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Protocol of Evaluating the Effects of the Reducing Cardiometabolic Diseases Risk Dietary Pattern in the Chinese Population with Dyslipidemia: a Single-center, Open-label, Dietary Intervention Study

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has been approved by the Medical Ethics Committee of the Second Hospital of Tianjin Medical University (approval number: KY2023020). The results from the study will be disseminated through publications in a peer-reviewed journal.

Trail registration number: Chinese Clinical Trial Registry (ChiCTR2300072472), http://www.chictr.org.cn.

Strengths and limitations of this study

This study developed the RCMDR dietary pattern that conforms to the dietary characteristics of Chinese people and will evaluate the effect on reducing the risk of CMDs.

This study will combine dietary feeding and dietary guidance for intervention, which is beneficial for participants to learn and master the RCMDR dietary pattern better and maintain healthy dietary habits even after the end of the study.

The study is an open trial, so the bias cannot be ruled out. By blinding data analysis and indicator detection, researchers minimized the bias.

Introduction

Cardiometabolic diseases (CMDs) is a kind of syndrome with cardiovascular damage and abnormal metabolism, including a series of diseases, such as cardiovascular disease (CVD), diabetes, dyslipidemia, and metabolic syndrome,[1] which is the leading cause of mortality in China[2] and worldwide.[3] Dyslipidemia is not only a type of CMDs, but also a significant risk factor for other CMDs. In China, the prevalence of dyslipidemia in adults has reached 35%, and has remained at a high level in recent years.[4] Several studies have found a potential association between lipid metabolism and the risk of CMDs.[5-7] It was plausible that dietary interventions might affect the risk of CMDs by regulating some lipid molecules.[8 9]

Diet is one of the ordinary and modifiable factors of CMDs. According to the Global Burden of Diseases Study 2017, nearly 1/5 of total death globally was attributed to diseases such as CVD, metabolic diseases, and cancers caused by unhealthy diet.[10] In 2019, China ranked first globally in terms of the absolute number of diet-related CVD death and disability-adjusted life-years burden.[11] In the past few decades, the dietary structure of Chinese has undergone notable changes. In China, from 1982 to 2012, cereals, tubers, and vegetable consumption decreased; fruits, milk, eggs, and nuts consumption remained low; animal foods consumption, primarily pork, increased rapidly, from 52.6 g/day to 137.7 g/day; cooking oil and salt consumption remained high; and the percentage of energy from fat increased from 18.4% to 32.9%.[12] High sodium, low fruit, and low marine n-3 fatty acids were leading dietary risk factors for CMDs mortality in China.[13] Therefore, in the prevention and treatment of CMD, it is

of great value to study the association between dietary factors and CMD, and to develop dietary patterns appropriate for Chinese.

Dietary pattern analysis is essential for exploring the relationship between the intake of various food groups or multiple nutrients and health outcomes from a holistic perspective, which is closer to daily diets.[14 15] Some Hesperian healthy dietary patterns have been demonstrated to be cardiometabolic protective, such as the Dietary Approaches to Stop Hypertension (DASH) and the Mediterranean diet.[16-18] However, because Chinese food culture differs from Hesperian food culture, healthy dietary patterns cannot simply be copied. The Chinese heart-healthy (CHH) diet is the first diet with a cardiovascular protective effect that fits Chinese food culture, but its effect on metabolic diseases is unclear.[19] To date, a healthy dietary pattern has not been found that can comprehensively prevent CMDs in line with the dietary characteristics of Chinese.

Consequently, a dietary pattern that might improve CMD health based on Chinese food culture was developed—the Reducing Cardiometabolic Diseases Risk (RCMDR) dietary pattern. This single-center, randomized, open-label, parallel-controlled dietary intervention study was designed to assess the effect of the RCMDR dietary pattern on reducing CMDs risk in people with dyslipidemia. Moreover, the secondary aim of this study is to assess the effect of RCMDR dietary pattern on improving blood lipids, blood pressure, blood glucose, insulin resistance, body composition indices, and 10-year CVD risk.

Methods

Study design

A single-center, open-label, randomized, controlled dietary intervention study was designed. In Tianjin, 100 eligible participants with dyslipidemia will be recruited. Participants will be randomized and allocated into the RCMDR dietary pattern intervention group or the general health education control group, with 50 participants in each group. After a one-week run-in period, participants in the intervention group will receive free standard breakfasts and lunches and dietary guidance based on the RCMDR dietary pattern. Standard breakfasts and lunches will be prepared and cooked by cooks based on menus and recipes developed by a nutritionist, using the ingredients available in local markets. The interventions will last 12 weeks, during which time standard breakfasts and lunches will be provided 5 working days per week, and dietary advice will be provided 2 rest days per week. Participants in the control group will receive dietary guidance based on the "Dietary Guidelines for Chinese Residents (2022)" for 12 weeks. The schedule of follow-up, physical examinations and indices measurements will be the same for both groups. Figure 1 shows the flowchart of the study.

Participant recruitment

Participants will be recruited by researchers using posters. After an initial understanding of the study, potential participants will enroll voluntarily. Men and women aged 35-45 years with dyslipidemia are eligible for the study according to the results of their latest physical examination. Subsequently, participants will be

randomized into intervention and control groups. The details of inclusion and exclusion criteria are listed.

Inclusion criteria

1. Aged 35-45, permanent resident in Tianjin;

2. With borderline elevation of blood lipids and low or moderate risk of atherosclerotic cardiovascular disease (ASCVD), meeting one of the following conditions:

(1) 5.2mmol/L ≤ TC < 7.2mmol/L;

(2) 3.4mmol/L ≤ LDL-C < 4.9mmol/L;

(3) 1.7mmol/L ≤ TG < 2.3mmol/L;

(4) HDL-C < 1.0mmol/L;

3. Without lipid-lowering therapy (the above dyslipidemic population);

4. Agree to participate in the study and sign an informed consent form.

Exclusion criteria

1. With high-risk or extremely high-risk of ASCVD,[4] meeting one of the following conditions:

(1) ASCVD patients;

(2) Diabetes patients ≥ 40 years old;

(3) Chronic kidney disease stages 3-4;

(4) Patients with grade 2 or above hypertension, or those with grade 1 hypertension who have two or more risk factors (risk factors include Smoking; HDL-C < 1.0mmol/L; Age of male ≥ 45 years old);

2. With a tumor or mental diseases (such as depression, mania, anxiety, schizophrenia);

3. With abnormal thyroid, liver or kidney function;

4. With cardiovascular or cerebrovascular diseases;

5. With active epilepsy or severe auditory perception disorders, which are difficult to cooperate with the investigation;

6. The researchers consider the person won't be able to cooperate in completing this study.

Run-in period and randomization

After signing informed consent forms, participants will enter a 1-week run-in period. The purpose of setting a run-in period is to help participants familiarize themselves with the intervention scheme and to allow the researchers to ascertain participants' intake of daily food and estimated energy requirement (EER), as well as to identify and exclude participants who are failing to adhere to the study protocol. During the run-in, participants in both groups will be requested to keep dietary records of the variety and intake of all foods consumed during the day. At the end of the run-in period, participants will be reassessed for eligibility and adherence to the study protocol. Any participant who fails to complete dietary records for two or more days a week for any reason will be excluded from further participation in the study.

Participants will be formally enrolled in the study if they pass the run-in period and complete the baseline data collection. Participants will be randomized into intervention and control groups with a 1:1 ratio by a statistical analyst unaware of the study, using a random sequence generated by SPSS 24.0. The randomized allocation concealment will be ensured using opaque sealed envelopes. The researchers

responsible for recruitment won't know the allocation sequence in advance.

Blinding

In the study, we will only provide the feeding intervention to participants in the intervention group, so it is not possible to implement blinding to the participants. Meanwhile, it is also infeasible to blind the cooks, nutritionists, and researchers in charge of preparing and measuring the standard meals. In addition, the researchers responsible for outcome assessment and data analysis will be blinded to the group allocation, and the data will be input into the computer by people outside the research team. On the other side, it might be easier to recruit participants in an open-label study than in a blinded study.

Intervention

Depending on the EER of the participants in the intervention group, the standard meals will be divided into three energy classes: 1400-1700 kcal, 1700-2000 kcal, and over 2000 kcal. Standard breakfasts and lunches will be prepared in the kitchen, then delivered to the fixed canteen, and distributed to each participant in the intervention group. Participants will be required to take photos of the leftovers from each standard meal and record the intake of all food consumed on weekdays (excluding standard meals) and rest days. Researchers will calculate the intake of daily nutrients based on these data and provide personalized dietary guidance to the participants. The frequency of dietary guidance will be once a week in the first month, once every two weeks in the second month, and once in the third month.

The participants in the control group won't be provided with standard meals. Only receive dietary guidance based on the "Dietary Guidelines for Chinese Residents (2022)". Participants in the control group will be required to choose any one working day and rest day every week for dietary records. The frequency of dietary guidance will be the same as the intervention group.

Recipes of standard meals will be developed by researchers, nutritionists, and cooks based on the nutritional composition of RCMDR dietary pattern. The nutrients composition of the RCMDR dietary pattern and the methods to reach it are shown in Table 1.

