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A Randomized Controlled Trial of Branched-Chain Amino Acid Supplementation for Preventing Myopia Progression

Study Protocol

Version No.: 04

Version Date: July 1, 2026

Document updated on July 1, 2026, to incorporate minor corrections and clarifications. This version reflects the protocol implemented since study initiation.

Project Title

A Randomized Controlled Trial of Branched-Chain Amino Acid Supplementation for Preventing Myopia Progression

I. Study Content

1. Study Objectives

1.1 Primary Objective

To evaluate the effect of BCAAs on the prevention of myopia progression through a randomized controlled trial (RCT).

1.2 Secondary Objective

To investigate whether the effect of BCAA supplementation varies according to age, sex, body build, and BCAA intake under different dietary patterns.

2. Study Content

2.1 Selection of Study Site

This is a randomized, double-blind clinical trial. Approximately 104 university students aged 18 to 25 years will be recruited from Nanjing to participate in the study. All participants will undergo vision examinations at the Department of Ophthalmology, Zhongda Hospital, with on-site guidance and assistance from the hospital's optometrists.

2.2 Selection of Study Participants

Source of participants: University students aged 18 to 25 years in Nanjing.

Inclusion criteria:

1) Healthy university students aged 18-25 years;

2) Spherical equivalent refraction (for eyes without prior corneal refractive surgery): SE ≤ -0.50 D;

3) Corrected visual acuity ≥ 0.8;

4) Corneal curvature 40 D-46 D;

5) Good compliance and ability to cooperate with follow-up and examinations;

6) Willingness and ability to participate in this study and sign the informed consent form.

Exclusion criteria:

1) Strabismus, amblyopia, or other pathological ocular changes;

2) Systemic diseases affecting refractive development and allergic constitution, such as diabetes, metabolic syndrome, etc.;

3) Active eye disease or history of ophthalmic surgery;

4) Use of orthokeratology lenses or other myopia-control interventions within the past 6 months;

5) Use of BCAA-related supplements within the past 6 months, or serum BCAA levels outside the reference range;

6) Considered by the responsible physician to be unable to participate or unable to comply with the study requirements;

7) Abnormal liver or kidney function.

Sample size

Assuming a two-sided significance level of 0.05, a statistical power of 80%, an expected between-group difference in refractive error change of 0.27 D, and a common standard deviation of 0.45 D, the required sample size was calculated to be approximately 44 participants per group. To allow for an anticipated 15% loss to follow-up, the sample size was increased to 52 participants per group. Therefore, a total of 104 participants will be recruited, with 52 participants allocated to the intervention group and 52 participants allocated to the control group.

$$N = 2 \times \frac{(Z_{\propto} + Z_{\beta})^2 \times \sigma^2}{d^2}$$

d: expected mean difference in refractive error between the intervention group and the control group, d = 0.27 D

σ: estimated standard deviation, σ = 0.45 D

Zα: corresponding standard normal deviate for $\alpha = 0.05$

Zβ: corresponding standard normal deviate for $\beta = 0.20$

r: loss-to-follow-up rate, r = 15%

Methods for participant recruitment and allocation

Recruitment process

Participants will be recruited from myopic university students aged 18–25 years in Nanjing through open recruitment strategies, including campus advertisements, electronic announcements, and social media platforms. Individuals expressing interest in the study will undergo a preliminary eligibility screening based on the predefined inclusion and exclusion criteria.

Potentially eligible individuals will be invited to attend a screening visit, during which comprehensive ophthalmic examinations and baseline assessments will be performed to confirm study eligibility. Eligible participants will receive detailed verbal and written information regarding the study objectives, procedures, potential risks, and expected benefits. Written informed consent will be obtained from all participants before any study-specific procedures are initiated.

Recruitment will continue consecutively until the target sample size of 104 participants has been enrolled.

Allocation method

Eligible participants will be stratified according to baseline myopia severity (low, moderate, and high myopia) before randomization. Within each stratum, participants will be randomly assigned in a 1:1 ratio to either the intervention group or the control group group using a computer-generated randomization sequence. The randomization schedule will be prepared by an independent statistician who is not involved in participant recruitment, clinical assessment, or outcome evaluation. Allocation concealment will be maintained using sequentially numbered, opaque, sealed envelopes until the time of assignment.

