

Original Article

Dietary camellia (*Camellia oleifera* Abel) seed oil in traditional Chinese cooking for high-risk cardiovascular disease: A three-arm double-blind randomized controlled feeding trial protocol

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Background and Objectives: As the Chinese economy has developed, dietary patterns have modernized, thereby increasing the incidence of chronic diseases such as cardiovascular disease (CVD). Many observational studies have shown that the Mediterranean diet based on olive oil is associated with a decreased incidence of CVD. This article aims to study the possible effects of dietary models by using three edible oils: olive oil, camellia seed oil (CSO), and soybean oil. CSO has a fatty acid composition similar to that of olive oil and is unique in China, and soybean oil is a dietary oil used in traditional Chinese cooking. **Methods and Study Design:** This intervention is designed based on a three-arm double-blind randomized controlled feeding trial. Three dietary models are established according to traditional Chinese cooking methods, each using one of the three plant edible oils mentioned above as a leading factor. Participants will be randomly assigned to each group and provided with a designated diet for 3 months. **Results:** The study population is planned to be women with a high risk of CVD and aged between 35 and 69 years. Weight and other CVD-related factors are treated as primary and secondary outcomes, respectively. **Conclusions:** This trial may inform dietary nutrition policies to a certain extent, especially concerning traditional Chinese cooking methods, for weight control and the improvement of cardiovascular-related risk factors in women with a high risk of CVD.

Key Words: Camellia seed oil, cardiovascular risk factors, three-arm double-blind randomized controlled feeding trial, traditional Chinese cooking, high risk of cardiovascular disease

INTRODUCTION

Chronic diseases, primarily heart disease, stroke, cancer, diabetes, and hypertension, have become major factors for global morbidity and mortality.^{1,2} Data from the “Healthy China Initiative” report indicated that in 2019, chronic diseases became the main cause of death for residents of China and accounted for more than 70% of total disease.³ However, these data also revealed significant changes over the past decade, during which chronic diseases accounted for more than 60% of deaths. Cardiovascular disease (CVD) was the main cause of death reported in 2008.^{4,5} The number of deaths caused by CVD was approximately 7.0 million (86% of all deaths) in 2010 and rose to 290 million in 2014,⁶⁻⁹ and similar results described in other articles have indicated that CVD has been the primary cause of death in China since 2006. Chinese government reports in 2015 also supported this trend, with mortality rates caused by CVD in rural and urban areas being 45.5% and 43.16%, respectively.¹⁰ The economic cost of CVD and other chronic diseases is high. Costs relating to CVD, hypertension, and diabetes pres-

ently account for approximately 4% of China’s gross domestic product. Studies have predicted that this percentage will be doubled by 2050 if countermeasures are not taken,¹¹ and poverty and inequality in health care will be a direct cause.¹² Therefore, reducing the incidence and mortality of CVD has become increasingly critical in China.

In the early 20th century, due to war and foreign invasions, China was one of the poorest countries in the world.^{13,14} The low level of economic development led to many health problems primarily caused by poverty-related diseases such as high infant mortality, infectious disease epidemics, and nutritional deficiencies.^{13,15}

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However, China's economy has undergone many changes after 1950, particularly in the past 20 years under the policy of "economic reform and opening up."¹⁶ With the growing economy, Chinese dietary patterns have changed substantially.¹³⁻¹⁵ Three national nutrition surveys were conducted in China in 1959, 1982, and 1992;^{17,18} they indicated that the average Chinese person's daily energy intake was 2,060 kcal in 1959, rose to a peak of 2,484 kcal in 1982, and then declined slightly to 2,328 kcal in 1992. The per capita protein intake was 57 g in 1959. This was subsequently improved upon, reaching 67 g and 68 g in 1982 and 1992, respectively. Furthermore, the per capita fat intake climbed from 49.3 g in 1982 to 58.3 g in 1992 (data from 1959 were lacking). These data confirm that Chinese people are progressively trending toward modern dietary patterns, indicated by a decreased intake of grains and an increased intake of animal-source foods and edible oil, as well as the rising consumption of tobacco and alcohol.^{14,19} Against the background of these changing dietary patterns, the primary cause of death in China has changed from poverty-related diseases to chronic diseases such as CVD.¹³⁻¹⁵ Research from Shanghai reported similar results, where grain consumption accounted for 80%–83% of total energy intake among Shanghai residents in 1950, dropping to 68%–72% in 1980. By contrast, energy intake from animal-source foods nearly tripled from 1950 to 1980. Because of this change, the mortality rate of CVD has risen sharply, and infectious diseases have rapidly decreased among residents.²⁰ Therefore, reducing the morbidity and mortality of CVD by changing dietary patterns has become a key concern for the field. In this trial, we established three dietary patterns (based on camellia seed oil [CSO], olive oil, and soybean oil) associated with traditional Chinese cooking styles to explore the relationship between diet and CVD.

A series of studies are currently aiming to determine the relationship between diet and CVD. The most notable of these studies have focused on the relationship between the Mediterranean diet and CVD. The Mediterranean dietary pattern (MDP) has been globally adopted and is currently the most widely recognized dietary pattern. This is because of its direct relationship with the prevention of noncommunicable diseases and the maintenance of good health.²¹⁻²⁵ A prospective study in 2009 investigated the correlation between Mediterranean dietary compliance and the risk of coronary heart disease. A total of 41,078 participants aged from 29 to 69 years were enrolled in the study. All the participants were recruited between 1992 and 1996, and the study was followed up until December 2004, with the average follow-up period of the participants reaching 10.4 years. The researchers used questionnaires and scoring systems to evaluate the compliance of participants and recorded and observed the occurrence of coronary heart disease events. The results revealed a significant association between high Mediterranean diet adherence and a low risk of coronary heart disease.²⁶ Another study surveyed 74,886 female participants aged between 38 and 63 years, and they were followed up for 20 years between 1984 and 2004. After the application of validated food frequency questionnaires and a Cox proportional hazards model, the conclusion was that women