The RCMDR dietary pattern was preliminarily developed based on previous studies' evidence, including food types, intake ranges, and explanations. Then, the Delphi method was used to optimize and modify the dietary pattern. In the end, a total of 23 experts completed two rounds of consultation, and the results showed that the active coefficients of the two rounds of expert consultation were 92.00% and 100%, the expert authority index of the two rounds of expert consultation were 0.751 and 0757; Kendall's W coefficient of the two rounds of expert consultation were 0.302 and 0.504, the consensus was obtained. The final RCMDR dietary pattern was established, including 9 domains, 20 sub-domains, and 6 elements. The detailed content of the RCMDR dietary pattern is shown in Table 2.

Specifically, the RCMDR dietary pattern includes a set of recipes of 2-week non-repeating and interchangeable standard breakfasts and lunches, as well as suggestions for dinners. These recipes were developed based on the nutrient composition and the

food group intake of the RCMDR dietary pattern. According to these recipes, the participants and their families can quickly learn and use this dietary pattern. A sample 1-day recipe is shown in Table 3.

Measurements and data collection

Formal baseline data collection will be conducted on the last 2 days of the run-in period, including a questionnaire interview on demography (age, gender, education, marital status), lifestyle (smoking, drinking, physical activity and sleep status), dietary habit (frequency questionnaire and 24-hour food record), history of diseases (hypertension and diabetes), family history of disease (hypertension, diabetes, hyperlipidemia, coronary heart disease, stroke and cancer), medication use (antihypertensive drugs and antidiabetic drugs), dietary supplement use (fish oil, vitamin C, vitamin B, calcium tablets, folic acid, multivitamins, iron, others), physical examinations (height, weight, blood pressure, waist-hip ratio, body composition indices), fasting blood test (fasting blood glucose (FBG), fasting insulin (FINS), glycated hemoglobin (HbA1c), fasting C-peptide, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), folic acid, homocysteine (Hcy), liver function (alanine aminotransferase (ALT), aspartate transaminase (AST)) and kidney function (Serum uric acid, urea, creatinine)).

Methods of physical measurements

Blood pressure measurement: the Omron J710 upper arm electronic blood pressure monitor measures systolic blood pressure (SBP) and diastolic blood pressure (DBP).

The third measurement will be required if the difference between SBP and DBP exceeds 10 mmHg.

Body composition indices measurement: the body composition analyzer and bioelectrical impedance method were used to measure body composition indices, such as height, weight, waist-to-hip ratio, body fat mass, body muscle mass and visceral fat mass, subcutaneous fat mass, basal energy expenditure, EER, etc.

Blood sample collection and tests

Blood samples will be collected at baseline and the end of the study. Before collecting blood samples, participants will be required to fast for at least 10 hours overnight. Fasting venous blood from participants will be collected by qualified nurses. The blood samples will be refrigerated and transported to the central laboratory of the School of Public Health, Tianjin Medical University. Analysis of FBG (using the glucose oxidase method), HbA1c (using the immunosuppressive turbidimetry), TG and TC (using the enzyme endpoint method), HDL-C (using the polyethylene sulfate precipitation method), LDL-C (using the polyethylene sulfate precipitation method), ALT, AST, serum uric acid, urea and creatinine (using the enzymatic method) will be carried out on a Roche Cobas 8000 automatic biochemistry analyzer. Fasting C-peptide, FINS (using the enzyme-linked immunosorbent assay (ELISA)) was measured using ELISA kit. Serum folic acid (using the chemiluminescence immunoassay method) was measured using a chemiluminescence immunoassay analyzer. Serum Hcy (using the enzyme cycling method) was measured using an automated chemical analyzer.

Dietary intake of foods and nutrients

Firstly, all raw materials will be weighed before cooking, including the amount of oil and salt used. After cooking, each dish will be weighed according to the energy level of the standard meal. Researchers will calculate the average daily energy and dietary nutrients each participant takes using the China Food Composition (6th edition) based on the photos of leftovers and dietary records submitted by the participants.[20]

Follow up schedules

At the mid-term of the study, the uninformed researchers will carry out the same questionnaire interview. At the end of the study, the uninformed researchers will conduct the same questionnaire interview and physical examination of the participants using the same method as the baseline. The frequency of dietary guidance for participants in both groups will be the same: once a week in the first month, once every two weeks in the second month, and once in the third month. Dietary data of participants in the intervention group will be collected daily, and in the control group will be collected twice a week. The schedule of measurements and visits of the study has been summarized in table 4.

Outcomes

The primary outcome is the change in cardiometabolic risk (CM-risk) score from baseline to the end of the study. The secondary outcomes include a change in blood lipid (LDL-C, TC, TG, HDL-C), blood pressure, FBG, insulin resistance measured by Homeostasis Model Assessment, body composition indices, and 10-year risk of CVD from the Prediction for Atherosclerotic Cardiovascular Disease Risk in China (China-PAR).[21]

Sample size

Although the CM-risk score is the primary outcome, the methods of calculating the cardiometabolic score in different studies are inconsistent. Participants in the study are dyslipidemia, and according to the 2023 Chinese guideline for lipid management, LDL-C is the primary target for lipid intervention.[4] Therefore, the sample size in the study was calculated using the change of LDL-C from baseline to the end of the study. According to the previous study that the calorie-restricted Mediterranean-style diet successfully reduced LDL-C by 0.23 mmol/L for hypercholesterolemic patients in 4 weeks, we conservatively assumed that the RCMDR dietary pattern will reduce LDL-C by 0.3 mmol/L in 12 weeks, and the standard deviation of LDL-C change is 0.5 mmol/L in the control group.[22] To have 80% power with a type I error rate of 5% to detect the assumed effect size, we would need 44 participants in each group. Assuming that 10% of study participants will be lost by the end of the study, 100 participants will be recruited.

Data management

Data will be collected in an electronic database. Participants will only be identified by a unique ID. Hard copies of data sheets containing participants' files and other data (including questionnaires, test results, records of informed consent, and other documents related to the conduct of the study) will be kept securely in a locked filing cabinet in a locked office, accessible only to key research team members. Blood samples will be kept in a locked freezer. All data and Blood samples will be stored in Tianjin Medical University.

Statistical analysis

Statistical analyses will be performed using SPSS 24.0 software. The analyses of outcomes will follow the intention-to-treat principle. Continuous variables conforming to normal distribution will be expressed as mean ± standard deviation, and non-normally distributed continuous variables will be expressed as median with limits of the interquartile range (IQR: 25th and 75th percentiles). Categorical variables will be shown as frequencies and proportions (percentages). The differences in baseline variables between groups will be evaluated by using a t-test, Wilcoxon rank test, or χ^2 test. Linear regression will be used to estimate the absolute differences between the two groups regarding primary and secondary outcomes and reported as the least squares mean after adjusting the center. Sensitivity analyses will be conducted to repeat the primary analyses with imputed missing values. We will use multiple imputations to impute missing values of outcomes. A two-sided *P* value <0.05 will be considered statistically significant.

Patient and public involvement

This research study didn't involve patients or the general public in its design, conduct, reporting, or dissemination.

Quality control

The reliability and validity of the questionnaire are high. According to the purpose of this study, based on previous studies, multiple collective discussions were conducted to unify the evaluation standards of the questionnaire, ensuring scientificity, consistency, and comparability. All researchers participating in this study must receive

uniform training and be tested after training, including standard operating procedures for participant data collection and methods for collecting and preserving biological samples to ensure accurate and complete information and to avoid measurement bias. All biological samples will be tested in the central laboratory of Tianjin Medical University. The researchers responsible for detecting relevant indicators will be blinded to the randomization grouping of participants.

Ethical and dissemination

The study complies with the Measures for Ethical Review of Life Sciences and Medical Research Involving Human Beings and the Declaration of Helsinki. Signed informed consent will be obtained from all participants. The study has been approved by the Medical Ethics Committee of the Second Hospital of Tianjin Medical University (approval number: KY2023020) and registered in the Chinese Clinical Trial Registry (ChiCTR2300072472). The results from the study will be disseminated through publications in a peer-reviewed journal.

Current status

The first participant was enrolled on 14 June 2023.

Discussion

This study is a randomized controlled dietary intervention trial to evaluate the effect of the RCMDR dietary pattern on reducing the risk of CMDs in people with dyslipidemia. Taking the evidence from the previous studies on the association between dietary factors and CMDs, we initially developed the RCMDR dietary pattern. Using

the Delphi method, the dietary pattern was optimized and modified based on the consultation and expert advice results, and the content of the dietary pattern was finalized.

The DASH diet has been proven to reduce the risk of CVD and improve cardiometabolic risk factors other than blood pressure, including LDL-C, HbA1c, FINS, and body weight.[23] The CHH diet, the first heart-healthy diet based on Chinese food culture, is similar in nutritional composition to the DASH diet, has an anti-hypertensive effect, and is predicted to lead to a 20% reduction in significant CVDs, 28% in heart failure, and 13% in all-cause mortality.[19] Moreover, the 2023 Chinese guideline for lipid management also recommends the CHH diet.[4] The Mediterranean diet has also been proven to have cardiometabolic protective effects, but its energy proportions of macronutrients are significantly different from the former two, with a relatively higher consumption of fat, even up to 40% of total energy intake.[16 24] The nutrient composition of the RCMDR dietary pattern was more similar to that of the CHH diet by calculating, including energy proportions of macronutrients and the amount of sodium, potassium, and fiber. Besides, considering the participants have dyslipidemia, we added a restriction on the intake of cholesterol.