Informed consent of participants

Researchers will provide a paper informed consent form and explain the main contents of the study. Participants will sign the informed consent form offline.

Outcome measures

1) **Myopia:** According to the diagnostic criteria proposed in the Guidelines for the Prevention and Control of Myopia (2024 Edition), myopia is classified into low, moderate, and high myopia based on cycloplegic spherical equivalent (SE) refraction (SE = sphere + 1/2 cylinder): low myopia, $-3.00\ D < SE \leq -0.50\ D$; moderate myopia, $-6.00\ D < SE \leq -3.00\ D$; and high myopia, $SE \leq -6.00\ D$.

2) **Main measurements in this study:** Including basic ophthalmic parameters, ocular structural parameters, and OCTA imaging parameters. Basic parameters include refractive status, axial length, visual acuity, and intraocular pressure. Structural parameters include retinal and choroidal thickness. OCTA parameters assess the optic disc and macular region, including retinal nerve fiber layer thickness, vessel density, retinal layer thickness, and vascular perfusion characteristics.

Intervention methods

Intervention group: Participants assigned to the intervention group will receive branched-chain amino acid (BCAA) supplementation. Each participant will take two oral capsules once daily after breakfast or dinner for 6 consecutive months. Each capsule contains 300 mg leucine, 150 mg isoleucine, and 150 mg valine, providing a total daily BCAA dose of 1.2 g. The BCAA capsules will be supplied by Life Extension (USA) and manufactured in accordance with Good Manufacturing Practice (GMP) standards. Participants will be instructed to maintain their usual diet and lifestyle throughout the intervention period and to avoid taking any additional BCAA-containing supplements during the study.

Control group: Participants assigned to the control group will receive matching placebo capsules containing medium-chain triglycerides (MCT). Each participant will take two oral capsules once daily after breakfast or dinner for 6 consecutive months. The placebo capsules will be manufactured in accordance with Good Manufacturing Practice (GMP) standards and will be identical to the BCAA capsules in appearance, size, color, odor, and packaging to ensure blinding throughout the study.

3. Study Methods

3.1 General Ophthalmic Examinations

1) **Spherical equivalent refraction (SE):** Cycloplegic autorefraction will be performed using the KR-800 automated kerato-refractometer (Topcon (Dongguan) Technology Co., Ltd., Dongguan, China) by certified optometrists. Cycloplegia will be induced with one drop of 0.5% proparacaine hydrochloride, followed 1 minute later by one drop of 1% cyclopentolate hydrochloride and a second drop 5 minutes later. Autorefraction will be performed at least 30 minutes after the first cyclopentolate instillation when the pupil diameter is ≥6 mm and the pupillary light reflex is absent. Three consecutive measurements will be obtained, with differences in both spherical and cylindrical power not exceeding 0.25 diopters (D). The mean of the three measurements will be used to calculate SER as spherical power plus one-half of the cylindrical power (SER = sphere + cylinder/2).

2) **Axial length (AL):** AL will be measured using the SUOER SW-9000 ophthalmic optical biometer. The height of the instrument will be adjusted according to the participant's height. Participants will be instructed to stabilize the chin and forehead on the chinrest and forehead rest, and eye position will be adjusted accordingly. During measurement, the participant will look at the fixation target and keep the eye still. Axial length will be measured three times and averaged.

3) **Retinal and choroidal microcirculation parameters:** Measurements will be performed using an ophthalmic optical coherence tomography angiography (OCTA) instrument. The height of the instrument will be adjusted according to the participant's height. Participants will be instructed to stabilize the chin and forehead on the support and adjust eye position appropriately. A macular fixation scanning mode will be used to scan a 12 mm × 12 mm area centered on the fovea. A disc scanning mode will be used to scan a 6 mm × 6 mm area centered on the optic disc. The built-in software automatically segments the retinal layers, choriocapillaris, and peripapillary related layers and generates blood-flow images for the corresponding layers. Based on automatic software analysis, superficial macular blood flow, deep blood flow, whole-retina blood flow, and choriocapillaris perfusion parameters will be obtained, including blood flow/perfusion percentages in the fovea, inner ring, and outer ring subregions. At the same time, blood-flow parameters of the retinal nerve fiber layer in the optic disc region will be obtained, including

average blood flow, four-quadrant blood flow, and blood-flow indices for each subregion. Meanwhile, parameters related to the foveal avascular zone (FAZ) will be recorded, including FAZ area, perimeter, and FAZ300. Images with poor automatic segmentation or identification will be checked and, if necessary, corrected by trained researchers before inclusion in the analysis.