with high Mediterranean diet adherence had a lower incidence of coronary heart disease and stroke.²⁷ However, MDP has been described in many variations. The concept of MDP originates from certain epidemiological studies primarily referring to the collection of traditional eating habits followed by people in different countries along the Mediterranean coast.²⁸ These studies have revealed that geographical differences were found in the incidence of CVD; for instance, southern European countries, such as France, Spain, Greece, and Italy, had lower incidences of CVD than did northern European countries and the United States.^{29,30} Dietary patterns are considered to be the main cause of such differences. After this discovery, the field of nutrition has gradually focused attention on the Mediterranean diet. The features of MDP include a high intake of monounsaturated fatty acids (MUFAs), plant proteins, whole grains, and seafood and a low intake of red meat and sugar.²⁷ Further research has indicated that MDP is largely characterized by the intake of olive oil, fruit, and vegetables, replacing red meat with fish and consuming small amounts of red wine and yogurt.²¹ Additionally, research has shown that eating plant-based foods and using olive oil as the main source of fat are the main characteristics of MDP.²² Although there is no uniform definition of MDP, olive oil is evident in all definitions as a major factor. A wide range of studies have confirmed that olive oil rich in MUFAs improves blood lipids, blood pressure, inflammation, and arterial wall function, being beneficial in the prevention of CVD through different mechanisms.³¹⁻³⁴

CSO applied in this trial is obtained from *Camellia oleifera* Abel seed and is the most common CSO, occupying 98% of the planting area in China.³⁵ CSO and tea seed oil are different because tea seed oil is extracted from the seeds of the tea tree (*C. sinensis* [L.] O. Ktze). The genus *Camellia* belongs to the Theaceae family and consists of over 200 woody evergreen species, of which *C. oleifera* is a major economic oil seed crop (Figure 1 [a], [b]).³⁶ The shape of the *C. oleifera* fruit is subspherical or spherical with a diameter of 2.3–3.3 cm, and seeds (characterized by a black hard shell and a yellow kernel) are contained at the base thereof in a circular manner (Figure 1 [c], [d]).^{37,38} *C. oleifera* is mainly cultivated in the southern provinces of China, including the 14 provinces of Hunan, Jiangxi, Guangxi, Zhejiang, Fujian, Guangdong, Hubei, Guizhou, Anhui, Yunnan, Chongqing, Henan, Sichuan, and Shanxi, occupying 90% of the global total (Figure 1 [e]).³⁹ CSO is an edible vegetable oil regarded as one of the four most important woody oils, along with olive oil, palm oil, and coconut oil. The earliest records of CSO in China can be traced back to 2,300 years ago in the "Shan Hai Jing," in which people are described using CSO for cooking purposes. CSO has been widely used in various fields such as food, cosmetics, and medicine. Nonetheless, its main function is still regarded to be its use as cooking oil.⁴⁰

The extraction of oil from *C. oleifera* seeds is a difficult process because they contain tea saponin (a foam stabilizer and emulsifier). Common methods for extracting oil include organic solvent, subcritical water, ultrasound-assisted, and microwave puffing-pretreated extractions.⁴¹ Data indicate that in Hunan province, more than