Compared to the CHH diet and "Dietary Guidelines for Chinese Residents (2022)", the RCMDR dietary pattern has some characteristics concerning the intake of food groups. Firstly, the intake of whole grains, vegetables, soy and its products, and nuts increased. Whole grains are rich in nutrients and phytochemicals, including dietary fiber, resistant starch, oligosaccharides, UFAs, minerals such as magnesium, zinc,

selenium, and potassium, B vitamins, antioxidants such as phenolic compounds, and phytosterols.[25 26] Whole grain intake is negatively associated with the risk of CMDs[27-30] and has the effect of controlling body weight, improving blood glucose, lipid profile, and other CM-risk factors.[31-33] To emphasize the importance of whole grains, we increased the intake of whole grains to 90-150 g/day, approximately accounting for 1/2 of cereals.

Vegetables and fruits are packed with dietary fiber, folate, potassium, magnesium, antioxidants, and various bioactive phytochemicals.[34] The protective effects of vegetables and fruits on CMDs were confirmed by many studies.[35-37] A meta-analysis showed that consuming up to 400g of vegetables per day reduced the risk of coronary heart disease by 12%, and consuming >400 g/day had a more substantial protective effect.[38] Hence, the intake of vegetables was increased to 350-550 g/day. Besides, some studies showed that green leafy and cruciferous vegetables might have better cardiometabolic protection.[39-41] Vegetables were divided into dark green leafy and cruciferous vegetables, other dark vegetables, and other vegetables.

Some large-scale prospective studies of Chinese population found that daily consumption of fresh fruit was associated with lower risks of CVD, diabetes and vascular complications compared to never or rarely consuming fresh fruit.[42 43] And increasing fruit intake appeared to be more effective than vegetables in preventing CVD and reducing the risk of all-cause mortality among Chinese.[44] A meta-analysis indicated that consuming 200-300g of fruit per day could decrease the risk of type 2 diabetes (T2D) by 10%, more than 300 g had no additional benefit.[45] Thus, the intake of fruits

is 200-350 g/day, which is consistent with "Dietary Guidelines for Chinese Residents (2022)".

Soybeans are rich in high-quality protein, UFAs, soybean isoflavones, and other phytochemicals, which might affect blood pressure, control blood glucose, lower l[38]ipids, anti-inflammatory, and improve body composition.[46] Based on the results of previous studies[47-49] and the target for the total energy percentage of protein in the RCMDR dietary pattern, we have increased the intake of soybeans to 30-40 g/day.

Nuts are a good source of UFAs, vitamin E, vitamin B, minerals, plant protein, and dietary fiber. Several studies have shown that the optimal intake of nuts reduces the risk of CVD, stroke, and coronary heart disease by 15-20 g/day.[50 51] Referring to this intake, the intake of nuts increased to 10-20 g/day.

Secondly, we reduced the intake of animal-based foods and divided meat intake into fish, poultry, and livestock. The intake of livestock and poultry was reduced, significantly limiting the intake of red meat and banning processed meat. Studies confirmed that red meat and processed meat increased the risk of CVD and T2D.[52 53] A study involving 195 countries assessing the global burden of disease in 2017 proposed dietary recommendations to minimize the risk of disease death, with recommendations for 18-27 g/day of red meat,[10] and the Planetary Health Diet recommends 14 g/day,[54] so this study the intake of red meat reduced to 100-125 g/week. However, the frequency of fish consumed per week was increased to 3-4 times per week, emphasizing the importance of consuming fish rich in n-3 PUFAs, consistent with the Mediterranean diet.

Meanwhile, considering the high content of lipids such as cholesterol, TGs, and phospholipids in egg yolk,[55] the intake of eggs was adjusted to 3-5 per week, which is consistent with the findings of the China-PAR.[56 57]

Thirdly, the RCMDR dietary pattern emphasized alternating consumption of various vegetable oils rich in UFAs and avoiding animal oils. In the past 30 years, the proportion of MUFAs in Chinese dietary fat consumption has decreased noticeably, and the ratio of n-6 to n-3 PUFAs has increased significantly.[58] Peanut oil, soy oil, and canola oil were the 3 main cooking oils consumed in China, while the recommended olive oil rich in oleic acid for the Mediterranean diet was rarely consumed.[59] Oleic acid is almost the only source of MUFAs in the Chinese diet.[58] Therefore, to achieve the target energy proportion of MUFAs, our standard breakfast and lunch will be cooked with olive oil. Moreover, compared to PUFAs, MUFAs are less prone to oxidation and react with reactive oxygen species, so olive oil is relatively stable and can be used for steaming, stewing, or sauteing.[60] Participants will be encouraged to use vegetable oils rich in n-3 PUFAs for low-temperature cooking at home to increase the proportion of n-3 PUFAs, such as flaxseed oil, and increase the intake of fish and nuts.[60] The above measures help the participants to alternately consume different types of vegetable oils and improve adherence and acceptability.

Considering the generalizability and affordability of the RCMDR dietary pattern, the daily cost was approximately RMB 50-60 per person per day, using common local food ingredients and cooking methods. In addition, the study will adopt a combination of feeding intervention and dietary guidance, making it easier for the participants to

learn and master the dietary pattern. Even after the experiment, the RCMDR dietary pattern is easy to grasp and stick to.

In summary, the study is a randomized controlled dietary intervention trial to evaluate the effect of the RCMDR dietary pattern on cardiometabolic health in people with dyslipidemia. The study not only comprehensively considered the effect of diet on CMDs, but also considered the generalizability and affordability of the RCMDR dietary pattern among the population. The study's findings will significantly prevent CMDs in China and improve Chinese residents' nutritional status and health levels.

Authors' contributors

G. Huang, W. Li and F. Ma conceived and designed the study. Q. Wu, L. Zhang, C. Cheng, X. Chen, L. Huang, and T. Li will perform the study, collect and analyse all experimental data. Q. Wu provided the first version of the manuscript. S. Bian, Z. Li, H. Liu, J. Yan, Y. Du, Y. Chen, M. Zhang, L. Cao, W. Li, F. Ma and G. Huang provided critical comments on the original manuscript. W. Li and G. Huang revised and finalised the manuscript. All authors read and approved the final version of the manuscript.

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Disclaimer

The funding has no role in the design of this trial and will not have any role during its execution, analyses, interpretation of the data or decision to submit results.

Competing interests statement

None declared.

Patient and public involvement

This research study didn't involve patients or the general public in its design, conduct, reporting, or dissemination.

Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer reviewed.

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Figure Legends

Figure 1. The flowchart of the study. RCMDR, reducing cardiometabolic diseases risk; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol.

Table 1. The nutrients composition of the RCMDR dietary pattern, and methods to reach the goals of nutrients

Nutrients	Intake	Methods to reach the goals
Energy (kcal)	1450-2350	Average intake was evaluated during the run-in period.
Energy distribution of three meals		
Breakfast: Lunch: Dinner	3: 4: 3	
Energy distribution of macronutrients		
Carbohydrates (kcal%)	56-58%	Increasing the use of whole grains and limiting added sugars (monosaccharide disaccharide).
Protein (kcal%)	17-19%	Increasing protein intake in legumes, dairy products, fish, and lean meat.
Fat (kcal%)	24-26%	Reducing the use of cooking oil by changing cooking methods, such as using steam instead of frying, and using low-fat or skim dairy products, choosing skinned lean meat.
Saturated	<7%	Banning animal cooking oils.
Monounsaturated	12%	Increasing the use of vegetable oil rich in monounsaturated fatty acid (MUFA).
Polyunsaturated	6-8%	Increasing the use of nuts and n-3 polyunsaturated fatty acid (PUFA)-rich fish.
Cholesterol (mg/day)	<300	Reducing the use of animal-based foods.
Fiber (g/day)	30	Increasing the use of foods high in dietary fiber, such as whole grains, legumes, vegetables, and fruits.
Sodium (mg/day)	3000	Reducing the use of salt and sodium seasoning during cooking.
Potassium (mg/day)	3700	Increasing the use of food with high potassium content.
Magnesium (mg/day)	500	Increasing the use of food with high magnesium content.
Calcium (mg/day)	1000	Increasing the use of dairy products, soy products, fish, nuts, and dark vegetables.
Folate (μg/day)	400-800	Increasing the use of plant-based foods that contain high amounts of folate.