3.2 Blood Biochemical Testing

Blood biochemical testing in this study will be completed by the Department of Laboratory Medicine, Zhongda Hospital affiliated to Southeast University. Fasting venous blood samples (3-5 mL, after at least 8 hours of fasting) will be collected in the early morning from all participants. After standing at room temperature, samples will be centrifuged (approximately 3000 rpm for 10 min) to separate serum, and the tests will be completed within the specified time frame or samples stored under standard conditions. Test indicators include liver function (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], total bilirubin, albumin), renal function (serum creatinine [Scr], blood urea nitrogen [BUN], uric acid [UA]), glucose metabolism (fasting plasma glucose [FPG]), and lipid indices (total cholesterol [TC], triglycerides [TG], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C]).

3.3 Measurement of Serum BCAA Levels

A 3 mL fasting blood sample will be drawn from the participant's antecubital vein. After centrifugation at 3000 rpm/min for 15 minutes, 1 mL of upper-layer serum will be transferred into a 10 mL stoppered centrifuge tube, and serum BCAA concentrations will be determined by liquid chromatography-mass spectrometry (LC-MS).

3.4 Physical Examination

Physical examination will be conducted in accordance with WS/T424-2013 *Anthropometric Methods*. Body weight, height, waist circumference, hip circumference, and blood pressure will be assessed. Height and weight will be measured to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) = weight (kg)/height^2 (m^2). Blood pressure will be measured twice at 5-minute intervals, and the average value will be used.

3.5 Measurement of Factors Associated With Myopia

Questionnaires will be used to collect baseline information from participants. Standardized case report forms will record demographic characteristics, medical history, and family history at baseline (e.g., parental myopia). At the same time, the questionnaire will collect information on lifestyle-related risk factors, such as outdoor activity and near-work time, visual habits, physical activity, and dietary patterns. It will also include a food frequency questionnaire to determine participants' reported intake of foods rich in BCAAs.

3.6 Statistical Methods

Statistical analyses will be performed using R software (version 4.5.1). Ocular parameters from both eyes will be included in the analyses. Participants with baseline outcome measurements and at least one post-randomization assessment will be included in the statistical analyses. Missing data will be handled using the last observation carried forward (LOCF) method. Continuous variables will be summarized as mean ± standard deviation (SD) or median (interquartile range [IQR]), as appropriate, and categorical variables will be presented as frequencies and percentages. Generalized estimating equations (GEE) will be used to evaluate changes in spherical equivalent refraction, axial length, uncorrected visual acuity, and scleral, choroidal, and retinal thickness over time, with time as the within-subject factor, intervention group as the between-subject factor, and the group-by-time interaction as the primary effect of interest. The GEE models will be adjusted for the corresponding baseline outcome values, age, sex, number of myopic parents, outdoor light exposure time, and near-work time. All statistical tests will be two-sided, and a *P* value < 0.05 will be considered statistically significant.