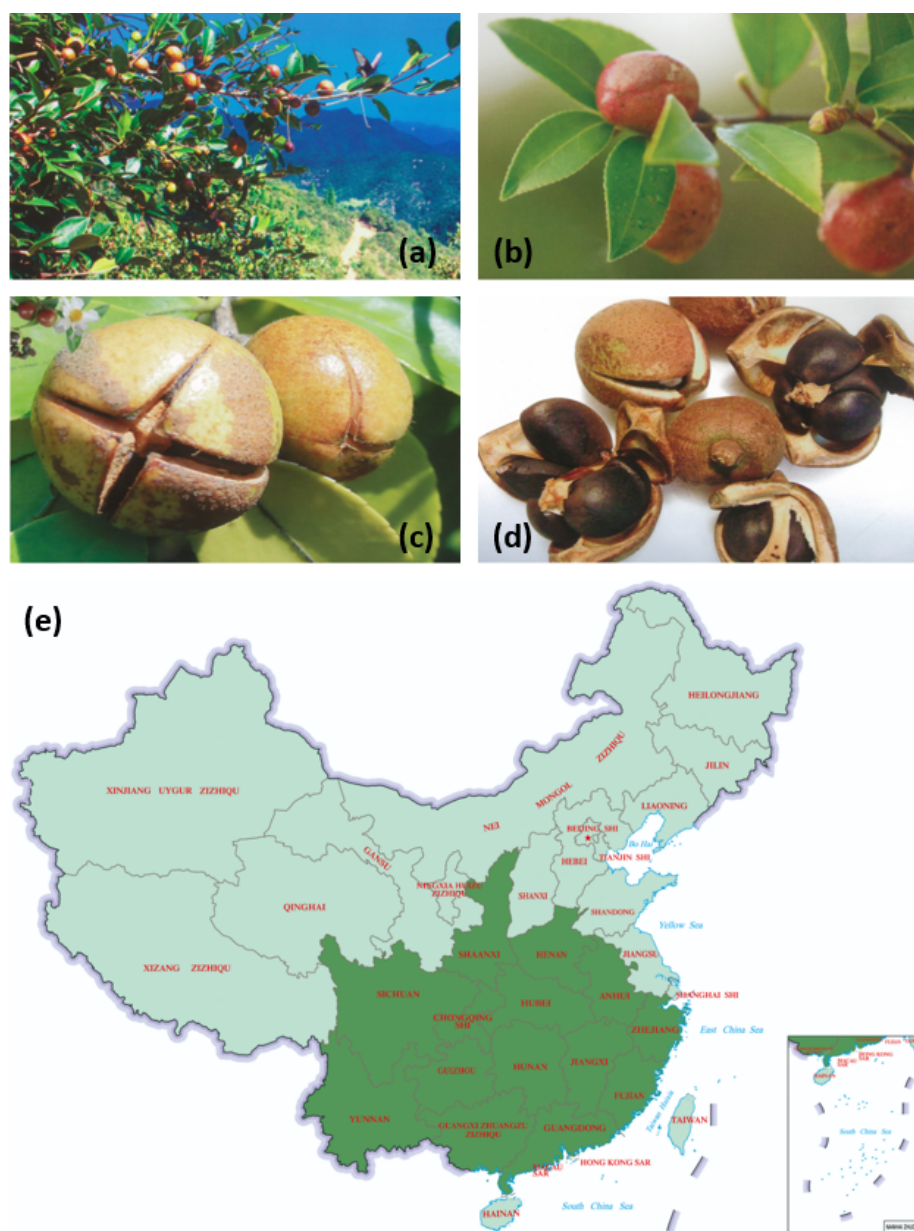


Figure 1. The characteristics and distribution of *Camellia oleifera* Abel. (a) The plant of *Camellia oleifera* Abel (a small evergreen broad-leaved tree with four seasons); (b) The foliage of *Camellia oleifera* Abel; (c) The fruit of *Camellia oleifera* Abel (subspherical or spherical with a diameter of 2.3–3.3 cm, glabrous, with 2–4 locules); (d) The seed of *Camellia oleifera* Abel (tips flattened or bluntly wedge-shaped, dark brown with darker patches); (e) The main distribution of *Camellia oleifera* Abel in China including 14 provinces: Hunan, Jiangxi, Guangxi, Zhejiang, Fujian, Guangdong, Hubei, Guizhou, Anhui, Yunnan, Chongqing, Henan, Sichuan, and Shanxi (shaded dark green).

50% of edible oil consumed is CSO. This represents approximately 14% of the population of China.⁴⁰ The production and cultivation of *C. oleifera* seeds in China exceeded 250 million kg in 2016 and 3.65 million ha in 2017.^{35,41} Data from the China Bureau of International Statistics revealed that 2.43 million tons of *C. oleifera* seeds were processed and 600,000 tons of CSO were produced in 2017.⁴² Furthermore, the annual output of CSO was approximately 260,000 tons in 2009 and was expected to exceed 2.5 million tons by 2020, accounting for 15%–25% of edible oil consumption among Chinese people.^{36,43} However, the edible oil industry is relatively underdeveloped in China due to a lack of technology, poor land resources, and an unbalanced structure. Therefore, the output of the edible oil industry is significantly lower in China than in developed countries, and the per capita

edible oil consumption among Chinese people is at approximately 70% of the world average.⁴⁴ With improvements in living standards and the modernization of dietary patterns among Chinese people, the demand for edible oil has increased substantially, such that imports of edible oil have increased in recent years. The high yield of the CSO industry may be the decisive factor in this improving situation.

In addition to its high output, the development of the CSO industry has other advantages; for instance, improving the economy, optimizing the environment, and reducing the proportion of the population in poverty. These advantages have prompted the Chinese government to provide a high level of support for the development of this industry. The planting conditions of *C. oleifera* are easy to achieve, and *C. oleifera* can achieve steady

growth even in barren lands with poor fertilization.³⁶ Therefore, promoting the cultivation of *C. oleifera* is feasible in many areas of China, particularly poor areas populated by farmers who lack scientific planting techniques. In general, *C. oleifera* begins to bear fruit after the eighth year of planting and maintains a high yield for 80 years.³⁶ In addition, planting can prevent soil erosion and protect local ecology. To improve the environment, increase farmers' incomes, and reduce China's dependence on imported oil, the Chinese government formulated relevant policies to support the development of the domestic CSO industry in 2009.³⁷

Numerous studies have focused on the composition of CSO. Research on triglyceride (TG) composition is the most prominent. The fatty acid composition of CSO is similar to that of olive oil (with a high oleic acid concentration, making up 76.0%–81.4%), and it is thus often referred to as “Eastern olive oil”.^{35,42,45} TGs are a major component of CSO, occupying approximately 95%–98%, with the remaining 2%–5% being a minority unsaponifiable component involving squalene, phytosterols, polyphenols, and lipid-soluble vitamins. Fatty acids are the main components of vegetable oil and are beneficial to human health. In CSO, the main fatty acids are oleic acid (approximately 80%), palmitic acid (8.37%–8.92%), linoleic acid (7.47%–9.19%), and stearic acid (1.96%–2.64%).⁴⁷ The dominant unsaponifiable components of CSO are squalene (110 mg/kg) and sterols (including β -amyryn [607.24–913.15 mg/kg], cycloartenol [578.87–1,093.67 mg/kg], and lanosterol [715.19–1,202.80 mg/kg]). These are notable for their abilities in reducing cholesterol absorption and preventing dementia, as well as their anticancer, anti-inflammatory, and immunomodulating effects.^{48,49} Other functional substances such as polar phenols (mainly phenolic acid, approximately 20.56–39.47 mg/kg) and carotenoids (mainly lycopene, β -carotene, and lutein) have also been detected in CSO.⁴⁷ In 2018, our team formulated standards for CSO (USP Monograph Submitted for Publication - Camellia Seed Oil, CAS: [225233-97-6]) for the United States Pharmacopoeia Food Chemistry Codex Standard based on fatty acid composition, inorganic impurities, organic impurities, and specific tests.⁵⁰

CSO has the highest MUFA concentration among all vegetable oils, excellent nutritional value, and stable storage qualities and is recommended by the Food and Agriculture Organization of the United Nations as a high-quality and healthy edible oil.⁴⁰ Various reports have highlighted the benefits of CSO in inhibiting peroxidation, delaying atherosclerosis, and regulating blood sugar.^{51–53} However, most studies of the correlation between CSO intake and CVD-related indicators have been conducted by establishing animal models (mainly including mice, rats, guinea pigs, and rabbits) rather than using human beings. Therefore, research on CSO diet interventions and related indicators of CVD in the human beings is necessary.