Table 2. The RCMDR dietary pattern

Food groups	Edible portion intake	Explanation
Cereals and tubers		
Cereals	200-300 g/day	
Whole grains	90-150 g/day	
Legumes*	10-30 g/day	
Tubers	50-100 g/day	
Vegetables and Fruits		
Vegetables	350-550 g/day	
Dark green leafy and cruciferous species vegetables	200-250 g/day	
Other dark vegetables	100-200 g/day	
Other vegetables	50-100 g/day	
Fruits	200-350 g/day	Complete fresh fruits
Foods of animal origin	90-150 g/day	Banning processed meat products
Fish	300-500 g/week	Eating 3-4 times a week and choosing fish rich in n-3 PUFAs.
Poultry	100-175 g/week	Skinned lean meat, fresh meat
livestock meat	100-125 g/week	Skinned lean meat, fresh meat
Eggs	3-5 per week	
Dairy products	300-500 g/day	Low-fat or skimmed milk, yogurt or other fermented dairy products
Soybeans and nuts		
Soybeans	30-40 g/day	The intake of soy products is calculated based on the same protein content. Individuals with hyperuricemia or gout are not recommended to increase their intake of soybeans or soy products.
Nuts	10-20 g/day	
Oil and salt		
Cooking Oil	15-25 g/day	Alternating consumption of various vegetable oils rich in UFA and avoiding animal oils.
Salt	<5 g/day	Low-sodium salt is recommended for those without contraindications.

Added sugar		
Added sugar (monosaccharide disaccharide	<150 g/week	Limiting the consumption of non-sugar sweeteners (NSS): All non-nutritional sweeteners that do not belong to the sugar category are low-calorie or calorie-free substitutes for free sugars. Common NSS include acesulfame, aspartame, edvante, cyclamate, neotame, saccharin, sucralose, steviol glycosides, and steviol derivatives.
Sugar-sweetened beverages	Eliminating or limiting the consumption of sugar-sweetened beverages.	Drinking sugary beverages should be calculated in the weekly intake of added sugar.
Other sugary foods (cakes, cookies, cold drinks and snacks, candy, etc)	Eliminating or limiting the consumption of other sugary foods.	Eating other sugary foods should be calculated in the weekly intake of added sugar.
Water and alcohol		
Water or tea	1700ml/day for males, 1500ml/day for females	Summer or excessive sweating can be increased as needed.
Alcohol	<15 g/day	Eliminating or limiting the consumption of alcohol.

*Legumes don't include soybeans.

Table 3. An example of a 1-day recipe for a diet

	RCMDR dietary pattern
Breakfast	Sweet potato-filled whole wheat steamed bun
	Millet congee
	Boiled spinach with olive oil
	Boiled egg
	Cherry tomatoes
	Walnuts and hazelnuts
	Skim milk
Lunch	Steamed rice with quinoa and chickpeas
	Stir-fried purple cabbage with colored pepper
	Stir-fried water spinach
	Stewed tofu with tomato
	Steamed fish
Snack	Sugar-free yogurt
	Apple
Dinner (suggestions)	Whole-grain cereal product
	Boiled or stir-fried vegetables
	Boiled or stir-fried soy products

Table 4. The schedule of measurements and visits of the study

	Follow-up (weeks)									
	Screening	Run-in*	1	2	3	4	6	8	10	12
Signed informed consent	√									
Eligibility confirmation	√	√								
Questionnaire interview	√	√					√			√
Dietary record#		√	√	√	√	√	√	√	√	√
Dietary guidance			√	√	√	√	√	√	√	√
physical examination		√								√
Fasting blood test		√								√
Blood sample		√								√
Reasons for withdrawal		√	√	√	√	√	√	√	√	√

*Baseline data are collected on the last 2 days of the run-in period.

#Dietary data of participants in the intervention group will be collected daily.

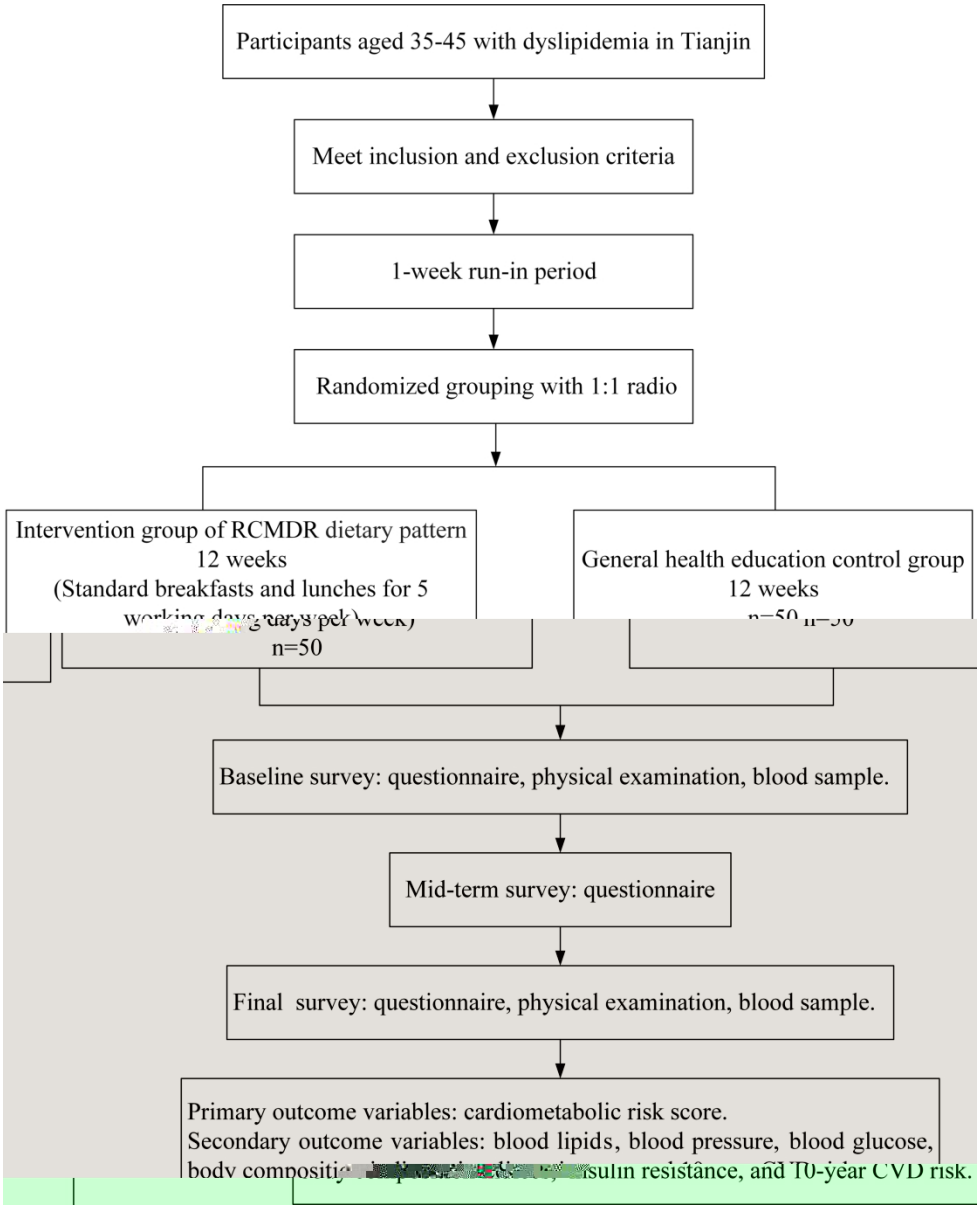

Figure 1. The flowchart of the study. RCMDR, reducing cardiometabolic diseases risk; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol.

224x276mm (300 x 300 DPI)

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3-4
	2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	3	Date and version identifier	3-4
Funding	4	Sources and types of financial, material, and other support	2 and 22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2 and 22
	5b	Name and contact information for the trial sponsor	2 and 22
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	22-23
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6

Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-11
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	14
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 4
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	15
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	7-8
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	9-10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	7 and 9
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12-14 and 16-17
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	15
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	16
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	NA
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	17
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	17
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	15
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	23
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	NA
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	NA

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	12-13

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

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Primary Subject Heading:	Public health

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Protocol of Evaluating the Effects of the Reducing Cardiometabolic Diseases Risk Dietary Pattern in the Chinese Population with Dyslipidemia: a Single-center, Open-label, Dietary Intervention Study

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Keywords: cardiometabolic diseases; dietary pattern; randomized controlled trial; protocol

Abstract

Introduction: Cardiometabolic disease (CMD) is the leading cause of mortality in China. A healthy diet plays an essential role in the occurrence and development of CMD. Although the Chinese heart-healthy (CHH) diet is the first diet with cardiovascular benefits, a healthy dietary pattern that fits Chinese food culture can effectively reduce the risk of CMD has not been found.

Methods/design: The study is a single-center, open-label, randomized controlled trial aimed at evaluating the effect of the Reducing Cardiometabolic Diseases Risk (RCMDR) dietary pattern in reducing the risk of CMDs in people with dyslipidemia and providing a reference basis for constructing a dietary pattern suitable for the prevention of CMDs in the Chinese population. Participants are men and women aged 35 to 45 with dyslipidemia in Tianjin. The target sample size is 100. After the run-in period, the participants will be randomized to the RCMDR dietary pattern intervention group or the general health education control group with a 1:1 ratio. The intervention phases will last 12 weeks, with a dietary intervention of 5 working days per week for participants in the intervention group. The primary outcome variable is the cardiometabolic risk score. The secondary outcome variables are blood lipid, blood pressure, blood glucose, body composition indices, insulin resistance, and 10-year risk of cardiovascular diseases.