4.Study Technical Roadmap

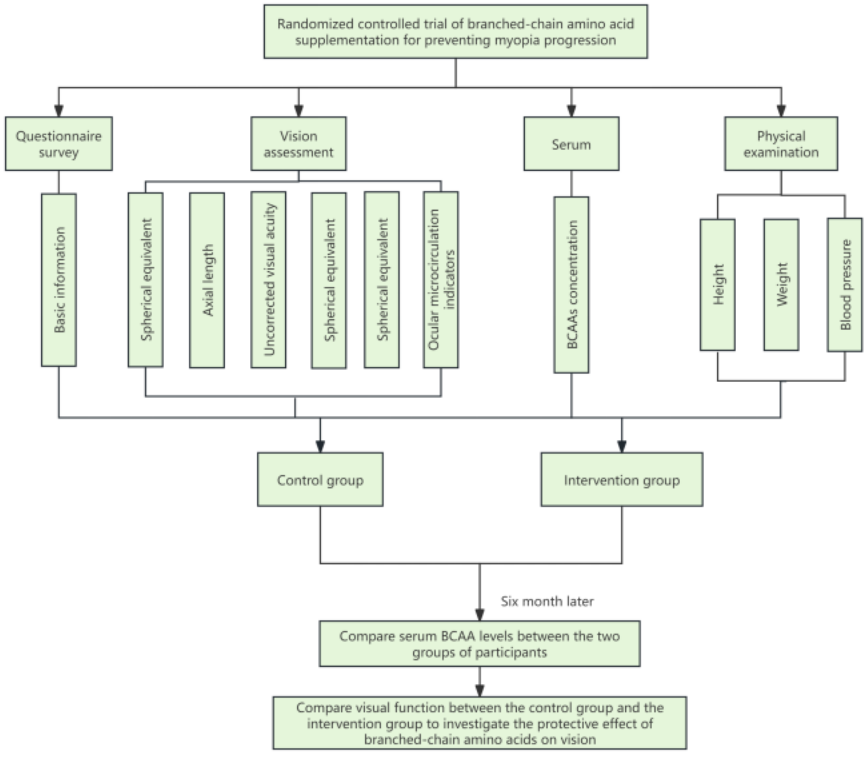


Figure 1. Technical Roadmap

5.Feasibility Analysis

5.1 Feasibility of the Research Concept

This study closely addresses the major challenge currently faced in public health—myopia—and, from a nutrition perspective, focuses on exploring the relationship between serum BCAAs and myopia progression. By evaluating the effect of BCAA supplementation on preventing myopia progression

through an RCT, the study proposes a new nutritional approach to myopia prevention and control and is both feasible and forward-looking.

5.2 Feasibility of the Human Study

The applicant has extensive experience in population-based health research and field investigations. Through previous myopia research projects, a stable collaborative platform has been established with the Affiliated Zhongda Hospital of Southeast University, providing strong support for participant recruitment, ophthalmic examinations, and study implementation. As the present study will be conducted among university students, who represent a relatively stable and accessible population with good compliance and follow-up feasibility, the proposed study is highly feasible.

5.3 Feasibility of the Research Team

Members of the project team come from the School of Public Health of Southeast University and Zhongda Hospital affiliated to Southeast University, including faculty members and researchers with rich experience in field organization, statistical analysis, laboratory research, and biomarker testing. With clearly assigned responsibilities and close collaboration, the team provides organizational and personnel support for the study design, implementation, testing of indicators, and smooth conduct of statistical analyses.

6. Innovation of the Project

Building on our previous animal studies suggesting a role of branched-chain amino acids (BCAAs) in myopia progression, this study is the first randomized controlled trial to evaluate the effect of BCAA supplementation on myopia progression in humans. By translating preclinical findings into clinical research, this study will provide clinical evidence for a novel nutrition-based strategy for myopia control.

7. Research Plan and Expected Progress

1) **August to October 2025:** Review the literature, further refine the study protocol, obtain ethics approval for the human study, develop standard operating procedures (SOPs), and provide standardized training for all study personnel to complete the preparatory work.

2) **December 2025 to March 2026:** Recruit university students in Nanjing, obtain written informed consent, conduct baseline assessments including questionnaire surveys, biological sample collection, and ophthalmic examinations, collect baseline data, complete randomization, and initiate the randomized controlled trial.

3) **April to October 2026:** Implement the randomized controlled trial according to the study protocol, complete scheduled follow-up visits, biological sample collection, and ophthalmic examinations, conduct final assessments and outcome measurements, and establish and verify the study database to ensure data completeness and quality.

4) **November 2026 to March 2027:** Perform data cleaning and statistical analyses, evaluate the effects of branched-chain amino acid supplementation on myopia progression and related ocular biological parameters, investigate the associations between BCAA levels and lifestyle factors and dietary patterns, summarize the study findings, prepare and submit SCI manuscripts, and complete the final project report.

