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

Study Protocol

Version No.: 03

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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 108 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) University students aged 18 to 25 years in Nanjing;

2) Spherical equivalent refraction (for eyes without prior corneal refractive surgery), -0.50 D to +0.50 D;

3) Uncorrected 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 informed consent.

Exclusion criteria:

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

2) Systemic diseases affecting refractive development, such as diabetes or fibrotic disorders;

3) Active eye disease or history of ophthalmic surgery;

4) Prior use of atropine, orthokeratology lenses, or other myopia-control methods;

5) Use of BCAA-related supplements within the past 6 months;

6) Any condition judged by the responsible physician to make the participant unsuitable for participation or unable to comply with study requirements.

Sample size

According to the following formula, assuming an estimated α of 5% and β of 20%, and a minimum intergroup difference in refractive change of 0.28 D in a two-sided study, the calculated initial effective sample size is approximately 46 participants per group. Considering a potential loss to follow-up of approximately 15%, the adjusted sample size is estimated to be about 54 participants per group. Accordingly, 54 participants will be allocated to the intervention group and 54 to the control group, resulting in a total sample size of 108 participants. The male-to-female ratio in both groups will be 1:1, with each group consisting of 27 males and 27 females.

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

d: expected mean difference in refractive error between the intervention group and the control group, d = 0.28 D

σ: estimated standard deviation, σ = 0.45 D

Zα: corresponding standard normal deviate for α = 0.05

Zβ: corresponding standard normal deviate for β = 0.20

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

Methods for participant recruitment and allocation

Recruitment process

Researchers will preliminarily screen potentially eligible participants through personal contacts, including the outpatient follow-up system, the previous research participant database, and cooperative community health records. During the first contact, only a general overview of the study will be provided. After oral agreement from the individual, the full study information sheet, including the draft informed consent form, will be sent by text message. Individuals who do not actively express willingness to participate will not be contacted a second time. Recruitment will continue until the target sample size is reached.

Allocation method

SPSS version 25.0 will be used to generate random numbers and a randomization list. According to the random sequence, slips of paper marked “intervention group” or “control group” will be placed into sealed opaque envelopes. Participants will be randomized in a 1:1 ratio to the intervention group or the control group. The randomization process will be completed by an independent third party unrelated to the study.

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 ≤ -0.50 D; moderate myopia, -6.00 D < SE ≤ -3.00 D; and high myopia, SE ≤ -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 in the intervention group will take 2 capsules daily, each with a net weight of 0.6 g and containing 300 mg leucine, 150 mg isoleucine, and 150 mg valine, for a total daily BCAA intake of 2400 mg. The capsules are provided by Life Extension, USA. The product meets Good Manufacturing Practice (GMP) quality standards and has passed heavy metal and microbial limit testing, ensuring consistency and safety of the intervention product. Participants will take the capsules once daily after breakfast or dinner for 6 consecutive months. According to the Recommended Daily Allowance (RDA) proposed by the Food and Nutrition Board of the Institute of Medicine (IOM), the daily recommended intake is 42 mg/kg/day for leucine, 19 mg/kg/day for isoleucine, and 24 mg/kg/day for valine. For a participant weighing 60 kg, the recommended daily intake would be approximately 2.52 g leucine, 1.14 g isoleucine, and 1.44 g valine, totaling 5.1 g BCAAs. The intervention dose in this study is

2.4 g, which is only about 47% of the recommended intake and is considered a low-dose supplementation level. This dose was determined based on multiple prior human studies and is far below the commonly used 5-15 g/day range in clinical interventions. No significant adverse reactions or hepatotoxicity/nephrotoxicity have been reported in healthy individuals aged 18-25 years consuming this dose of BCAAs daily. Therefore, the selected dose in this study has good safety and tolerability and is suitable for human intervention research.

Control group: The control group will receive two 0.6 g placebo capsules containing medium-chain triglycerides daily for 6 consecutive months. The ingredient content of the placebo capsules is provided by California Gold Nutrition. All capsules are identical in appearance, shape, odor, and packaging. The hygienic indices of the capsules have been tested by the National Food Quality Supervision and Inspection Center (Shanghai), and odor, appearance, colony count, and other indicators all met testing standards.

3. Study Methods

3.1 General Ophthalmic Examinations

1) **Spherical equivalent refraction (SE):** SE will be measured using the KR-800 automated kerato-refractometer manufactured by Topcon (Dongguan) Technology Co., Ltd. Before measurement, one drop of 0.5% proparacaine hydrochloride eye drops (Alaine) will be instilled, followed 1 minute later by one drop of 1% cyclopentolate hydrochloride eye drops (CYCLOGYL), and a second drop of 1% cyclopentolate hydrochloride eye drops 5 minutes later to induce cycloplegia in preparation for autorefraction 30 minutes later. Measurements will be taken three consecutive times. The maximum-minimum differences for sphere and cylinder will each be ensured to be no greater than 0.25 D, and the average of the three measurements will be recorded. SE is defined as spherical power plus one-half of cylindrical power.