Ethics and dissemination: The study complies with the Measures for Ethical Review of Life Sciences and Medical Research Involving Human Beings and the Declaration of Helsinki. Signed informed consent will be obtained from all participants. The study

has been approved by the Medical Ethics Committee of the Second Hospital of Tianjin Medical University (approval number: KY2023020). The results from the study will be disseminated through publications in a peer-reviewed journal.

Trail registration number: Chinese Clinical Trial Registry (ChiCTR2300072472), http://www.chictr.org.cn.

Strengths and limitations of this study

The RCMDR dietary pattern is designed based on the dietary habits of Chinese people, and following it can help reduce the risk of cardiometabolic diseases (CMDs).

The pattern considers dietary feeding and guidance, which can help participants learn and maintain healthy eating habits even after the study ends.

The blinding data analysis and indicator detection techniques used during the study helped minimize any bias that could have affected the results of the open trial.

Introduction

Cardiometabolic diseases (CMDs) is a kind of syndrome with cardiovascular damage and abnormal metabolism, including a series of diseases, such as cardiovascular disease (CVD), diabetes, dyslipidemia, and metabolic syndrome [1], which is the leading cause of mortality in China [2] and worldwide [3]. Dyslipidemia is not only a type of CMDs, but also a significant risk factor for other CMDs. In China, the prevalence of dyslipidemia in adults has reached 35%, and has remained at a high level in recent years [4]. Several studies have found a potential association between lipid metabolism and the risk of CMDs [5-7]. It was plausible that dietary interventions might affect the risk of CMDs by regulating some lipid molecules [8,9].

Diet is one of the ordinary and modifiable factors of CMDs. According to the Global Burden of Diseases Study 2017, nearly 1/5 of total death globally was attributed to diseases such as CVD, metabolic diseases, and cancers caused by unhealthy diet [10]. In 2019, China ranked first globally in terms of the absolute number of diet-related CVD death and disability-adjusted life-years burden [11]. In the past few decades, the dietary structure of Chinese has undergone notable changes. In China, from 1982 to 2012, cereals, tubers, and vegetable consumption decreased; fruits, milk, eggs, and nuts consumption remained low; animal foods consumption, primarily pork, increased rapidly, from 52.6 g/day to 137.7 g/day; cooking oil and salt consumption remained high; and the percentage of energy from fat increased from 18.4% to 32.9% [12]. High sodium, low fruit, and low marine n-3 fatty acids were leading dietary risk factors for CMDs mortality in China [13]. Therefore, in the prevention and treatment of CMD, it

is of great value to study the association between dietary factors and CMD, and to develop dietary patterns appropriate for Chinese.

Dietary pattern analysis is essential for exploring the relationship between the intake of various food groups or multiple nutrients and health outcomes from a holistic perspective, which is closer to daily diets [14,15]. Some Hesperian healthy dietary patterns have been demonstrated to be cardiometabolic protective, such as the Dietary Approaches to Stop Hypertension (DASH) and the Mediterranean diet [16-18]. However, because Chinese food culture differs from Hesperian food culture, healthy dietary patterns cannot simply be copied. The Chinese heart-healthy (CHH) diet is the first diet with a cardiovascular protective effect that fits Chinese food culture, but its effect on metabolic diseases is unclear [19]. To date, a healthy dietary pattern has not been found that can comprehensively prevent CMDs in line with the dietary characteristics of Chinese.

Consequently, a dietary pattern that might improve CMD health based on Chinese food culture was developed—the Reducing Cardiometabolic Diseases Risk (RCMDR) dietary pattern. This single-center, randomized, open-label, parallel-controlled dietary intervention study was designed to assess the effect of the RCMDR dietary pattern on reducing CMDs risk in people with dyslipidemia. Moreover, the secondary aim of this study is to assess the effect of RCMDR dietary pattern on improving blood lipids, blood pressure, blood glucose, insulin resistance, body composition indices, and 10-year CVD risk.

Methods

Study design

A single-center, open-label, randomized, controlled dietary intervention study was designed. In Tianjin, 100 eligible participants aged 35-45 with dyslipidemia will be recruited. Enhanced management of dyslipidaemia in this age group may better reduce the risk of incident CVD and diabetes and contribute to the primary prevention of CMDs [20-22]. Participants will be randomized and allocated into the RCMDR dietary pattern intervention group or the general health education control group, with 50 participants in each group. After a one-week run-in period, participants in the intervention group will receive free standard breakfasts and lunches and dietary guidance based on the RCMDR dietary pattern. Standard breakfasts and lunches will be prepared and cooked by cooks based on menus and recipes developed by a nutritionist, using the ingredients available in local markets. According to the 2023 Chinese guideline for lipid management, the lipid level should be reviewed in the first 3–6 months for those receiving non-pharmacological treatment such as diet control [4]. And consistent with previous studies [23,24], the interventions will last 12 weeks. During intervention phases, standard breakfasts and lunches will be provided 5 working days per week, and dietary advice will be provided 2 rest days per week. Participants in the control group will receive dietary guidance based on the “Dietary Guidelines for Chinese Residents (2022)” for 12 weeks. The schedule of follow-up, physical examinations and indices measurements will be the same for both groups. Figure 1 shows the flowchart of the study.

Participant recruitment

Participants will be recruited by researchers using posters. After an initial understanding of the study, potential participants will enroll voluntarily. Men and women aged 35-45 years with dyslipidemia are eligible for the study according to the results of their latest physical examination. Subsequently, participants will be randomized into intervention and control groups. The details of inclusion and exclusion criteria are listed.

Inclusion criteria

1. Aged 35-45, permanent resident in Tianjin;

2. With borderline elevation of blood lipids and low or moderate risk of atherosclerotic cardiovascular disease (ASCVD), meeting one of the following conditions:

(1) 5.2mmol/L ≤ TC < 7.2mmol/L;

(2) 3.4mmol/L ≤ LDL-C < 4.9mmol/L;

(3) 1.7mmol/L ≤ TG < 2.3mmol/L;

(4) HDL-C < 1.0mmol/L;

3. Without lipid-lowering therapy (the above dyslipidemic population);

4. Agree to participate in the study and sign an informed consent form.

Exclusion criteria

1. With high-risk or extremely high-risk of ASCVD [4], meeting one of the following conditions:

(1) ASCVD patients;

(2) Diabetes patients ≥ 40 years old;

(3) Chronic kidney disease stages 3-4;

(4) Patients with grade 2 or above hypertension, or those with grade 1 hypertension who have two or more risk factors (risk factors include Smoking; HDL-C < 1.0mmol/L; Age of male ≥ 45 years old);

Participants will be formally enrolled in the study if they pass the run-in period and complete the baseline data collection. Participants will be randomized into intervention and control groups with a 1:1 ratio by a statistical analyst unaware of the study, using a random sequence generated by SPSS 24.0. The randomized allocation concealment will be ensured using opaque sealed envelopes. The researchers responsible for recruitment won't know the allocation sequence in advance.

Blinding

In the study, we will only provide the feeding intervention to participants in the intervention group, so it is not possible to implement blinding to the participants. Meanwhile, it is also infeasible to blind the cooks, nutritionists, and researchers in charge of preparing and measuring the standard meals. In addition, the researchers responsible for outcome assessment and data analysis will be blinded to the group allocation, and the data will be input into the computer by people outside the research team. On the other side, it might be easier to recruit participants in an open-label study than in a blinded study.

Intervention

Depending on the EER of the participants in the intervention group, the standard meals will be divided into three energy classes: 1400-1700 kcal, 1700-2000 kcal, and over 2000 kcal. Standard breakfasts and lunches will be prepared in the kitchen, then delivered to the fixed canteen, and distributed to each participant in the intervention group. Participants will be required to take photos of the leftovers from each standard meal and record the intake of all food consumed on weekdays (excluding standard

meals) and rest days. Researchers will calculate the intake of daily nutrients based on these data and provide personalized dietary guidance to the participants. The frequency of dietary guidance will be once a week in the first month, once every two weeks in the second month, and once in the third month.

The participants in the control group won't be provided with standard meals. Only receive dietary guidance based on the "Dietary Guidelines for Chinese Residents (2022)". Participants in the control group will be required to choose any one working day and rest day every week for dietary records. The frequency of dietary guidance will be the same as the intervention group.

Recipes of standard meals will be developed by researchers, nutritionists, and cooks based on the nutritional composition of RCMDR dietary pattern. The nutrients composition of the RCMDR dietary pattern and the methods to reach it are shown in Table 1. Compared to other dietary patterns with cardiometabolic benefits, such as the DASH diet and the Mediterranean diet [25,26], the nutrient composition of the RCMDR dietary pattern was more similar to that of the CHH diet, including energy proportions of macronutrients and the amount of sodium, potassium, and fiber. Besides, considering the participants have dyslipidemia, we added a restriction on the intake of cholesterol.

The RCMDR dietary pattern was preliminarily developed based on previous studies' evidence, including food types, intake ranges, and explanations. Then, the Delphi method was used to optimize and modify the dietary pattern. In the end, a total of 23 experts completed two rounds of consultation, and the results showed that the active coefficients of the two rounds of expert consultation were 92.00% and 100%, the

to use vegetable oils rich in n-3 PUFAs for low-temperature cooking at home, such as flaxseed oil [32].