8. Expected Research Outcomes

(1) Based on animal experiment results, this randomized controlled trial aims to clarify the differences between the BCAA intervention group and the control group in changes in refractive error, axial length, choroidal and retinal thickness, and serum BCAA concentration, and to evaluate the intervention effect.

(2) Provide a scientific basis for the clinical application of BCAAs to improve myopia progression.

II. Foundation and Conditions for the Work

1. Research Foundation

The project team has long been engaged in human and animal research on branched-chain amino acids, particularly the effect of BCAAs on scleral thickness during myopia progression, and has conducted multiple cohort and metabolomics studies. The main research foundations related to this project are as follows:

1.1 Abnormal Serum BCAA Metabolism and Myopia Progression in Adolescents

Under the support of the National Natural Science Foundation of China General Program led by the applicant, entitled *Abnormal Serum Branched-Chain Amino Acid Metabolism and Adolescent Myopia Progression and the Mechanism of mTORC1-Regulated Scleral Remodeling*, a previously established adolescent myopia cohort was used. At baseline, questionnaires and blood samples were collected from 304 senior high school students. Using incident myopia in previously non-myopic students or progression in already myopic students after two years as outcomes, a nested case-control study was conducted (Group A as controls and Group B as cases). Untargeted metabolomics was performed on serum samples from the case and control groups. After normalized statistical processing of the serum testing data, a total of 311 annotated metabolic compounds were identified. In the case group, leucine (Figure 2A), isoleucine (Figure 2B), and valine (Figure 2C) levels were significantly lower than those in the control group ($P < 0.05$). Thus, it was preliminarily concluded that serum BCAA metabolism is negatively associated with myopia progression, pending further targeted metabolomics verification in an expanded adolescent cohort. Changes in serum BCAAs in the control and case groups are shown in Figure 2.

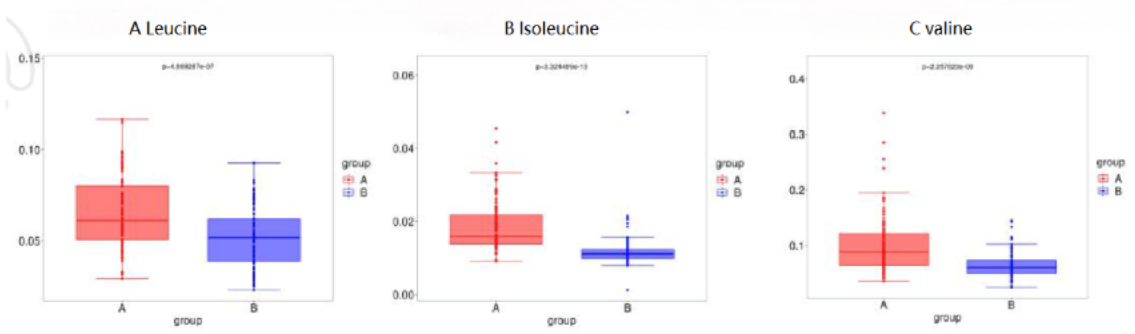


Figure 2. Boxplots comparing BCAA metabolites between the myopia progression group (Group B) and the non-myopia progression group (Group A)

1.2 Study of the Mechanism by Which BCAAs Regulate Scleral Remodeling Through mTORC1

Under the support of the same National Natural Science Foundation of China General Program led by the applicant, a series of preliminary cell experiments confirmed that BCAAs can affect the transdifferentiation of scleral fibroblasts. The research group treated mouse scleral fibroblasts with different concentrations of BCAAs and identified 5 mg/mL as the optimal concentration. Cell viability testing was then performed. CCK-8 cell viability assay results showed that treatment of primary mouse scleral fibroblasts with 5 mg/mL BCAAs for 24 hours did not significantly reduce cell viability (Figure 3A), indicating that BCAAs are not cytotoxic under the proposed treatment conditions of this project. In addition, experiments on scleral fibroblast-to-myofibroblast transdifferentiation showed that after 12 hours of treatment with 5 mg/mL BCAAs, mTORC1 activity in scleral fibroblasts increased (Figure 3B), while α-SMA expression decreased significantly (Figure 3C). These results suggest that BCAAs can affect the transdifferentiation of scleral fibroblasts and may be related to mTORC1, which requires further verification.

