“Evaluation of the efficacy of calcium α -ketoglutarate in improving human aging”

Study Protocol and StatisticalAnalysis Plan;

Informed Consent Form

2025/6/13

Evaluation of the efficacy of calcium α -ketoglutarate in improving human aging

Ca-AKG (calcium -α -ketoglutarate)

Aging is a progressive physiological decline that occurs over time, characterized by the accumulation of cellular damage, imbalance of tissue homeostasis, and progressive decline in the function of multiple organ systems, ultimately leading to increased individual vulnerability and a significantly higher risk of death. As the global aging process accelerates, this biological phenomenon is posing a serious challenge to human society. According to statistics from the World Health Organization (WHO), the proportion of people aged 60 and above in the world will exceed 20 percent by 2050. At the same time, there is a significant increase in the incidence of age-related chronic diseases such as neurodegenerative diseases, cardiovascular diseases, metabolic syndromes and bone and joint diseases. This trend is particularly prominent in China, the world's most populous country, where the population aged 60 and above is projected to reach 300 million by the end of 2024, accounting for 21.1% of the total population; It is projected that the proportion of people aged 65 and above will increase to 15.6 percent by 2050. Given the significant public health challenges posed by an aging population, anti-aging research and the prevention and treatment of related degenerative diseases have become a key area of modern medical and life science research.

At present, existing anti-aging intervention strategies mainly include dietary adjustments (such as calorie restriction, intermittent fasting and antioxidant diets), anti-aging drugs (such as antioxidant drugs, metformin, rapamycin, etc.) and cell reprogramming (such as induced pluripotent stem cells and partial reprogramming). Although these regimens have shown some anti-aging potential in animal models, their application in humans still faces many problems. For example, dietary adjustments are difficult to adhere to in the long term and the effects vary greatly from individual to individual; Antioxidant drugs have shown some effect in animal models, but the evidence in humans is insufficient; Metformin and rapamycin have potential anti-aging properties, but their long-term effects and safety need to be further verified; Although cell reprogramming technology shows great potential in restoring a youthful

state, there are safety risks such as causing cancer. Therefore, it is of great scientific and social value to explore anti-aging interventions based on new targets that are well tolerated.

α-Ketoglutarate (AKG), as a core metabolic intermediate in the tricarboxylic acid cycle (TCA cycle), plays a key role in cellular energy metabolism, amino acid synthesis and REDOX balance. Studies have shown that AKG can simulate caloric restriction by inhibiting ATP synthase and TOR kinase and activating the AMPK pathway, and has demonstrated longevity effects in a variety of model organisms such as nematodes, fruit flies, yeasts and mice. However, the half-life of oral AKG is less than 5 minutes, and only 40 percent of oral AKG can be absorbed and metabolized by the intestinal mucosa, which limits its potential for clinical application.

Calcium Alpha-Ketoglutarate (CaAKG), as a stable salt form of AKG, not only enhances the chemical stability and intestinal absorption efficiency of the compound, but also synergistic calcium supplementation has potential intervention value for osteoporosis, which is common in the elderly population. Studies have shown that CaAKG intervention can significantly reduce the biological aging level of middle-aged and elderly subjects, but the mechanism of action has not been fully elucidated and there is a lack of large-scale, long-term follow-up randomized controlled trial (RCT) data to support it. At the population level, there is a lack of systematic assessment studies on the effects of CaAKG on age-related indicators in the human body, such as biological age, mitochondrial dysfunction, and age-related gene expression.

For this purpose, this study intends to conduct a randomized, double-blind, placebo-controlled trial to comprehensively evaluate the effects of CaAKG intervention on Phenoage*age, age-related indicators, kinematic indicators, glycolipid metabolism levels, etc. in middle-aged and elderly populations, in order to clarify the health improvement effect of CaAKG intervention on middle-aged and elderly populations To provide population evidence and strategies for the development of new anti-aging intervention strategies.

l. Trial registration and subject inclusion

(1) Trial registration

Registration for this trial should be submitted at https://clinicaltrials.gov in accordance with the randomized controlled study protocol.

(2) Inclusion and exclusion of study subjects

Thirty middle-aged and elderly subjects were recruited and divided into a control group and an experimental group, with 15 subjects in each group.