Specifically, the RCMDR dietary pattern includes a set of recipes of 2-week non-repeating and interchangeable standard breakfasts and lunches, as well as suggestions for dinners. These recipes were developed based on the nutrient composition and the food group intake of the RCMDR dietary pattern. Considering the generalizability and affordability of the RCMDR dietary pattern, the daily cost was approximately RMB 50-60 per person per day, using common local food ingredients and cooking methods. In addition, the study will adopt a combination of feeding intervention and dietary guidance, making it easier for the participants and their families to learn and master the dietary pattern according to these recipes. Even after the experiment, the RCMDR

indices), fasting blood test (fasting blood glucose (FBG), fasting insulin (FINS), glycated hemoglobin (HbA1c), fasting C-peptide, total cholesterol (TC), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), folic acid, homocysteine (Hcy), liver function (alanine aminotransferase (ALT), aspartate transaminase (AST)), kidney function (Serum uric acid, urea, creatinine)), apolipoprotein E (APOE) and methylenetetrahydrofolate reductase (MTHFR) genotype polymorphism.

Methods of physical measurements

Blood pressure measurement: the Omron J710 upper arm electronic blood pressure monitor measures systolic blood pressure (SBP) and diastolic blood pressure (DBP). The third measurement will be required if the difference between SBP and DBP exceeds 10 mmHg.

Body composition indices measurement: the body composition analyzer and bioelectrical impedance method were used to measure body composition indices, such as height, weight, waist-to-hip ratio, body fat mass, body muscle mass and visceral fat mass, subcutaneous fat mass, basal energy expenditure, EER, etc.

Blood sample collection and tests

Blood samples will be collected at baseline and the end of the study. Before collecting blood samples, participants will be required to fast for at least 10 hours overnight. Fasting venous blood from participants will be collected by qualified nurses. The blood samples will be refrigerated and transported to the central laboratory of the School of Public Health, Tianjin Medical University. Analysis of FBG (using the

glucose oxidase method), HbA1c (using the immunosuppressive turbidimetry), TG and TC (using the enzyme endpoint method), HDL-C (using the polyethylene sulfate precipitation method), LDL-C (using the polyethylene sulfate precipitation method), ALT, AST, serum uric acid, urea and creatinine (using the enzymatic method) will be carried out on a Roche Cobas 8000 automatic biochemistry analyzer. Fasting C-peptide, FINS (using the enzyme-linked immunosorbent assay (ELISA)) was measured using ELISA kit. Serum folic acid (using the chemiluminescence immunoassay method) was measured using a chemiluminescence immunoassay analyzer. Serum Hcy (using the enzyme cycling method) was measured using an automated chemical analyzer. ApoE and MTHFR genotype polymorphism testing will be commissioned by a gene sequencing company.

Dietary intake of foods and nutrients

Firstly, all raw materials will be weighed before cooking, including the amount of oil and salt used. After cooking, each dish will be weighed according to the energy level of the standard meal. Researchers will calculate the average daily energy and dietary nutrients each participant takes using the China Food Composition (6th edition) based on the photos of leftovers and dietary records submitted by the participants [33].

Follow up schedules

At the mid-term of the study, the uninformed researchers will carry out the same questionnaire interview. At the end of the study, the uninformed researchers will conduct the same questionnaire interview and physical examination of the participants using the same method as the baseline. The frequency of dietary guidance for

participants in both groups will be the same: once a week in the first month, once every two weeks in the second month, and once in the third month. Dietary data of participants in the intervention group will be collected daily, and in the control group will be collected twice a week. The schedule of measurements and visits of the study has been summarized in table 4.

Outcomes

The primary outcome is the change in cardiometabolic risk (CM-risk) score from baseline to the end of the study. The secondary outcomes include a change in blood lipid (LDL-C, TC, TG, HDL-C), blood pressure, FBG, insulin resistance measured by Homeostasis Model Assessment, body composition indices, and 10-year risk of CVD from the Prediction for Atherosclerotic Cardiovascular Disease Risk in China (China-PAR) [34].

Sample size

Although the CM-risk score is the primary outcome, the methods of calculating the cardiometabolic score in different studies are inconsistent. Participants in the study are dyslipidemia, and according to the 2023 Chinese guideline for lipid management, LDL-C is the primary target for lipid intervention [4]. Therefore, the sample size in the study was calculated using the change of LDL-C from baseline to the end of the study. According to the previous study that the calorie-restricted Mediterranean-style diet successfully reduced LDL-C by 0.23 mmol/L for hypercholesterolemic patients in 4 weeks, we conservatively assumed that the RCMDR dietary pattern will reduce LDL-C by 0.3 mmol/L in 12 weeks, and the standard deviation of LDL-C change is 0.5

mmol/L in the control group [35]. To have 80% power with a type I error rate of 5% to detect the assumed effect size, we would need 44 participants in each group. Assuming that 10% of study participants will be lost by the end of the study, 100 participants will be recruited.

Data management

Data will be collected in an electronic database. Participants will only be identified by a unique ID. Hard copies of data sheets containing participants' files and other data (including questionnaires, test results, records of informed consent, and other documents related to the conduct of the study) will be kept securely in a locked filing cabinet in a locked office, accessible only to key research team members. Blood samples will be kept in a locked freezer. All data and Blood samples will be stored in Tianjin Medical University.

Statistical analysis

Statistical analyses will be performed using SPSS 24.0 software. The analyses of outcomes will follow the intention-to-treat principle. Continuous variables conforming to normal distribution will be expressed as mean ± standard deviation, and non-normally distributed continuous variables will be expressed as median with limits of the interquartile range (IQR: 25th and 75th percentiles). Categorical variables will be shown as frequencies and proportions (percentages). The differences in baseline variables between groups will be evaluated by using a t-test, Wilcoxon rank test, or χ^2 test. Linear regression will be used to estimate the absolute differences between the two groups regarding primary and secondary outcomes and reported as the least squares

mean after adjusting the center. Sensitivity analyses will be conducted to repeat the primary analyses with imputed missing values. We will use multiple imputations to impute missing values of outcomes. A two-sided *P* value <0.05 will be considered statistically significant.

Quality control

The reliability and validity of the questionnaire are high. According to the purpose of this study, based on previous studies, multiple collective discussions were conducted to unify the evaluation standards of the questionnaire, ensuring scientificity, consistency, and comparability. All researchers participating in this study must receive uniform training and be tested after training, including standard operating procedures for participant data collection and methods for collecting and preserving biological samples to ensure accurate and complete information and to avoid measurement bias. All biological samples will be tested in the central laboratory of Tianjin Medical University. The researchers responsible for detecting relevant indicators will be blinded to the randomization grouping of participants.

Patient and public involvement

This research study didn't involve patients or the general public in its design, conduct, reporting, or dissemination.

Ethical and dissemination

The study complies with the Measures for Ethical Review of Life Sciences and Medical Research Involving Human Beings and the Declaration of Helsinki. Signed informed consent will be obtained from all participants (Supplemental file 2). The study

has been approved by the Medical Ethics Committee of the Second Hospital of Tianjin Medical University (approval number: KY2023020) and registered in the Chinese Clinical Trial Registry (ChiCTR2300072472). The results from the study will be disseminated through publications in a peer-reviewed journal.

Current status

The first participant was enrolled on 14 June 2023.

In summary, the study is a randomized controlled dietary intervention trial to evaluate the effect of the RCMDR dietary pattern on cardiometabolic health in people with dyslipidemia. The study not only comprehensively considered the effect of diet on CMDs, but also considered the generalizability and affordability of the RCMDR dietary pattern among the population. The study's findings will significantly prevent CMDs in China and improve Chinese residents' nutritional status and health levels.

Authors' contributors

G. Huang, W. Li and F. Ma conceived and designed the study. Q. Wu, L. Zhang, C. Cheng, X. Chen, L. Huang, and T. Li will perform the study, collect and analyse all experimental data. Q. Wu provided the first version of the manuscript. S. Bian, Z. Li, H. Liu, J. Yan, Y. Du, Y. Chen, M. Zhang, L. Cao, W. Li, F. Ma and G. Huang provided critical comments on the original manuscript. W. Li and G. Huang revised and finalised the manuscript. All authors read and approved the final version of the manuscript.

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Disclaimer

The funding has no role in the design of this trial and will not have any role during its execution, analyses, interpretation of the data or decision to submit results.

Competing interests statement

None declared.

Patient consent for publication

Not required.

Provenance and peer review

Not commissioned; externally peer reviewed.

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Figure Legends

Figure 1. The flowchart of the study. RCMDR, reducing cardiometabolic diseases risk; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol.