The lipid composition of soybean oil differs greatly from those of CSO and olive oil, and there is little research supporting the suggestion that a soybean oil diet can improve the relevant indicators of CVD. Because soybean oil is the most consumed edible oil among Chi-

nese people,⁴⁴ it will be used in this trial for the control group. Thus, the characteristics of Chinese people's daily diets were accurately reflected. Traditional Chinese cooking methods were used in this dietary intervention. Therefore, determining whether the edible oil used in the intervention is suitable for traditional Chinese cooking methods (the usage of soybean oil and CSO has a long history in China) is necessary. For olive oil, one study compared composition changes under traditional Chinese cooking methods (stir frying, pan frying, and deep frying), and the results revealed that the MUFA concentration in olive oil remained at a relatively high level.⁵⁴ Research has also determined whether polyphenol compounds in olive oil will be destroyed under high temperature conditions. Food cooked with olive oil (stir frying and deep frying) was found to contain an increased concentration of oleuropein and hydroxytyrosol, meaning that cooking did not reduce the nutrient concentration in olive oil.⁵⁵ This study investigates whether a dietary pattern with CSO as the main ingredient exerts effects similar to those of a dietary pattern using olive oil under highly feasible traditional Chinese cooking methods.

Most studies of dietary patterns and CVD are prospective trials with long-term follow-up characteristics or utilize questionnaires and evaluation models to study the relationship between dietary patterns and CVD in specific areas. For example, in the Jiangsu region of China, the eating habits of 2,518 adults (1,146 men and 1,372 women) were surveyed. The final results indicated that the traditional Jiangsu dietary pattern was associated with a higher-than-average incidence of hypertension, which may be due to high salt intake.⁵⁶ A total of 3,591 participants in Southwest China (including Han Chinese and ethnic minorities) participated in the same type of dietary investigation where traditional minority dietary patterns (grassland dietary patterns) were prevalent. Grassland dietary pattern adherence had a positive correlation with a low risk of hypertension.⁵⁷ However, research on dietary interventions in the population is scant. Therefore, this study establishes a three-arm double-blind randomized controlled feeding trial to analyze the relationship between the CSO dietary pattern (CDP) and CVD risk. Three dietary modes, applying olive oil, CSO, and soybean oil (traditional Chinese cooking oil) as cooking fat sources, respectively, will be followed using traditional Chinese cooking methods (including frying, boiling, and steaming), and interventions will be applied on participants on week days for three months.

Outcome and hypothesis

The primary outcome of the study is to determine whether the three dietary patterns will be beneficial for controlling the weight of people with a high risk of CVD. The secondary outcome is to clarify the effect of the three dietary patterns on cardiovascular metabolic risk factors, such as blood pressure, blood lipid profiles (total cholesterol, TGs, high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C], apolipoprotein A1 [APO-A1], apolipoprotein B [APO-B], and lipoprotein (a) [Lp (a)]), enzymes (aspartate aminotransferase [AST], lactate dehydrogenase [LDH], creatine kinase, creatine kinase isoenzyme, alanine aminotransferase

[ALT], and homocysteine [HCY]), and inflammatory factors (hypersensitive C-reactive protein [hs-CRP], myoglobin [Mb], high-sensitivity troponin T [hs-TnT], tumor necrosis factor- α [TNF- α], interleukin [IL]-6, IL-8, N-terminal pro-brain natriuretic peptide [NT-proBNP], and hypersensitive troponin I [hs-TnI]). The assumption is that the CDP and olive oil diet pattern (ODP) would have similar effects on cardiovascular risk factors and surpass the soybean oil diet pattern (SDP).

METHODS

Experimental design overview

This trial is a three-arm double-blind randomized controlled feeding study that will assess three dietary patterns (CDP, ODP, and SDP) and examine their effects on cardiovascular risk factors. For the three dietary models, the food type, cooking methods, and food quantity will be kept consistent. By contrast, the cooking oil used for each model will be different (using CSO, olive oil, and soybean oil, respectively). Traditional Chinese cooking methods will be used in this experiment, including stir frying and pan frying (deep frying will be excluded because of the difficulty involved in controlling and calculating oil intake). The intervention will last 3 months during which time all participants will be requested to consume the specified food (provided by the research kitchen) for lunch and dinner on work days. During the intervention, a questionnaire will be used to record what was eat-

en for breakfast during weekdays, and the typical weekend diet will also be recorded as supplementary material. The intervention will be conducted in four different communities in Hangzhou, Zhejiang Province: Gudang, Yile, Jiulongcang, and Zijingang. The communities will provide public activity centers or public restaurants where food can be distributed smoothly. The primary outcome is weight, with blood pressure, blood profiles, and enzyme and inflammatory factors as secondary outcomes. The Ethics Committee of the College of Biosystem Engineering and Food Science at Zhejiang University has approved this project (ID number ZJU-BEFS-2019001). The trial has been registered in the Chinese Clinical Trial Registry (<http://www.chictr.org.cn>, registration number ChiCTR1800020078). Figure 2 presents the particulars of processes conducted in the trial.

