1. Administrative information

1.1 Title

The Short-term Effects of Sunlight Exposure on Fundus Blood Flow Perfusion in Children: A Randomized Controlled Trial

1.2 Trial registration

ClinicalTrials.gov identifier NCT05594732

1.3 Protocol version

Completed and approved in August, 2022.

1.4 Funding

Sponsor: Shanghai Eye Disease Prevention and Treatment Center

1.5 Roles and responsibilities

Profs. Jun Chen, Xun Xun and Xiangui He are the PIs of this project. Dr. Jingjing Wang is responsible for particpants recruiting. Mr. Bo Zhang is responsible for the eye examination. Drs. Lingyi Zhao are responsible for the study design, data management, data anlaysis and drafting the paper.

2. Introduction

2.1 Background

Myopia has emerged as a widespread global concern. According to the World Health Organization (WHO) report of 2023, approximately 2.2 billion people worldwide were affected by vision impairment, with over 1 billion cases being potentially preventable, and the leading cause being refractive errors [1]. Notably, the prevalence of myopia among Chinese school-aged children in 2020 was 53% [2]. In 2018, eight departments including the Ministry of Education jointly issued the Implementation Plan for Comprehensive

Prevention and Control of Myopia in Children and Adolescents. As of 2020, the overall myopia rate among children and adolescents in China was 52.7%.[3] The COVID-19 pandemic has increased students' time of indoor eye-using, and it showed that the sunlight exposure of myopic students is lower than that of non-myopic students.[4] This increasing trend highlights the pressing need for immediate attention and proactive preventive strategies.

Studies have found that sunlight exposure has a protective effect on the occurrence of myopia in children and adolescents, but the immediate effects of sunlight exposure on ocular parameters for myopia prevention remain incompletely understood.[4] Various randomized controlled trials (RCTs) conducted in different regions of the world have provided compelling evidence of the protective role of prolonged sunlight exposure [5–10]. Altered fundus blood flow is considered to play a role in the pathogenesis of myopia and can serve as a predictive indicator for myopia development [11,12]. This critical physiological parameter is intricately linked to ocular health and visual function, playing a pivotal role in maintaining fundus homeostasis and facilitating the delivery of essential nutrients and oxygen [13].

Clinical studies have demonstrated that individuals with myopia often exhibit diminished choroidal and retinal blood flow perfusion, contributing to scleral hypoxia and subsequent myopia progression [14,15]. Animal studies also observed a change in the myopic eye compared to the fellow eye, and actively control the fundus vessels could affect the course of myopia development, which add to the evidence of the role of fundus blood flow played in myopia pathogenesis [16,17]. Although the focuses of these studies also were the long-term change of myopia, they at least pointed to the fact that fundus blood flow was a promising direction in exploring myopia pathogenesis. The gap that studies on the immediate change of fundus blood flow after myopic stimulus and sunlight exposure was lacking should be filled. Therefore, it is postulated that fundus blood flow perfusion (BFP) could serve as a short-term predictive indicator for myopia. Investigating the correlation

between sunlight exposure and fundus BFP can aid in identifying effective sunlight exposure patterns for myopia prevention.

Therefore, this study selected blood flow perfusion as the primary outcome, aiming to compare the effects of different sunlight exposure modes on fundus blood flow perfusion after 1 hour of near work, and provide clues for the prevention and control of myopia.

2.2 Objectives

This study is to explore the effects of different sunlight exposure patterns, specifically the sunlight exposure duration and sunlight exposure intensity, on fundus blood flow perfusion.

2.3 Trial design

A randomized controlled clinical trial.

3. Methods

3.1 Participants, interventions, and outcomes

3.1.1 Study setting

Participants were asked to read books at a distance of 33 centimeters for 1 hour in an indoor environment with 2000 lux after basic eye examinations and then had sunlight exposure for 15 minutes by randomly grouping into two groups, the low-illuminance (4k lux) group or high-illuminance (10k lux) group.

3.1.2 Eligibility criteria

Inclusion Criteria:

a. School students aged from 7 to 15, regardless of sex or gender;
b. Diopter between -2.0D and 3.0D, and astigmatism not exceed 0.75D;
c. No organic disease and in good general condition;
d. Have obtained the consent of their parents or guardians, and can

cooperate.