Table 1. The nutrients composition of the RCMDR dietary pattern, and methods to reach the goals of nutrients

Nutrients	Intake	Methods to reach the goals
Energy (kcal)	1450-2350	Average intake was evaluated during the run-in period.
Energy distribution of three meals		
Breakfast: Lunch: Dinner	3: 4: 3	
Energy distribution of macronutrients		
Carbohydrates (kcal%)	56-58%	Increasing the use of whole grains and limiting added sugars (monosaccharide, disaccharide).
Protein (kcal%)	17-19%	Increasing protein intake in legumes, dairy products, fish, and lean meat.
Fat (kcal%)	24-26%	Reducing the use of cooking oil by changing cooking methods, such as using steam instead of frying, and using low-fat or skim dairy products, choosing skinned lean meat.
Saturated	<7%	Banning animal cooking oils.
Monounsaturated	12%	Increasing the use of vegetable oil rich in monounsaturated fatty acid (MUFA).
Polyunsaturated	6-8%	Increasing the use of nuts and n-3 polyunsaturated fatty acid (PUFA)-rich fish.
Cholesterol (mg/day)	<300	Reducing the use of animal-based foods.
Fiber (g/day)	30	Increasing the use of foods high in dietary fiber, such as whole grains, legumes, vegetables, and fruits.
Sodium (mg/day)	3000	Reducing the use of salt and sodium seasoning during cooking.
Potassium (mg/day)	3700	Increasing the use of food with high potassium content.
Magnesium (mg/day)	500	Increasing the use of food with high magnesium content.
Calcium (mg/day)	1000	Increasing the use of dairy products, soy products, fish, nuts, and dark vegetables.
Folate (μg/day)	400-800	Increasing the use of plant-based foods that contain high amounts of folate.

Table 2. The RCMDR dietary pattern

Food groups	Edible portion intake	Explanation
Cereals and tubers		
Cereals	200-300 g/day	
Whole grains	90-150 g/day	
Legumes*	10-30 g/day	
Tubers	50-100 g/day	
Vegetables and Fruits		
Vegetables	350-550 g/day	
Dark green leafy and cruciferous species vegetables	200-250 g/day	
Other dark vegetables	100-200 g/day	
Other vegetables	50-100 g/day	
Fruits	200-350 g/day	Complete fresh fruits
Foods of animal origin	90-150 g/day	Banning processed meat products
Fish	300-500 g/week	Eating 3-4 times a week and choosing fish rich in n-3 PUFAs.
Poultry	100-175 g/week	Skinned lean meat, fresh meat
livestock meat	100-125 g/week	Skinned lean meat, fresh meat
Eggs	3-5 per week	
Dairy products	300-500 g/day	Low-fat or skimmed milk, yogurt or other fermented dairy products
Soybeans and nuts		
Soybeans	30-40 g/day	The intake of soy products is calculated based on the same protein content. Individuals with hyperuricemia or gout are not recommended to increase their intake of soybeans or soy products.
Nuts	10-20 g/day	
Oil and salt		
Cooking Oil	15-25 g/day	Alternating consumption of various vegetable oils rich in UFA and avoiding animal oils.
Salt	<5 g/day	Low-sodium salt is recommended for those without contraindications.

Table 4. The schedule of measurements and visits of the study

	Follow-up (weeks)									
	Screening	Run-in*	1	2	3	4	6	8	10	12
Signed informed consent	√									
Eligibility confirmation	√	√								
Questionnaire interview	√	√					√			√
Dietary record#		√	√	√	√	√	√	√	√	√
Dietary guidance			√	√	√	√	√	√	√	√
physical examination		√								√
Fasting blood test		√								√
Blood sample		√								√
Reasons for withdrawal		√	√	√	√	√	√	√	√	√

*Baseline data are collected on the last 2 days of the run-in period.

#Dietary data of participants in the intervention group will be collected daily.

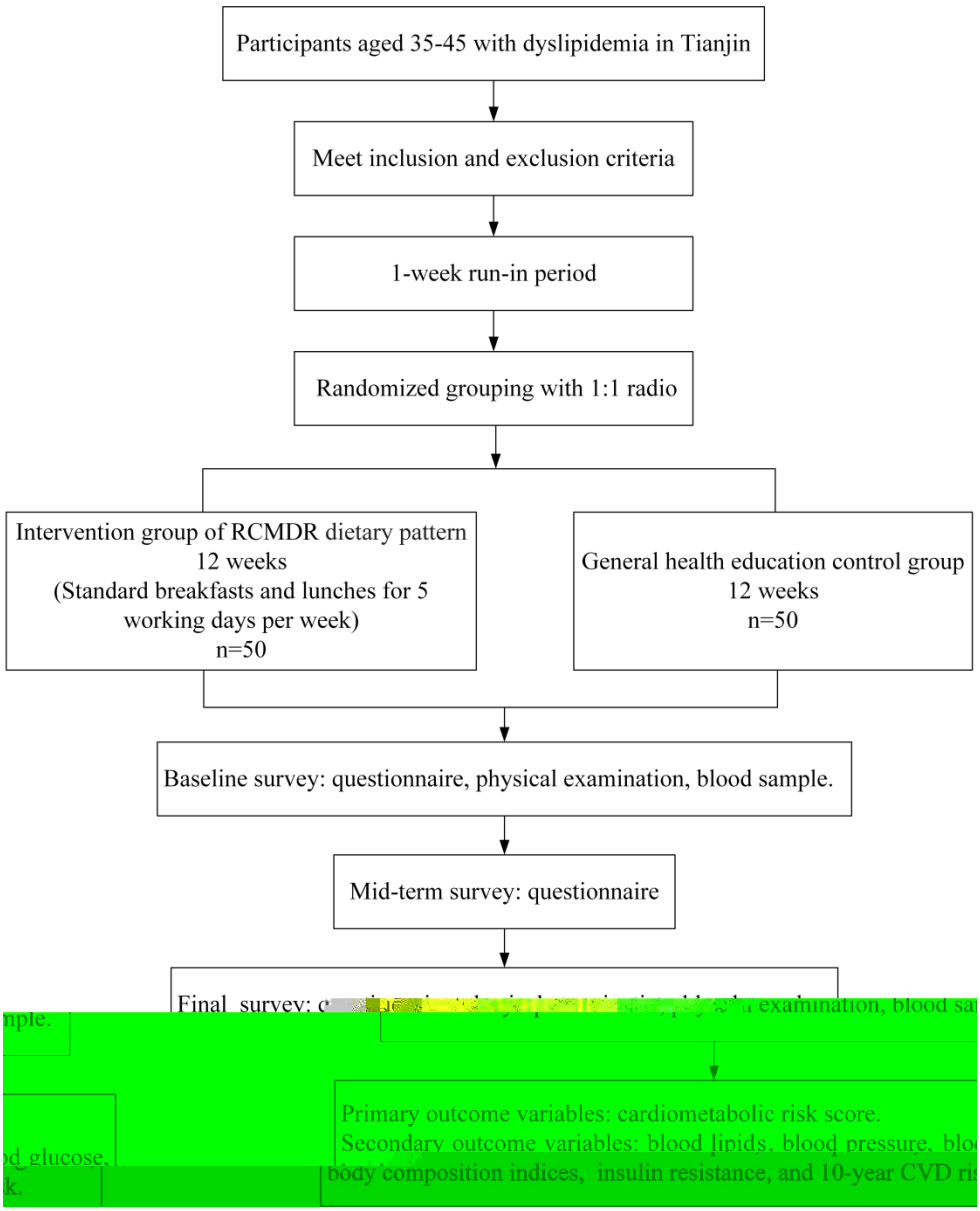

Figure 1. The flowchart of the study. RCMDR, reducing cardiometabolic diseases risk; CVD, cardiovascular disease; LDL-C, low-density lipoprotein cholesterol.

224x276mm (600 x 600 DPI)

Delphi process and results of developing the RCMDR dietary pattern

1. Delphi Process

1.1 The panel of experts

1.2 Data collection

1.3 Data Analysis

2. Results

2.1 Characteristics of the expert panel

2.2 Active degrees of the expert consultation

2.3 Authority degrees of the expert consultation

2.4 Coordination degrees of the expert consultation

2.5 Screening and modification of indices

Indices	Intake	Explanation	Mean	SD	*CV*	Result of discussion
Cerealr revie□only						

Seasonings

Water and beverages

≤

Energy distribution of three meals

Physical activity

Indices	Intake	Explanation	Mean	SD	*CV*	Result of discussion
Cereals and tubers						
Vegetables and Fruits						
Foods of animal origin						
Dairy products						
Soybeans and nuts						
Oil and salt						

Added sugar

)

Water and alcohol

Energy and distribution

(English Version)

Study title: Construction and application of a healthy dietary pattern for controlling and reducing the risk of cardiometabolic diseases

Principal Investigator: Shanshan Bian

Dear participant:

You are invited to participate in the study Construction and application of a healthy dietary pattern for controlling and reducing the risk of cardiometabolic diseases, which is supported by the National Nutrition Science Research Grant of Chinese Nutrition Society.