Target population and inclusion/exclusion criteria

The target population of this study is the group with a high risk of CVD. This target group has two significant advantages. First, the population of the group is also the target population for global CVD prevention strategies. Second, such a population has a wide range and a large base that is helpful for screening participants. A total of 90 women aged 35–69 years will be included in the study, taking into account compliance and inclusion and exclusion requirements for high-risk cardiovascular populations. The inclusion and exclusion criteria are listed in

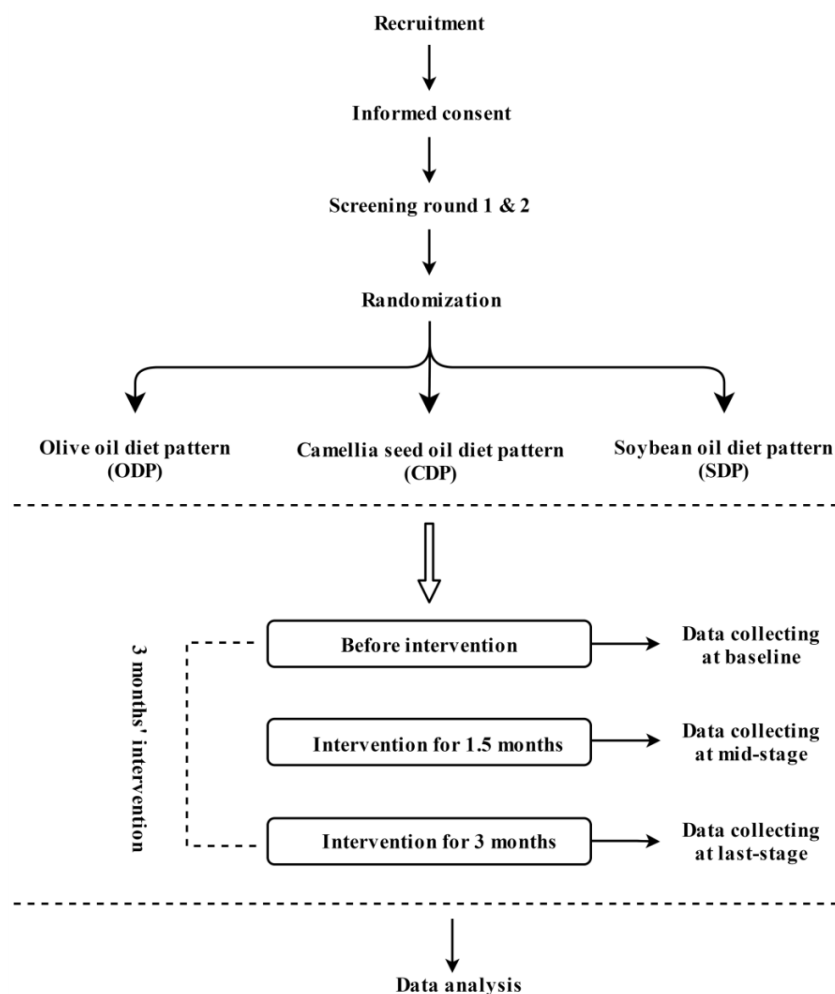


Figure 2. Flow chart for the whole feeding trial.

Table 1.

Recruitment

Recruitment will be mainly achieved using community announcements, fliers, newspaper advertisements, social media platforms (WeChat and QQ), and Internet forum posts. Health knowledge popularization activities will be conducted in communities to stimulate the enthusiasm and participation of potential participants and to expand influence.

Informed consent, population screening, and randomization

The chair of the project will explain the research content, form, and precautions to each potential participant, allowing them a whole day to fully consider their decision to participate. The rights and interests of potential participants will also be indicated by the chair. For instance, participation in the project will be entirely voluntary, and withdrawal will be allowed at any time during the intervention and would not have any adverse effects on potential participants. Potential participants who provide informed consent will be required to sign an informed consent form, followed by two rounds of screening.

The main purpose of the first round of screening is to determine the conformity of participants in nonclinical test indicators for inclusion and exclusion criteria (such as age and history of malignant diseases). Furthermore, a questionnaire will be used to determine potential participants' eating habits and preferences so as to determine their suitability for the specified dietary pattern and to ascertain any allergens to prevent allergic reactions. The second round of screening will be mainly achieved by testing the clinical indicators of all potential participants, meaning that potential participants will be requested to

provide blood samples after 12-h fasting, as well as random urine, feces, and hair and nail samples.

Data research personnel will randomly assign participants who pass the screening to one of the three dietary patterns according to a number list that is randomly generated by a computer. The results of the randomized groupings will be known only to food supply personnel. Data analysis personnel, sample collectors, follow-up research personnel, and participants will all be blinded to these results. This is to ensure that the trial remains double blind. To ensure the accuracy of the food supply under the random grouping, the food given to each participant will be marked with the name and location of the point of distribution, which will be selected by the participants themselves.

Composition of dietary patterns and food preparation

The three dietary modes consist of three vegetable oils: olive oil, CSO, and soybean oil. Olive oil used in this trial will be virgin olive oil produced by Shiguang Olive Oil Technology Co., Ltd., in Longnan, Gansu Province, China. Virgin CSO produced by Changfa Cereals Oils and Foods Co., Ltd. in Zhejiang Province will be used for the CDP. Soybean oil will be purchased from the first-class Arawana brand refined soybean oil sold by Fengyi Trading Pte., Ltd. The fatty acid compositions of each of the three trial oils are listed in Table 2 and are measured through gas chromatography (GC-2014, Shimadzu Instruments Co., Ltd.; chromatographic column purchased from Agilent, model DB-23 with a length of 60 m, diameter of 0.25 mm, and film of 0.25 μ m). The concentrations of other micronutrients such as squalene, β -sitosterol, tocopherols (α -tocopherol, β -tocopherol, γ -tocopherol, and δ -tocopherol), and total phenolic compounds are also listed in Table 2 (data collected from other research).