Inclusion criteria:

1. Age 40-75 years, gender not limited
2. Have not taken any other anti-aging research products in the last two months
3. Be able to use a smartphone
4. The subjects were informed, voluntarily signed the informed consent form, and agreed to participate in all visits and treatments as required by the trial protocol

Exclusion criteria:

1. Having cardiovascular disease, severe/uncontrolled hypertension, rheumatic heart disease, congenital heart disease, deep vein thrombosis, pulmonary embolism
2. Having type 1 / Type 2 diabetes (treated with oral metformin or insulin), diabetes complications
3. Cancer or having undergone surgery, systemic drug therapy, radiotherapy, etc. within the last 3 years
4. Suffering from chronic obstructive pulmonary disease (COPD), severe asthma (daily medication)
5. Suffering from multiple sclerosis, autoimmune/immunodeficiency diseases
6. Recent history of sepsis or infection (hospitalization in the last 3 months)
7. Any mental illness or neurodegenerative disorder
8. Any metal implants in the body
9. Hepatitis/cirrhosis
10. Severe kidney disease (GFR <30 mL/min/1.73 m^2)
11. Having other diseases that are not suitable for participation in this trial (as determined by the investigator)

Ⅱ. Randomization grouping design

Based on community service centers in the urban area of Hangzhou, 30 middle-aged and elderly subjects were screened and recruited. Random block assignment was performed using Stata 13.0 software to evenly distribute age and gender, and the subjects were divided into two groups of 15 people each.

III. Intervention trial process

The researchers explained the research plan to the subjects in detail through oral and written means, and gave the subjects sufficient opportunities to understand the detailed process of the research. During the intervention period, the subjects were required to maintain their original dietary habits and lifestyle.

Subjects were required to have a physical examination before the start of the intervention to obtain baseline information on all subjects included.

Using Stata 13.0 statistical software, 30 subjects were randomly divided into two groups according to the inclusion/exclusion criteria based on the principle of informed consent for patients. On day 0 of the trial, the researchers gave each participant one dose of the intervention. The intervention for the experimental group was calcium α -ketoglutarate tablets, while for the control group it was starch.

The researchers reminded the subjects to take two tablets of the intervention daily with lunch via wechat group chat at noon. Blood and fecal samples were taken on an empty stomach at the beginning of the study intervention and at the end of the intervention phase to test biochemical indicators. Bone density changes in the subjects were measured using a human bone density meter. Subjects were required to maintain the same diet and lifestyle as before during the trial.

IV. Anthropometric measurement, collection, testing and processing of body fluid samples

(1) Anthropometric measurement

Basic information and dietary status of the included subjects were collected as baseline information:

A. questionnaire on demographic characteristics and behavioral information of the subjects: including age, gender, marital status, education, occupation, economic income, basic family information, smoking, drinking, physical activity, and drug use beyond the exclusion criteria restrictions, for analysis and control of confounding factors.

B. Dietary Survey: A 3-day 24-hour dietary survey was used to collect dietary information at baseline and for 3 days before the end of the intervention (including

two non-consecutive working days and one weekend), and a nutrition calculator was used to calculate the intake of various nutrients.

C. Physical Activity Questionnaire: Physical activity assessment was conducted using the Global Physical Activity Questionnaire recommended by the World Health Organization. During the intervention, the number of steps taken by the volunteers was monitored daily via wechat Exercise.

D. Measurements of body composition, height, waist circumference, blood pressure and heart rate: All included subjects were measured for body composition, height, waist circumference, blood pressure and heart rate on days 0 and 90 of the trial, with weight accurate to 0.1 kg; Height and waist circumference measured to 0.1 cm; Blood pressure and heart rate were measured after the subjects sat still for 10 minutes, twice with a 10-minute interval, and the average of the two measurements was taken.

(2) Collection and processing of fluid samples

On the 0th and 90th days of the trial, fasting blood samples of the subjects after fasting for 12 hours were collected and placed in three EDTA anticoagulant tubes (5 mL) and two procoagulant tubes (5 mL) respectively. Plasma and serum samples were separated by centrifugation at 3000 rpm/ min and 4 ° C for 10 min, and monocytes were isolated and stored at -80 ° C. It was used for subsequent tests.