Please read this informed consent form carefully and make a cautious decision on whether to participate in this study. Your participation in this study is completely voluntary. As a participant, you must sign the informed consent form before joining this study. When your study doctor or investigator discusses the informed consent form with you, you can ask him/her to explain any questions that you do not understand. We encourage you to have a full discussion with your family and friends before you decide to take part in this study. You have the right to refuse to participate in this study and to withdraw from the study at any time without penalty or losing any rights to which you are entitled. If you are taking part in other studies, please tell your study doctor or the investigators. The background, purpose, study procedures, and other important

information about this study are as follows:

Cardiometabolic diseases (CMDs) is a kind of syndrome with cardiovascular damage and abnormal metabolism, including a series of diseases, such as cardiovascular disease, diabetes, dyslipidemia, and metabolic syndrome. CMDs is one of the main chronic non-communicable diseases affecting human health, accounting for more than half of the global disease burden in adult. Dietary intervention, as an important part of comprehensive lifestyle intervention, plays an important role in all stages of the occurrence and development of CMD and its comorbidities. Dietary pattern studies, which consider the relationship between certain or several types of food or nutrient intake and health as a whole, are closer to what people eat in the real world. Dietary intervention is also known as a preferred strategy for intervening in the clinical progression of CMDs because of its low risk, low cost, and high benefit. Although dietary patterns characterized by vegetables, fruits, whole grains, fish, legumes and nuts are protective factors against the disease, such as the Dietary Approaches to Stop Hypertension and the Mediterranean diet. However, a healthy dietary pattern that fits Chinese food culture can effectively reduce the risk of CMD has not been found. Therefore, it is urgent to construct a healthy dietary pattern with Chinese characteristics for controlling and reducing the risk of CMDs.

The aim of this study is to assess the effect of the Reducing Cardiometabolic Diseases Risk (RCMDR) dietary pattern on reducing CMDs risk in people with dyslipidemia, and to provide a reference for the construction of a dietary pattern suitable for the prevention of CMDs in China.

100 participants will be enrolled in this study.

If you agree to participate in this study, please sign this informed consent form. Fasting venous blood samples will be collected before and after the intervention respectively, with 18 ml of blood samples each time, approximately 36 ml in total. Your blood samples collected during the physical examination will be measured for liver and kidney function, blood glucose, blood lipids, and apolipoprotein E (APOE) and methylenetetrahydrofolate reductase (MTHFR) genotype polymorphism, which affect lipid and folate metabolism. Except for the results of the APOE and MTHFR genotype polymorphism testing, the results of other indicators in two physical examinations will be informed to you in the form of an electronic report.

Before you are enrolled in the study, your medical history will be asked, and you will be screened according to the results of your latest physical examination.

After determining that you are eligible to participate in the study, you will be randomized and allocated into the RCMDR dietary pattern intervention group or the

general health education control group. This study will last for 12 weeks. Before and after the intervention, questionnaires and physical examinations will be carried out. Food frequency questionnaires, physical activity and sleep status will be assessed once a month. In addition, we will also provide you with free dietary guidance once a week in the first month, once every two weeks in the second month, and once in the third month.

If you are assigned to the intervention group, you will be provided with standard breakfasts and lunches 5 working days per week (60 working days in total) for free. And we will develop a personalized dietary plan for you based on your dietary habits and the results of physical examination. You will be required to take photos of the leftovers from each standard meal and record the intake of all food consumed on weekdays (excluding standard meals) and rest days. We will calculate the intake of daily nutrients based on these data and provide personalized dietary guidance to you.

If you are assigned to the control group, you will be provided with professional dietary guidance based on “Dietary Guidelines for Chinese Residents (2022)”. You will be required to choose any one working day and rest day every week for dietary records. We will give you guidance based on the problems in your diet.

This study will last for 12 weeks. Food frequency questionnaires, physical activity and sleep status will be assessed once a month. Questionnaires as well as physical examinations will be conducted before and after the intervention. The frequency of dietary guidance will be once a week in the first month, once every two weeks in the

second month, and once in the third month.

You will be allowed to leave the study at any time without losing any of the benefits you would have received. However, if you decide along the way to withdraw from the study, we encourage you to discuss this with your study doctor first.

If you withdraw from the study for any reason, you may be asked about the details of your participation in the study. You may also be asked to undergo the physical examination if your study doctor considers it necessary.

18 ml of fasting venous blood samples will be collected from you before and after the intervention respectively. The blood samples will be refrigerated, and will be destroyed after all indices have been measured. All examinations are performed by trained healthcare professionals.

There are no additional risks to participating in this study.

If you experience any discomfort, a new change in your condition, or anything unexpected during the study, whether it is related to the study or not, you should tell your doctor immediately.

You will be required to follow up the questionnaires on a regular basis during the study, which will take up some of your time and may be troublesome or inconvenient for you.

Direct benefits: If you participate in this study, you may have direct medical benefits.

1) Your blood lipid levels may be decreased to some extent, but there is no guarantee that it will definitely work for you.

2) Whether you are in the intervention group or the control group, we will provide you with a free medical examination and report.

3) If you are in the intervention group, we will provide you with free standard breakfasts and lunches on weekdays (5 days per week) during the intervention period. After you have carefully completed the questionnaire, we will design a personalized dietary plan for you based on your eating habits and physical examination report, and you will receive free scientific dietary guidance at least once a month. You are welcome to consult us at any time if you have any questions about diet and nutrition.

If you are in the control group, we will provide you with free scientific dietary guidance at least once a month after you complete the questionnaires, and you can ask us any questions about diet and nutrition at any time. In addition, at the end of the study, we will provide you with the appropriate meal compensation of RMB 20 per day for 60 days. If you are unable to complete the entire study due to special circumstances, we will compensate you according to the number of days you participate in the study. All compensation will be paid in a one-time payment to your bank card by the study institution at the end of the study.

Potential benefit: This study may control or reduce your risk of developing other

CMDs and stop or slow the progression of your dyslipidemia.

We hope that the information gained from your participation in this study will benefit you or others with the same disease in the future.

Your medical records (medical records, laboratory examination report sheets, etc.) will be kept intact in Tianjin Medical University. The investigator, the sponsor's representative and the ethics committee will have access to your medical records. Your personal identity will not be revealed in any public reports of the results of this study. Every effort will be made to protect the privacy of your personal medical information to the extent permitted by law. It is possible that your medical records may be used again in future studies. You may also choose to refuse the use of your medical records in studies other than this one.

Except for your private information, the study data will be available for public access and sharing. Sharing of study data will be limited to web-based Electronic Data Capture only and will ensure that no private information about you will be disclosed.

There is no extra cost for you to participate in this study. There will be no charge for the examinations that the investigators will do to check your health and no charge for the dietary guidance that will be given.

Routine treatments and examinations for other diseases you have at the same time will not be free.

If you are assigned to the control group, you will be compensated for the cost of meals at the end of the study.

As you are not taking any additional risk by participating in this study, there is no compensation or indemnity.

Your participation in the study is voluntary throughout the entire process. If you decide not to participate in this study, it will not affect other treatments you should receive. If you decide to participate, you will be asked to sign this written informed consent. You have the right to withdraw from the study at any stage without discrimination or unfair treatment, and your medical treatment and rights will not be affected.

As a participant, you will be required to provide truthful information about your medical history and current medical condition; to inform the study doctor of any discomfort you notice during this study; not to take any restricted medications, foods,

and so on that have been communicated to you by your study doctor; and to inform your study doctor that if you have recently or are currently participating in other studies.

If there is any significant new information during the study that may affect your willingness to continue participating in the study, your doctor will inform you promptly. If you are concerned about your own study data, or you would like to know the findings after this study, you may ask any questions about this study at any time and receive answers accordingly, please contact Qi Wu at **********.

The Ethics Committee has approved this study. If you have any questions related to your rights/interests, or if you would like to reflect the difficulties, dissatisfaction and concerns encountered during this study, or if you would like to provide comments and suggestions about this study, please contact the Medical Ethics Committee of the Second Hospital of Tianjin Medical University at ***-********.

Participant Consent Statement:

I have been informed of the purpose, background, process, risks and benefits of this study. I have plenty of time and opportunity to ask questions, and the answers to the questions have been to my satisfaction.

I have also been told who to contact when I have questions, want to report difficulties, concerns, suggestions for the study, or want further information, or help with the study.

I have read this informed consent and agree to participate in this study.

I understand that I may choose not to participate in this study or withdraw from this study at any time during the study without any reason.

I already know that if I get worse, or if I have a serious adverse event, or if my study doctor decides it's not in my best interest to continue, he/she will decide to withdraw me from the study. The funder or regulatory agency may also terminate during the study without my consent. If this happens, I will be promptly notified by my doctor and the study doctor will discuss other options with me.

I will be provided with a copy of the informed consent which contains my signature and that of the investigator.

Participant Signature: Date:

(NOTE: If participant has no capacity/limited capacity, legal representative signature and date will be required)

Tel:

Legal Representative's Signature: Date:

(NOTE: If participant has no capacity/limited

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	3-4
	2b	All items from the World Health Organization Trial Registration Data Set	4
Protocol version	3	Date and version identifier	3-4
Funding	4	Sources and types of financial, material, and other support	2 and 22
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1-2 and 22
	5b	Name and contact information for the trial sponsor	2 and 22
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	22-23
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	NA
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	5
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	6

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8-9
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-13
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	NA
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	11
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	NA
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	16
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Table 4
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	16
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	8

Methods: Assignment of interventions (for controlled trials)

Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	9-10

Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	10
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	8 and 9-10
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	10
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	NA
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	13-15 and 18
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	NA
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	17
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	17-18
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	17-18
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	17-18
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	NA
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	NA

Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	NA
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	NA
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	19
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	NA
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	19
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	NA
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	17
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	20
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	NA
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	NA
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	18
	31b	Authorship eligibility guidelines and any intended use of professional writers	NA
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	NA
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Supplemental file 2

Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	14-15

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.