Table 1. Major inclusion and exclusion criteria

Major inclusion criteria
Age 35-69 years old (including 35, 69 years old)
Signing informed consent
Meet any three of the high-risk population criteria:
Body mass index (BMI) female ≥ 24 kg/m ² , male ≥ 26 kg/m ²
Waist to height ratio (WHtR) ≥ 0.5
Waist hip ratio (WHpR) ≥ 0.90 (male) / 0.85 (female)
Ankle Brachial Index > 1.3 or < 0.9
Abdominal circumference (AC) > 90 (male) / 85 (female)
Cholesterol (TC) > 5.2 mmol/L
Triglyceride (TG) ≥ 250 mg/dL
Blood pressure (BP) SBP > 140 mmHg, DBP > 90 mmHg
Fasting blood glucose (FBG) > 6.1 mmol/L [Adjust FBG to > 7.0 mmol/L considering the situation of subjects with elevated fasting blood glucose]
2-h postprandial blood glucose > 7.8 mmol/L
High density lipoprotein < 1.56 mmol/L
Low density lipoprotein > 3.38 mmol/L
Smoking
Major exclusion criteria
Patient diagnosed with cardiovascular and cerebrovascular diseases
Taking or injecting CVD related drugs (e.g.: antihypertensive drugs, insulin)
Have a history of malignant disease (e.g.: tumor)
There are malignant infectious diseases (e.g.: AIDS, hepatitis B, hepatitis C, tuberculosis)
Planned surgery during the trial
Subjects with cognitive impairment or who are unable to take care of themselves
Participating in other clinical trials
Do not sign the informed consent

Food supply personnel will be preparing and supplying food according to the groups, with the diet content mainly referring to the guidelines of the Chinese Diet Pagoda to control the amounts of salt and oil as well as the combinations of meat and vegetables. The salt and edible oil intakes will be controlled at 6 g and 30 g, respectively, requiring accurate weighing by food supply personnel. Dietary macronutrients will be distributed as 45%–65% of carbohydrates, 10%–35% of proteins, and 20%–35% of lipids.⁶⁵ To achieve a nutritional balance and diversified flavors, the menu will be updated every Monday, ensuring that participants would not become bored of the same foods, thus increasing their compliance. However, to reduce differences in the composition of food raw materials and cooking methods and to strictly maintain double-blinded conditions, ingredients in each meal will be exactly the same among the three groups. All ingredients used in the trial will be traditional Chinese foods, including green vegetables, pork, beef, seafood, and rice. The cooking methods used will be traditional Chinese cooking methods such as frying, steaming, boiling, and stewing. Because this measure is in line with Chinese dietary habits and characteristics, participants will likely accept the provided food.

Dietary intervention and follow-up racking

The dietary intervention will last for 3 months, during which all participants will be provided with lunch and dinner corresponding to the dietary pattern group to which they belong. Because the focus of the dietary intervention is to compare possible differences among diets

using different vegetable oils, there will be specific requirements and restrictions for breakfast (which are not provided), snacks, and extra meals. For breakfast, a questionnaire will be used to record food types, and participants will be asked to avoid vegetable oil fats. In addition, snacks and extra meals are discouraged, with those containing vegetable oils especially restricted. However, the intakes of water, fruit, yogurt, nuts, and foods of personal preference (excluding vegetable oil) are unrestricted.

Food supply personnel will place labels on meal boxes including the name and location (location of distribution and consumption) of each participant. Meal delivery personnel will be dispatched to activity centers and public canteens in four communities in Hangzhou according to the locations indicated on the meal boxes. Each community will have 2–3 research personnel who will be responsible for diet supervision. This primarily involves the distribution of meals and the recording of food intake for each participant. Each participant is required to have at least one meal (lunch or dinner) on site every day.

During the dietary intervention period, the follow-up research personnel will conduct satisfaction surveys with participants every 2 weeks (the locations of the surveys are at the four community sites that distribute food) and record participants' views and suggestions on the trial. Afterward, all follow-up research personnel will conduct a summary meeting and adjust the details of the dietary interventions based on feedback from participants (such as the taste of the food and the meal distribution times). In accordance with the results of the survey, participants who fail to comply with the protocols will be questioned

Table 2. Fatty acid compositions and other micronutrients (squalene, β -sitosterol, tocopherol and total phenolic compounds) of CSO, olive oil, and soybean oil[†]

Compositions	Camellia seed oil	Olive oil	Soybean oil
Fatty acid			
C14:0	-	-	-
C16:0	8.76±0.05	12.9±0.04	10.9±0.06
C16:1	-	1.14±0.04	-
C18:0	0.93±0.48	1.04±0.17	3.57±0.16
C18:1n-9	79.5±0.33	74.5±0.12	22.4±0.00
C18:2n-6	9.9±0.04	8.91±0.00	56.1±0.33
C18:3n-6	-	-	-
C18:3n-3	0.34±0.05	0.68±0.04	7.03±0.10
C20:0	-	0.27±0.02	-
C20:1n-9	0.62±0.08	0.22±0.01	-
SFA	9.86±0.43	14.5±0.15	14.6±0.23
USFA	90.1±0.43	85.5±0.15	85.4±0.23
MUFA	79.9±0.34	75.4±0.26	22.4±0.00
PUFA	10.2±0.09	10.0±0.33	63.1±0.23
n-6/n-3	33.3±0.01	12.5±0.00	7.69±0.00
Tocopherols			
α -tocopherol	153-771 ³⁵	10.2-208 ⁵⁸	0.69-0.92 ⁶⁰
β -tocopherol	-	0.75-1.05 ⁵⁸	0.11-0.13 ⁶⁰
γ -tocopherol	9.4-59 ³⁵	0.7-2.1 ⁵⁸	8.07-9.46 ⁶⁰
δ -tocopherol	0.27-28 ³⁵	4.9-15.1 ⁵⁹	1.86-2.46 ⁶⁰
Squalene	110-847 ^{35,46}	200-8260 ⁵⁸	320-750 ⁶¹
Total phenolic compounds	20.6-39.5 ⁶²	213-450 ⁵⁸	480-520 ⁶³
β -sitosterol	107-240 (mg/kg) ³⁵	530-2639 (mg/kg) ⁵⁸	39.9±1.65 (%) ⁶⁴