Morning feces of the subjects were collected on days 0 and 90 of the trial. The process of collecting fresh fecal samples: Use a sterile spoon to scoop the inside of the feces (avoiding the part exposed to air) and quickly transfer them to a sterile, enzyme-free fecal storage container (collect 2 samples), immediately freeze them in liquid nitrogen, and then transport them with dry ice to a laboratory -80 ° C refrigerator for future use.

Ⅴ. Outcome indicators

(1) Primary outcome measures:

Biological age (Phenoage*age) is calculated by the Phenoage formula.

Test cycles: 0 days, 90 days

(2) Secondary outcome measures:

A. Bone density measurement: Dual-energy X-ray absorptiometry (DXA/DEXA)

B. Secondary indicators of aging: β -galactosidase activity, superoxide dismutase 2/ catalase (SOD2/CAT), mRNA expression of age-related genes (e.g., Sirt1-7, TERT/TERC, p21 (CDKN1A), p16INK4a(CDKN2A) PDGFRB, PLOD1, MAP4K4, NFKBIA, GFAP, F2RL3, HERPUD1, PPP1R15A, PCBP1, TFAM, MMP7)

C. Body composition: body weight, body mass index (BMI), body fat mass, body fat percentage, fat-free body mass, muscle mass, body water, protein, inorganic salts, etc

D. T cell mitochondrial dysfunction tests: total T lymphocytes ($CD3^+$), helper T cells ($CD4^+$), cytotoxic T cells ($CD8^+$), $CD4^+/CD8^+$ ratio.

E. Kinematic tests: grip strength, physical activity questionnaire, number of finger taps per minute

F. Glucose metabolism: Glycated hemoglobin (HbA1c), fasting blood glucose, fasting insulin, insulin resistance index (HOMA-IR)

G. Lipid metabolism: Serum triglycerides (TG), total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A-i (APOA-i), apolipoprotein B (Apo B)

Test cycles: 0 days, 90 days

(3) Exploratory analysis indicators:

A. Levels of inflammation: Hypersensitive C-reactive protein (hs-CRP), tumor necrosis factor (TNF-α), interleukin-1 β(IL-1β) Interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tumor necrosis factor -α (TNF-α), interleukin-17A Interleukin-12p70 (IL-12p70), interferon -α (IFN-α), interferon -γ (IFN-γ)

B. Gut microbiota: 16s rRNA sequencing

Test cycles: 0 days, 90 days

(4) Safety indicators:

A. Kidney function: UREA (UREA), serum creatinine (CREA), potassium (K), sodium (NA), chlorine (CL), calcium (CA), carbon dioxide binding capacity (CO2-CP), anion gap (AG)

B. Blood routine: White blood cell count (WBC), lymphocyte count (LYM),

monocyte count (MONO), neutrophil count (GRAN), lymphocyte ratio (LYM%), neutrophil ratio (GRPR%), monocyte ratio (MONO%), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean corpuscular hemoglobin concentration (MCHC), corpuscular volume distribution width (RDW), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelet pressure product (PCT), etc

C. Liver function: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), albumin (ALB), total protein (TP), globulin (GLB), albuglobulin ratio (A/G) The ratio of aspartate to alanine (AST/ALT).

D. Vital signs: blood pressure, blood oxygen, pulse, electrocardiogram, lung function, arterial hardness

Test cycles: 0 days, 90 days

VI. Quality control

(1) Test management and data monitoring

The project team conducts periodic summaries and discussions through meetings every two weeks to analyze the problems existing in the trial process and make timely improvements. Double data entry is used to ensure the completeness and accuracy of the data entry. Each test indicator (baseline and follow-up period) of each subject should be carefully recorded and archived. Data should be stored in triplicate (one in the cloud database, one on the work computer, and one in paper form) to prevent data loss. To protect the privacy of the subjects, all staff who have access to the data should sign a confidentiality agreement, and unauthorized staff are not allowed to browse or use the data.

(2) Compliance assessment

The researchers established wechat contacts with each volunteer and reminded the subjects daily to take Ca-AKG (calcium -α -ketoglutaric acid) tablets/placebo on time, and the subjects were required to finish taking them within the prescribed time.