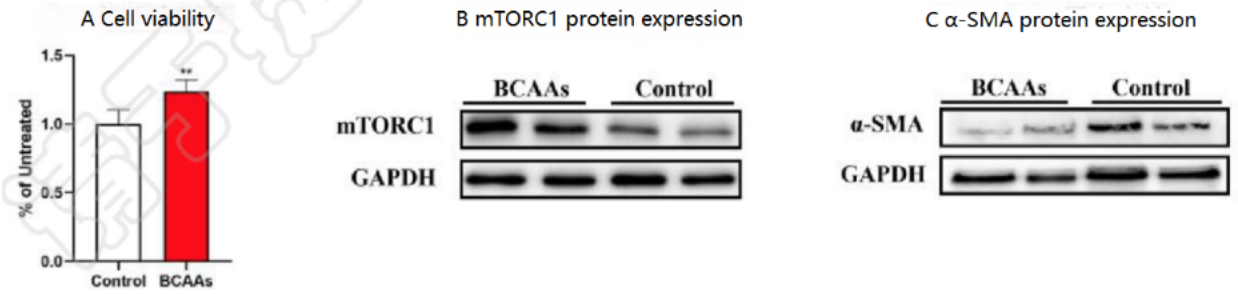
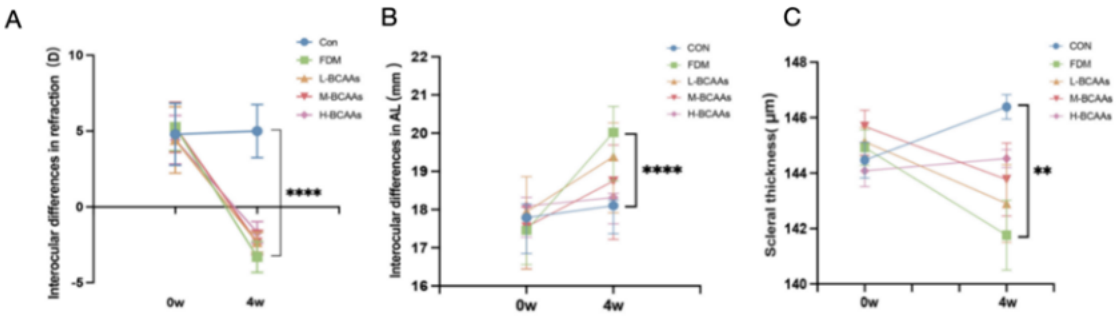


Figure 3. Effects of BCAA treatment on survival of mouse scleral fibroblasts (A), mTORC1 protein expression (B), and α-SMA protein expression (C)

1.3 Effects of BCAAs on Ocular Biometric Indices in Guinea Pigs With an FDM Model

Under the support of the same National Natural Science Foundation of China General Program led by the applicant, a series of previous animal experiments confirmed that BCAAs can reduce myopia-induced changes in refractive error, axial length, and scleral thickness. The research group randomly assigned ninety 4-week-old guinea pigs into a control group, a model group, and low-, medium-, and high-dose BCAA groups. The low, medium, and high BCAA intervention doses were 412 mg/kg, 2060 mg/kg, and 4120 mg/kg, respectively. This study aimed to compare the effects of different BCAA doses on ocular biometric parameters in guinea pigs with form-deprivation myopia (FDM). After 4 weeks of intervention, the effects of different BCAA doses on refractive error in FDM guinea pigs are shown in Figure 4A. As shown in Figure 4A, the refractive power in the high-dose intervention group was higher than that in the model group ($P < 0.05$). Although there was no difference between the medium- and low-dose intervention groups and the model group, there was still an upward trend. The effects of different BCAA doses on axial length (AL) in FDM guinea pigs are shown in Figure 4B. As shown in Figure 4B, AL in the medium- and high-dose intervention groups was lower than that in the model group ($P < 0.05$); although there was no difference between the low-dose intervention group and the model group, there was still a downward trend. The effects of different BCAA doses on scleral thickness in FDM guinea pigs are shown in Figure 4C. As shown in Figure 4C, scleral thickness in the high-dose intervention group was higher than that in the model group ($P < 0.05$); although the medium- and low-dose intervention groups

showed no difference from the model group, there was still an increasing



trend.