SFA: saturated fatty acid; USFA: unsaturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acids; n-6/n-3: n-6 polyunsaturated fatty acids/n-3 polyunsaturated fatty acids.

[†]Fatty acid were expressed as relative content in total acid (Mean±SD (%), tocopherols, squalene and total phenolic compounds were expressed as mg/kg and β -sitosterol are expressed as content (mg/kg) / relative content (Mean±SD (%)).

and recorded. Furthermore, participants will be encouraged and guided to improve compliance. If participants continue to deviate from the research protocol, they will be asked to withdraw from the trial. Throughout the intervention, three clinical measurements will be performed at baseline, midterm (intervention for 1.5 months), and late stage (after 3 months' intervention). When midterm and late-stage clinical measurements are taken, the specific details of clinical tests (such as the date, location, and measurement requirements) will be confirmed with participants 1 week in advance, ensuring that each participant can attend on time and in accordance with the rules. The food interventions will be processed as usual during clinical measurements.

Adherence assessment

Compliance with the dietary intervention will be evaluated from two perspectives: those of research staff responsible for follow-up and participants themselves. The follow-up research personnel will assess the compliance of participants based on daily records, including the number of uneaten meals and the remaining quantity of each meal per participant. This will contribute 60% toward the final compliance assessment results. Participants themselves will also conduct self-assessments that will account for 40% of the final assessment results. Participant self-assessments will concern missed meals and food intake quantity. Participants' self-assessments will be collected and recorded in the form of questionnaires.

Data analysis plan

Body weight is the primary outcome of this trial. The trial will be conducted among middle-aged and elderly women living in urban communities in Hangzhou, Zhejiang Province, China (men will be included in follow-up research). According to a previous study, the average weight of the target population is 60 kg.⁶⁶ Therefore, the minimum clinical difference in weight change between the two groups is 1.8 kg. This is because the calculation is generally performed using 3% of the body weight.⁶⁷ The standard deviations (SDs) of the weight changes, projected based on previous research on olive oil dietary interventions, is 3.2 kg.⁶⁵ One-way analysis of variance (ANOVA) will be performed using the G.power software program (version 3.1). Using a power of 90% and a two-tailed significant difference level set at 0.05, the total sample size will be 66. To avoid demolding, the sample size will be raised to 90.

Baseline variables (age, height, and sex) and CVD-related risk factors (body weight, waist circumference, arm circumference, ankle circumference, hip circumference, total cholesterol, TGs, HDL-C, LDL-C, APO-A1, APO-B, Lp (a), AST, LDH, creatine kinase, creatine kinase isoenzyme, ALT, HCY, NT-proBNP, hs-TnI, hs-CRP, Mb, hs-TnT, TNF- α , IL-6, and IL-8) will be presented using descriptive statistics (according to frequencies, means, and SDs) throughout the entire trial (at baseline, midterm, and late stage). Furthermore, the results of the participant compliance analysis and evaluation (including the researcher evaluation and participant self-evaluation) will be expressed as percentages. The analyses of the three intervention groups at different periods

will be performed using Fisher's exact test, the Pearson chi-square test, and ANOVA. Participants will be allocated into the three intervention groups using randomization. A hypothesis test will be performed during the analysis, which means that there will be no significant differences in dietary intake or other possible influencing factors among the three groups of participants.

An intention-to-treat approach will be applied to analyze the primary outcome (weight) and secondary outcomes (lipid components, enzymes, inflammatory factors, and typical biomarkers) to avoid the effects of crossover and withdrawal between the groups, because crossover and withdrawal may cause failed randomization. Thus, participants will be analyzed based solely on their random assignments and without consideration of whether they complied with the entire intervention. The aforementioned analysis will include only participants who completed the intervention. To explore other possible outcomes of the intervention, in future studies, a sensitivity analysis will be performed for all outcome variables, and an analysis of the correlation between different outcome variables will be attempted.

RESULTS

Measurement plan of prevention

The dietary consumption of edible oil and salt will be strictly controlled and recorded before the start of the intervention and daily during the intervention. All detection indicators (physical and biochemical indicators) will be collected three times at baseline, midterm, and late stage. Data collection personnel will be masked from the grouping information during the entire intervention to achieve fairness and blindness. The specific measurement plan is presented in Table 3.

Diet evaluation questionnaire

Participants' diet before participating in the intervention and weekend diets during the intervention will be recorded using a 24-h diet review method. Questionnaire survey personnel will assist all participants to conduct the questionnaire survey twice. This means that the typical dietary content of each participant will be recorded for 2 days.