Checks were conducted at irregular intervals during the intervention, and if there were any remaining drugs, the remaining amounts were recorded. Subjects who consumed less than 85% of the drug throughout the trial period were ultimately excluded. Meanwhile, at baseline (day 0) and at the end of the intervention (day 90), researchers will measure the bone density of volunteers using dual-energy X-ray absorptiometry (DXA/DEXA) to verify the population's adherence to Ca-AKG (calcium -α -ketoglutaric acid) tablets/placebo by comparing the changes in bone density levels before and after the intervention between the two groups.

Ⅶ Reporting of adverse events

no

ⅧInformed Consent

The project leader should explain the research content, form and precautions to the research subjects. In addition, the project leader should make it clear to the research subjects that participation in this study is voluntary and entirely up to the subjects themselves. If a patient decides to participate, they also have the right to withdraw at any time without any adverse effect on current and future treatment. Once a study subject withdraws from the study, no items for the study will be provided to him/her.

Ⅸ. Primary endpoint events, secondary endpoint events

Primary endpoint event: Abnormal physical indicators in the subjects.

Ⅹ . Data processing and statistics

The trial data included in this study were analyzed using the intention-to-treat principle (ITT).

Statistical analysis of the data was performed using Stata 15.0 and R 4.3.2 software. The data of this study were divided into count and measurement data. For continuous variables that met the normal distribution, statistical description was conducted using mean ± standard deviation or confidence interval. For categorical variables, statistical description was conducted using frequency and percentage. Independent sample t-tests were used to assess the differences between groups in baseline characteristics. Data that did not meet the normal distribution were statistically described using the median (interquartile range), and the Wilcoxon rank sum test was used to assess the between-to-group differences in baseline characteristics. The primary outcome variables were analyzed using the analysis of

covariance (ANCOVA) to compare the differences in outcome measures between the two groups after the intervention. The corrected covariates included age, biological age of the subjects, and baseline values; Other outcome variables were analyzed using repeated measures analysis of variance, adjusted for age and baseline values to avoid confounding bias. Use Hierarchical Test Procedures to control the inflation of a class of errors. A Marginal structural model was used to analyze the sensitivity of the results. The changes in gut microbiota between groups were evaluated using analyses such as PCA, followed by screening for significant differences using analyses such as PLS-DA, OPLS-DA, LEfSe, and metabolic pathway analysis of the differences using Cytoscape v.3.2.1 software, combined with related gene and metabolic database (KEGG) Multi-omics association analysis, multi-pathway extension analysis, interaction network and bioregulatory model construction analysis were performed. $P < 0.05$ was considered statistically significant.

XI. Sample pretreatment and preservation

During the testing of all indicators of the subjects, some indicators need to be tested or preprocessed immediately. Blood, urine and feces are all required to be retained. The sample size should be the same as the normal test volume and should be stored in a -80 ° C refrigerator.

Informed consent form. Notification page

Dear volunteers:

We are going to conduct a study on the efficacy evaluation of calcium α -ketoglutarate in improving human aging. Your specific circumstances meet the inclusion criteria for this study. Therefore, we invite you to participate in this study. Please read carefully the following information to understand the purpose, procedures and duration of the study, as well as the benefits, risks and discomforts that participating in the study may bring to you.

Here is an introduction to the study:

I. **Research Background:**

Aging is the physiological decline that occurs with age, accompanied by the accumulation of cell damage and the decline of multiple organ functions, significantly increasing the risk of death. The global aging trend is severe, and the WHO predicts that more than 20 percent of the population will be over 60 years old by 2050. China's aging population is particularly prominent, with 300 million people aged 60 and above projected for 2024 (21.1%). Given the major public health challenges posed by an aging population, anti-aging research and the prevention and treatment of related degenerative diseases have become a key area of modern medical and life science research.

Existing anti-aging strategies mainly include dietary interventions (such as calorie restriction), drugs (such as metformin and rapamycin), and cell reprogramming, but these strategies have problems such as large individual differences and unverified safety. α -ketoglutaric acid (AKG), as a metabolite of the TCA cycle, mimics the calorie restriction effect and shows longevity in animal models, but has low oral bioavailability. Its stable form, calcium α -ketoglutarate (CaAKG), enhances absorption and also serves as a calcium supplement. Studies have shown that CaAKG intervention significantly reduces biological aging levels in middle-aged and elderly subjects, but the mechanism of action has not been fully elucidated and there is a lack of data from large-scale, long-term follow-up randomized controlled trials (RCTS) to support it. At the population level, there is a lack of systematic assessment studies on the effects of CaAKG on age-related indicators in the human body, such as biological age, mitochondrial dysfunction, and age-related gene expression.