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

SPSS version 25.0 will be used for data processing. Statistical analyses will include ocular parameters from both the left and right eyes. Analyses will be conducted according to the modified intention-to-treat principle and will include only participants who have baseline outcome measurements and at least one post-randomization observation. The last observation carried forward (LOCF) method will be used to replace missing data. Measurement data will be expressed as mean ± standard deviation or median (interquartile range), and count data will be described as number of cases (percentage). Generalized estimating equations (GEE), including one within-subject factor (time), one between-subject factor

(intervention), and their interaction, will be used to compare changes in spherical equivalent, axial length, uncorrected visual acuity, and scleral, choroidal, and retinal thickness. Covariates in the GEE model will include the corresponding baseline measurement values, age, sex, number of myopic parents, outdoor light exposure time, and near-work time. All effect tests will be performed at a two-sided α level of 0.05.

4.Study Technical Roadmap

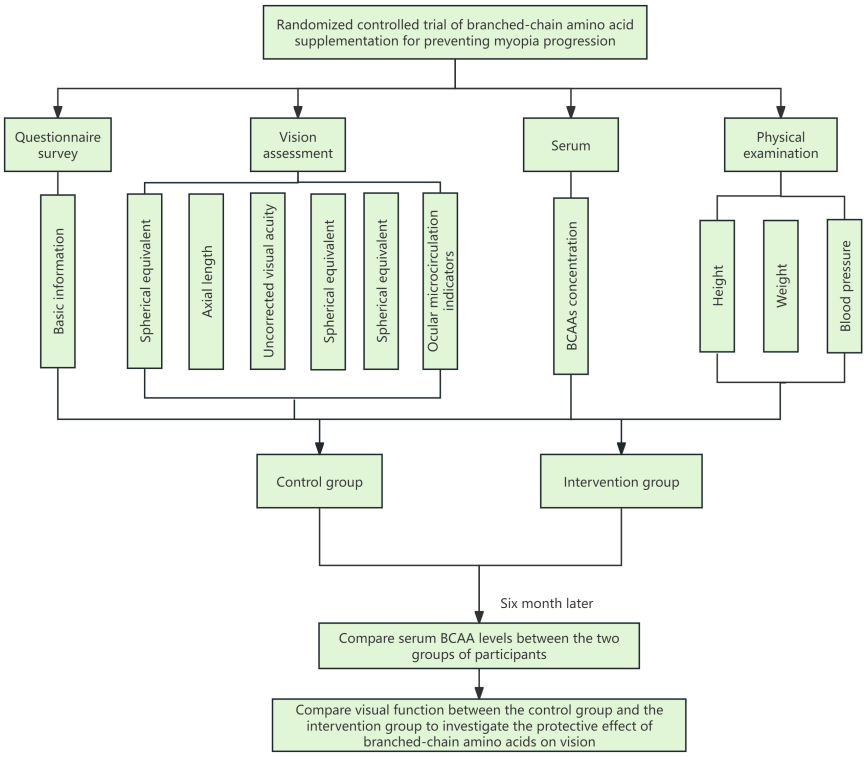

Figure 1. Technical Roadmap

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 long been engaged in population health monitoring and has extensive experience in field investigations among human populations. Through the previously established myopia cohort project, a strong foundation and platform for collaboration have been built with Zhongda Hospital affiliated to Southeast University and the local Center for Disease Control and Prevention. This project plans a validation study in university students and is therefore 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

6.1 Innovation in Topic Selection

Based on prior animal studies, this study is the first to identify a close association between serum BCAAs and scleral thickness, introducing a new nutritional perspective into strategies for myopia prevention and control.

6.2 Innovation in Research Concept

Based on prior animal studies of BCAA intervention for preventing myopia progression, this project is the first randomized controlled trial in humans to investigate the relationship between BCAAs and myopia. It will further analyze whether BCAAs can prevent myopia progression in humans, provide a new scientific basis for clinical application, and broaden the scope of nutritional interventions for myopia.

7. Research Plan and Expected Progress

1) **August to September 2025:** Review the literature, refine the study protocol, and apply for ethics approval for the human study; train personnel participating in the project; conduct questionnaire surveys and sample collection among university students in Nanjing; distribute informed consent forms; complete baseline investigations and data collection; and complete testing of BCAA levels in samples.

