up of serious adverse events

Serious adverse events should be examined and treated according to clinical judgment, including necessary clinical laboratory examination and physical examination. The results of any inspection or other updated SAE-related information obtained must be reported in a follow-up report within the same time frame and process as the initial report.

Test termination/suspension criteria

Sponsor reserves the right to terminate/suspend this experiment. Before terminating/suspending a clinical trial, the sponsor must notify the investigator, the Ethics Committee and the State Food and Drug Administration and state the reasons. After the early termination/suspension of the study, the restart of the study must be reviewed and approved by the Ethics Committee;

Provision to end clinical trials

The trial ends when all subjects meet the following conditions:
1) All subjects completed 13-month clinical study and follow-up;
2) or the death, loss of follow-up, or withdrawal of informed consent of all subjects.

14. Data management

Data Management

1) The researcher must ensure that the data is true, complete and accurate;
2) When any correction is made to the test record, the revised data can only be underlined, annotated by the margin, and the reason should be stated. The original record should not be erased or overwritten.
3) Complete laboratory inspection items.

Data recording and file saving

Subject data on the case report form should be recorded in subject code and subject can only be identified by subject code or their initials.

According to the requirements of the study protocol, the researcher shall fill in the collected data in a timely, complete, correct and clear Case Report Form (CRF), and send the signed CRF to the clinical research data manager in a timely manner. The corresponding database system is used for two-person and two-machine input, and then the database is compared twice. During the period, if any problem is found, the inspector is notified in time and the researcher is asked to answer. The exchange of questions and answers between them shall be in the form of a question table, which shall be kept for future reference. All steps involved in data management are documented so that data quality and test execution can be checked;

After all the CRFS have been entered and verified correctly, the data manager will write out the database inspection report, which includes the study completion status (including the list of dropped subjects), inclusion/exclusion criteria check, integrity check, logical consistency check, outlier data check, time window check, etc.

At the audit meeting, the main researcher, the representative of the bidding unit, the ombudsman, the data administrator and the statistician will make a decision on the informed consent signed by the subjects and the problems raised in the database inspection report, write the audit report, and the database will be locked at the same time.

After completing data entry and verification as required, CRF shall file and save the data in

numbered order, and fill in the retrieval catalog for reference. Electronic data files, including databases, inspection programs, analysis programs, analysis results, code books and explanatory files, etc., should be saved separately, and there are multiple backups saved on different disks or recording media, properly stored to prevent damage. All original files shall be kept for the period specified accordingly.