Ⅱ. Study Objectives:

For this purpose, this study intends to conduct a randomized, double-blind, placebo-controlled trial to

comprehensively evaluate the effects of CaAKG intervention on Phenoage*age, age-related indicators, kinematic indicators, glycolipid metabolism levels, etc. in the middle-aged and elderly population, in order to clarify the health improvement effect of CaAKG intervention on the middle-aged and elderly population To provide population evidence and strategies for the development of new anti-aging intervention strategies.

Ⅲ. Specific Procedures and Processes:

(1) Population inclusion and exclusion criteria

Main inclusion criteria: 1. Age 40-75 years, gender not limited; 2. Have not taken any other anti-aging research products in the last two months; 3 Be able to use a smartphone; 4. The subject was informed, voluntarily signed the informed consent form, and agreed to participate in all visits and treatments as required by the trial protocol.

Main exclusion criteria: 1. Having cardiovascular disease, severe/uncontrolled hypertension, rheumatic heart disease, congenital heart disease, deep vein thrombosis, pulmonary embolism; 2. Having type 1 / Type 2 diabetes (treated with oral metformin or insulin), diabetic complications; 3. Cancer or having undergone surgery, systemic drug therapy, radiotherapy, etc. within the last 3 years; 4. Suffering from chronic obstructive pulmonary disease (COPD), severe asthma (daily medication); 5. Suffering from multiple sclerosis, autoimmune/immunodeficiency diseases; 6. Recent history of sepsis or infection (hospitalization within the last 3 months); 7. Any mental illness or neurodegenerative disorder; 8. Any metal implants in the body; 9. Hepatitis/cirrhosis; 10. Severe kidney disease (GFR <30 mL/min/1.73 m^2); 11. Having other diseases that are not suitable for participation in this trial (as determined by the investigator).

(2) Specific implementation steps

1. Collection of basic information and health status information of the research subjects

The researchers explained the study plan to the volunteers in detail both orally and in writing, and gave them ample opportunities to learn about the detailed process of the study. During the intervention, the volunteers were required to maintain their original dietary habits and lifestyle.

Before the intervention trial begins, the subjects need to undergo a physical examination once to obtain the baseline information of all included subjects.

Main survey contents: (a) Conduct a dietary survey study on the subjects using the 3-day 24-hour dietary record method; (b) Survey of demographic characteristics and behavioral information of volunteers: age, gender, marital status, education, occupation, economic income, basic family situation, smoking and fitness activities, etc. (c) Physical activity survey: The WHO Global Physical Activity Questionnaire was used for assessment, and during the intervention period, the number of steps taken was monitored daily via wechat Exercise; (d) Measure basic physical indicators such as body composition, height, waist circumference, blood pressure and heart rate. With weight accurate to 0.1kg and height and waist circumference accurate to 0.1cm, wear light clothing and remove shoes and hats during the measurement. Blood pressure and heart rate were measured after the volunteers sat still for minutes, with a 10-minute interval between the second and first measurements, and the average of the two measurements was taken.

2. Bone density test

3. Blood tests

Age-related indicators: β -galactosidase activity, superoxide dismutase 2/ catalase (SOD2/CAT), mRNA expression of age-related genes (e.g., Sirt1-7, TERT/TERC, p21 (CDKN1A), p16INK4a(CDKN2A) PDGFRB, PLOD1, MAP4K4, NFKBIA, GFAP, F2RL3, HERPUD1, PPP1R15A, PCBP1, TFAM, MMP7)

T cell mitochondrial dysfunction detection: total T lymphocytes (CD3+), helper T cells (CD4+), cytotoxic T cells (CD8+), CD4+/CD8+ ratio.