Figure 4. Effects of BCAA treatment on refractive error (A), axial length (B), and scleral thickness (C) in guinea pigs with an FDM model

2.Working Conditions

This project is supported by the School of Public Health of Southeast University. The platform has all equipment and laboratory conditions required for the project, including biosafety cabinets, microscopes, flow cytometers, microplate readers, bioanalyzers, fluorescence quantitative PCR instruments, and liquid chromatography-mass spectrometry systems. Ophthalmic examinations and venous blood collection in this project will be technically supported by the Departments of Ophthalmology and Pediatrics of Zhongda Hospital affiliated to Southeast University, respectively. The implementing institution will provide basic ophthalmic examinations for participants, including spherical equivalent refraction (SE), axial length (AL), choroidal, and retinal thickness, as well as preliminary screening for basic ocular diseases and professional clinical opinions. The host institution possesses the necessary instrumentation, equipment, and technical personnel to complete the project and thereby provides assurance for its implementation. The principal investigator has long been engaged in population health monitoring, has accumulated many years of experience in field organization and implementation, and has established long-term and sound cooperative relationships with collaborating institutions, thus providing a strong foundation for the smooth conduct of the randomized controlled trial in humans.

3.Research Team Member

Name	Sex	Age	Position/Title	Institution and Department	Professional Specialty	Role in Project
Weina Liu	Female	39	Associate Professor	Department of Nutrition, School of Public Health, Southeast University	Child and Adolescent Health	Principal Investigator
Zhonghong Zhang	Female	53	Associate Chief Physician	Zhongda Hospital	Ophthalmology	On-site examinations
Xiaoyu Wei	Female	24	Student	School of Public Health, Southeast University	School of Public Health	Population intervention
Fen Chen	Female	24	Student	School of Public Health, Southeast University	School of Public Health	Population intervention
Qinye Liu	Female	24	Student	School of Public Health, Southeast University	School of Public Health	Population intervention
Yingyun Shi	Female	24	Student	School of Public Health, Southeast University	School of Public Health	Population intervention
Lin Jiang	Female	36	Nurse	Zhongda Hospital	Pediatrics	Blood collection

4.Budget

Funding source for this project: National Natural Science Foundation of China, project approval number 82404291.

No.	Budget Item	Amount (10,000 CNY)	Basis and Rationale
1	Total direct project costs	5	
2	Research operational expenses	1	For data collection and literature review
3	Instrument/equipment costs	0	
4	Experimental materials	1	Purchase of experimental reagents, consumables, and special tools, such as BCAA capsules, blood collection needles, and blood collection tubes
5	Laboratory renovation expenses	0	
6	Collaboration expenses	1	Payment for technical support from collaborating institutions
7	Project implementation expenses	1	Salaries of project staff and routine office expenditure, such as printing of survey questionnaires
8	Travel/meeting/international collaboration and exchange	0	
9	Publication/literature/information dissemination/intellectual property affairs	2	
10	Labor costs	1	Compensation for personnel participating in the project
11	Expert consultation fees	0	
12	Compensation for blood collection from study participants	1	Nutritional subsidy for blood collection provided to participants
13	Other expenses	0	

Other Content

Applicant's Commitment

As the applicant for the research project "A Randomized Controlled Trial of Branched-Chain Amino Acid Supplementation for Preventing Myopia Progression", I hereby solemnly undertake that:

1. This study will strictly comply with the Declaration of Helsinki, the Measures for the Ethical Review of Biomedical Research Involving Humans issued by the National Health Commission, and the regulations of this institution's Ethics Committee.

2. All participants must sign an informed consent form and will be clearly informed of the purpose, procedures, potential risks, and rights related to the study, and their right to withdraw from the study at any time will be safeguarded.

3. Participants' personal information and study data will be encrypted and used only for analysis in this study, and shall not be disclosed or used for any other purpose without authorization.

4. Neither I nor the research team has any financial conflict of interest related to this study, and all funding sources have been truthfully declared.

5. We accept full-process supervision by the Ethics Committee.