2) **October to November 2025:** Complete the randomized controlled experiment; organize and analyze baseline and end-point data; carry out multivariable regression analyses of BCAAs and related lifestyle and dietary patterns; and draft and publish an SCI paper.

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.

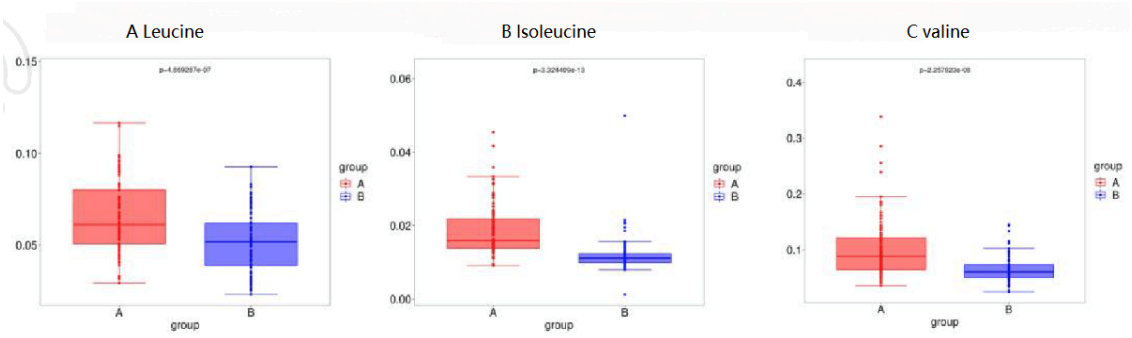

Figure 2. Boxplots comparing BCAA metabolites between the myopia progression group (Group B) and the non-myopia progression group (Group A)

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.

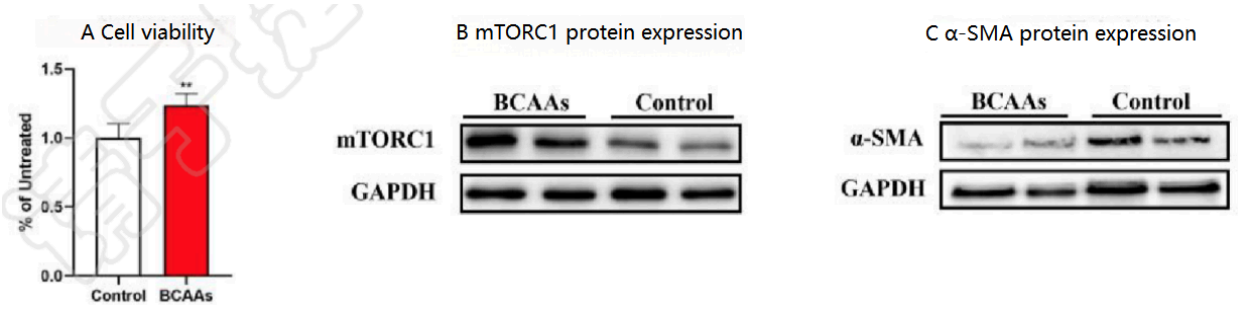

Figure 3. Effects of BCAA treatment on survival of mouse scleral fibroblasts (A), mTORC1 protein expression (B), and α-SMA protein expression (C)

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.

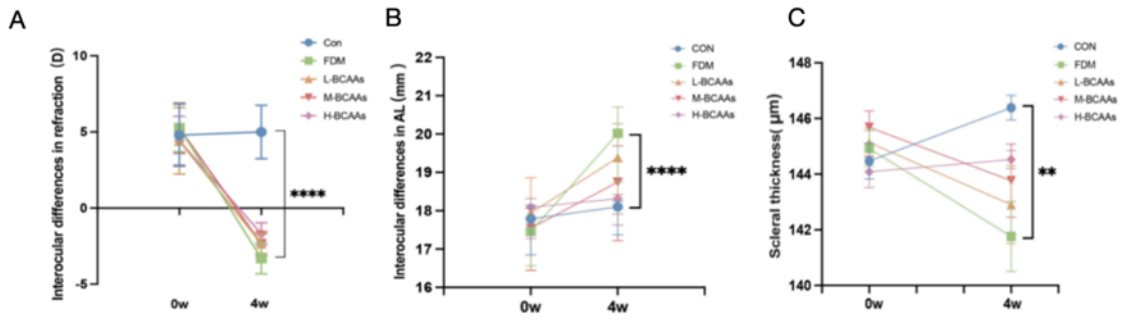

Figure 4. Effects of BCAA treatment on refractive error (A), axial length (B), and scleral thickness (C) in guinea pigs with an FDM model

2.Working Conditions

This project is supported by the School of Public Health of Southeast University and the Key Laboratory of Environmental Medicine Engineering of the Ministry of Education. 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 the Prevention of 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.