Anthropometry

Weights will be measured using an electronic scale (accurate to 0.1 kg); weighing time will be arranged after morning urine and before breakfast, and participants will be required to take off their shoes and wear light clothing. Height will be measured by a mechanical height measuring instrument. Waist circumference, arm circumference, ankle circumference, and hip circumference will be measured using an anthropometric measuring tape (150 cm). During measurement, participants will be requested to wear less clothing. The waist circumference will be measured at the end of the participant's exhalation and before the beginning of inhalation. Measurement of the circumference of the midpoint will be performed between the lowest point of the rib and the upper edge of the iliac crest. During arm circumference measurement, the participant's arm is required to sag naturally, and the most protruding part of the upper arm will be taken for measurement. For hip measurement, participants will be asked to

Table 3. Measurement timetable

Trial phases	Measurement
Pre-intervention period	
Screening period	Informed consent 12-h fasting blood [†] Questionnaire of diet preference Questionnaire of typical breakfast pattern Questionnaire of typical diet pattern on weekends A
Intervention period	
Baseline	Anthropometry Blood pressure 12-h fasting blood [†] Fecal sample [‡] Urine sample [§] Hair sample [¶] Nails sample [¶]
Mid-term	Anthropometry Blood pressure 12-h fasting blood [†] Fecal sample [‡] Urine sample [§] Hair sample [¶] Nails sample [¶]
Last-stage	Anthropometry Blood pressure 12-h fasting blood [†] Fecal sample [‡] Urine sample [§] Hair sample [¶] Nails sample [¶]
Each day of intervention	Food record
Each 2 weeks of intervention	Satisfaction survey
Post-intervention period	
Evaluation feedback	Participant compliance self-assessment questionnaire Questionnaire of typical diet pattern on weekends B

[†]Blood samples after 12-h fasting will be collected during the screening period (the same sample will be used at the baseline to avoid a high blood collection frequency), and the midterm and the late stage of the intervention. Plasma and serum (separated from whole blood) will be aliquoted and stored at -80 °C for related testing. Blood cells will be used for further studies related to DNA.

[‡]Fresh fecal samples will be collected under anaerobic conditions, quickly frozen (using liquid nitrogen), and stored at -80 °C, and may be analyzed for gut microbes in follow-up research.

[§]Random urine samples will be stored at -40 °C and indicators in urine related to CVD will be detected in a future study.

[¶]Hair and nail samples will be collected in a sterile sample bag and stored at -40 °C, and some exploratory trials related to CVD will be conducted.

stand with their legs upright, and the most prominent part of the gluteus maximus will be taken for measurement. At the time of measuring the ankle circumference, the participants will be required to stand as wide as their shoulders, and the measurement will be performed at the ankle bone. Blood pressure will be measured using an upper-arm blood pressure monitor (U80EH, Shenzhen Youruian Technology Co., Ltd.). Participants will be required to rest for 10 minutes before measurement, and they must remain in a seated position and maintain their arm (usually the right arm) in a cuff at the same level as the heart.

Detection of biochemical indicators

Biochemical indicators will be determined using blood samples provided by participants after 12-h fasting, and the collection volume will be approximately 15 mL. Detection indicators will mainly include lipid components, enzymes, inflammatory factors, and typical biomarkers. Lipid components, total cholesterol, TGs, HDL-C, LDL-C, APO-A1, APO-B, and Lp (a) will be included. Five enzymes will be detected: AST, LDH, creatine kinase, creatine kinase isoenzyme, and ALT. Furthermore, HCY, NT-proBNP, hs-TnI, hs-CRP, Mb, hs-TnT, TNF- α , IL-6,

and IL-8 are linked with inflammatory factors, as well as being typical biomarkers. All of the aforementioned indicators are related to CVD and can also be altered through the target diet (as supported in this trial), which will be detected in the specific laboratory.

Candidate index detection

The remaining blood samples (serum and plasma separated from whole blood) will be stored at -80 °C, and the extracted blood cells will be cryopreserved in liquid nitrogen. The aforementioned samples may be analyzed in the future, for example by metabolomics, lipidomics, and proteomics, or for CVD-related biomarkers. Fresh fecal samples (collected from participants before breakfast) will be collected under anaerobic conditions. The fecal samples will be quickly frozen using liquid nitrogen and stored at -80 °C and may be analyzed for gut microbes in follow-up research, such as through 16S rDNA sequencing. The collection volume of random urine samples will be approximately 10 mL, stored at -40 °C. At the time of collection, participants will be required to use a sterile urine collection cup to collect midstream urine during urination to avoid contamination. In future studies, indi-

cators related to CVD may be detected in urine. The hair and nail samples will be collected in a sterile sample bag and stored at -40 °C for testing for any content that may be related to CVD.

Data and sample supervision

Data collection personnel will type and arrange original data into the table/questionnaire by using a specific randomized number (to ensure a double-blinded study) that will be sent to data supervision personnel. Original data will be backed up and stored in database A, which will be specific to original data. Data supervision personnel will check the completeness and validity of the data. This means that missing or unclear data will be rejected and returned to data collection personnel until they meet reception standards. Basic information, physical indicators, clinical indicators, questionnaires, and adherence recording are included in data collection. Apart from trial statisticians and data supervisors, the remaining staff will not be allowed to access data and results until the end of the trial. The supervision of sample storage and use will be the responsibility of the sample supervision personnel. The sample supervision personnel will be responsible for ensuring that the storage conditions of various samples complied with the rules, managing the use of the samples and assuring that the remaining sample residues are harmless.

Research management and schedule

The technical staff members of the research team are all from the School of Biosystem Engineering and Food Science of Zhejiang University, with Professor Shen Lirong from Zhejiang University as the chairman of the management system. In addition, four leaders were set up in the communities where the experiment will be conducted. Under the auspices of Professor Shen, research personnel and community leaders will hold a meeting every 2 weeks to verify the progress of the project and notify any possible problems that may arise.

Conclusion

The trial is expected to provide evidence for weight control and improvement of CVD-related metabolic indicators in high-risk cardiovascular populations (especially women) and to provide some reference values for traditional Chinese dietary patterns and nutrition policies.

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AUTHOR DISCLOSURES

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