Glucose metabolism: Glycated hemoglobin (HbA1c), fasting blood glucose, fasting insulin, insulin resistance index (HOMA-IR)

Lipid metabolism: Serum triglycerides (TG), total cholesterol (CHOL), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A-i (APOA-i), apolipoprotein B (Apo B)

Levels of inflammation: Hypersensitive C-reactive protein (hs-CRP), tumor necrosis factor (TNF-α), interleukin-1 β(IL-1β) Interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-5 (IL-5), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tumor necrosis factor -α (TNF-α), interleukin-17A Interleukin-12p70 (IL-12p70), interferon -α (IFN-α), interferon -γ (IFN-γ)

Kidney function: UREA (UREA), serum creatinine (CREA), potassium (K), sodium (NA), chlorine (CL), calcium (CA), carbon dioxide binding capacity (CO2-CP), anion gap (AG)

Liver function: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyl transpeptidase (γ-GT), alkaline phosphatase (ALP), total bilirubin (TBIL), direct bilirubin (DBIL), indirect bilirubin (IBIL), albumin (ALB), total protein (TP), globulin (GLB), albuglobulin ratio (A/G) The ratio of oryzol-alanine (AST/ALT)

Blood routine: White blood cell count (WBC), lymphocyte count (LYM), monocyte count (MONO), neutrophil count (GRAN), lymphocyte ratio (LYM%), neutrophil ratio (GRPR%), monocyte ratio (MONO%), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin content (MCH), mean corpuscular hemoglobin concentration (MCHC), corpuscular volume distribution width (RDW), platelet (PLT), mean platelet volume (MPV), platelet distribution width (PDW), platelet pressure product (PCT), etc.

4. Detection of vital signs

Blood pressure, blood oxygen, pulse, electrocardiogram, lung function, arterial hardness, etc.

5. Fecal tests

Gut microbiota: 16s rRNA sequencing

6. Kinematic detection

Grip strength, physical activity questionnaire, number of finger taps in one minute, etc.

7. Body composition tests

Weight, body mass index (BMI), body fat mass, body fat percentage, fat-free body mass, muscle mass,

body water, protein, inorganic salts, etc.

8. Intervention strategies

The researchers explained the study protocol to the subjects in detail both orally and in writing, and gave them ample opportunities to understand the detailed process of the study. During the intervention, the subjects were required to maintain their original dietary habits and lifestyle. Subjects were required to have a physical examination before the start of the intervention to obtain baseline information on all subjects included.

Using Stata 13.0 statistical software, 30 subjects were randomly divided into two groups according to the inclusion/exclusion criteria based on the principle of informed consent for patients. On day 0 of the trial, the researchers gave each participant one dose of the intervention. The intervention for the experimental group was calcium α -ketoglutarate tablets, while for the control group it was starch.

The researchers reminded the subjects to take two tablets of the intervention daily with lunch via wechat group chat at noon. Blood and fecal samples were taken on an empty stomach at the beginning of the study intervention and at the end of the intervention phase to test biochemical indicators. Bone density changes in the subjects were measured using a human bone density meter. Subjects were required to maintain the same diet and lifestyle as before during the trial.

Ⅳ. Benefits you may have from participating in this study:

Calcium α -ketoglutarate supplements may lower your biological age, increase your bone density and muscle mass, improve your motor function, lower your inflammatory levels, improve glycolipid metabolism, and reduce the risk of age-related diseases.

If you participate in the study, your doctor will closely monitor your condition, and a registered dietitian will educate you on anti-aging knowledge and guide you on dietary habits.

For this study, calcium α -ketoglutarate supplements and medical examinations as per the trial protocol will be provided free of charge.

We hope that the information from your participation in this study will benefit more people.

To fully protect your rights, we have developed a detailed intervention study protocol, which has been reviewed and approved by the Medical Ethics committee, and we will implement this nutritional supplement intervention protocol strictly in accordance with the protocol.

Ⅴ . Adverse reactions, risks and risk prevention measures that may arise from your participation in this study

The intervention was well tolerated and no adverse reactions were found.

The execution of this research project will be strictly carried out in accordance with the norms. However, this process may cause some adverse reactions, which are hereby informed as follows: Patients participating in the trial will undergo blood drawing during the trial period. The risks of blood drawing from the arm include temporary discomfort and/or cyanosis. Infection, bleeding, clotting or fainting may occur, though the likelihood is low. Once such risks and accidents occur, physicians will take active response measures

If you experience any adverse reactions between trials, please call your study physician for advice promptly. If your health does suffer from research-related impairments as a result of your participation in this

study, the sponsor will cover the cost of treatment and provide you with corresponding financial compensation in accordance with national regulations.

We solemnly promise to minimize the probability of the risks and adverse consequences described above.

Ⅵ. Explanation of Costs

Subjects will participate free of charge in all project segments of this trial.

Ⅶ Compensation for participation in this study, including damages

If you suffer an injury related to this trial study, Zhejiang Chinese Medical University will receive corresponding treatment, compensation and indemnity in accordance with relevant national laws and regulations.

Ⅷ Confidentiality of your Personal Information

Information about your participation in this study will be recorded in the study record/case report form. All results of trials that appear in the original medical records (including personal data, test reports, etc.) will be kept strictly confidential to the extent permitted by law. Your name will not appear in the report form; only the number assigned to you when you participated in the trial will be shown. Your number will only appear in research summaries, articles, and public publications if necessary.

When necessary, drug regulatory authorities, ethics committees, or project funding departments may, in accordance with regulations, access the information of the participants in the study. But they will not use the information of the participants for any other purpose or disclose it to any other group without permission.

Ⅸ. Do you have to participate in this study?

Whether to participate in this study is entirely up to your own will, and you may refuse to participate in this study.

Ⅹ . Can you withdraw from this trial halfway?

You have the right to withdraw from the study at any time during the course of the study. If you opt out of this study, your health rights outside of this study will not be affected, nor will you be discriminated against or treated unfairly as a result.

Your doctor or investigator may discontinue your participation in this trial at any time for your best interests.

If you withdraw from the trial for any reason, you may be consulted about your use of the investigational drug. You may also be required to undergo laboratory tests and physical examinations if your doctor deems it necessary. You may also refuse without being discriminated against or treated unfairly as a result.

XI. Ethics Committee

If you have any doubts about this study, you can contact the person in charge of this study at the phone number provided below.

Contact 1:Chenxiang Shi Tel: 086 13685867718

Contact 2: Jiaomei Li Tel: 086 18158519707

Please keep this information.

Informed consent. Consent signature page

Statement of Consent

1. I have read this informed consent form and the person responsible for the project has provided me with a detailed explanation of the purpose, content, risks and benefits of this trial.

2. I have discussed and asked questions related to this study, and I am satisfied with the answers to these questions.

3. I had plenty of time to make a decision.

4. I voluntarily agree to participate in the scientific research described herein and consent to the use of my research data for the publication of this research.

5. I consent to the drug regulatory authority, the ethics committee, or the project funding department having access to my research materials.

6. I will receive a copy of the signed and dated informed consent form.

Finally, I decided to agree to participate in this trial study.

Subject Signature: Date:_____Year_____Month_____Day

Contact number of the subject:

I confirm that I have explained the details of this study to the subjects, including their rights as well as possible benefits and risks, and provided them with a copy of the signed informed consent form.

Researcher's Signature: Date:_____Year_____Month_____Day

Contact information of the researcher:

Ethical review opinion of Zhejiang University of Traditional Chine se Medicine

Application number	20250613-2
Project name	Evaluation of the efficacy of calcium α -ketoglutarate in improving human aging
Project origin	Horizontal topics
Research Unit	School of Public Health, Zhejiang University of Traditional Chinese Medicine
Lead researcher	Jiaomei Li
Review modalities	Quick review
Review date	June 13,2025
Audit-revie w file	1. Application form for ethical review of scientific research projects by medical Ethics Committee; 2. Project contract; 3. Informed consent form

Review comments

In accordance with the Ethics Review Methods for Human Life Sciences and Medical Res earch (2023), SFDAs Good Clinical Practice for Drug Clinical Trials (2020), WMAs Declaration of Helsinki, and CIOMS International Ethical Guidelines for Human Biomedical Research, the Ethics Committee has reviewed the case and provides the following opinions:

The project complies with the principles of the Helsinki Declaration and relevant Chinese policies and regulations, and agrees to carry out the research.

Medical Ethics Committee of Zhejiang University of Traditional Chinese Medicine

2025 June 13