Exclusion Criteria: Suffering from amblyopia, strabismus, color weakness, congenital cataract, glaucoma and other eye diseases;

3.1.3 Interventions

Participants were randomized into the following two groups:

Group 1: 4000 Lux sunlight exposure

Group 2: 10,000 Lux sunlight exposure

The two groups of subjects read paper books in the same indoor light environment for 60 minutes and then were exposed into the corresponding outdoor natural light environment. The fundus blood flow perfusion were examined before reading, after reading and at 5^{th} minute and 15^{th} minute of sunlight exposure. The lighting conditions were assessed using an illuminance meter (SIS-20, EVERFINE, China).

3.1.4 Outcomes

a. Primary outcome measure: The difference of change in fundus blood flow perfusion after sunlight exposure for 15 minutes.
b. Secondary outcome measure: The change trend of fundus blood flow perfusion between each time point of sunlight exposure.

3.1.5 Sample size

There were few studies reported the values of fundus BFP under different sunlight exposures. Based on a pilot test [18], it was determined that the estimated sample size needed for the study was 46 participants at least, with 23 individuals allocated to each group. This calculation was based on a significance level of 0.05, a desired statistical power of 90%, a minimum expected difference in superficial retina BFP changes of 1.5% (2.5% vs 1.0%), the standard deviation within the group is 1.5, and an equal allocation ratio of 1:1.

3.1.7 Recruitment

Participants will be recruited through both online and offline postings. Study introduction and purpose, eligibility criteria, study contents, intervention details, participants' benefit, as well as contact information are made clear on the postings.

3.2 Assignment of interventions

Simple randomization is adopted, with random numbers set in advance. Each enrolled subject is randomly divided into two groups in a 1:1 equal ratio.

3.3 Data collection, management, and analysis

3.3.1 Data collection methods

Data will be collected using questionnaires, eye examinations, and light environment testing.

i. Questionnaire survey
   a. Basic information: age, gender, etc.
   b. Medical history: history of previous eye disease or trauma, eye surgery, and other systemic diseases, etc.

ii. Eye examination
   a. Visual acuity (VA): ETDRS Log MAR E eye chart (Precision Vision, Villa Park, Illinois, USA) is used, with measuring distance being 4m; the right eye is tested first and followed by the left eye, recognizing the visual mark from top to bottom. Best VA is recorded as the line of the smallest visual marks that can be recognized. The recognition time of each visual mark is 2 to 3 seconds, and the subject is required to open his eyes naturally, and avoid squinting or tilting the head, or stretching the neck and peek. VA is recorded in decimals. Those who do not cooperate or cannot understand should be recorded. Test results should be accurate to number. Five visual targets in each line all need to be

recognized; wrongly pointing 1 to 2 visual marks in each row is recorded as -1, -2, pointing out 3 wrong visual marks or more will be record the visual acuity +1, +2 in the upper line; if the subject cannot recognize the largest visual marks in the first line at a distance of 4m, he or she should move forward and VA is recorded as 3m, 2m, 1m, or 0.5m respectively. If the largest visual target cannot be seen at 0.5m, then record FC (finger count), HM (hand move), LP (light perception), or NLP (no light perception).

b. Axial length measurement: IOL Master (version 700, Carl Zeiss Meditec, Germany) is used for axial length measurement. Simulated eye calibration is done before measurement. Each eye is measured 5 times and the mean is recoded. The difference each time should not exceed 0.05mm. Otherwise, re-measurement is required. Corneal curvature, axial length, and anterior chamber depth are measured in sequence. At least 5 consecutive measurements are made to obtain the combined signal and composite measurement value. The green light appears when the SNR value is over 2.0, indicating a credible result; otherwise, the measuring time should be increased until a credible combined signal appears.
c. Intraocular pressure measurement: Intraocular pressure is measured using a non-contact tonometer (NT-510, Nidek Company, Japan). Each eye is measured three times and averaged, and the difference between the two times should be less than 5 mmHg.
d. Auto-refraction (Topcon KR8900, Japan): Standard simulated eye correction is done before the test. Mode of refraction + keratometry is used, and the cylinder is set to a negative value. Each eye is measured at least 5 times, with difference in spherical lens power not exceeding 0.25DS, otherwise the measurement will be restarted to obtain a stable average value. Take the average of 3 consecutive measurements.
e. Eye surface health assessment: Use OCULUS77000 to detect tear film

break-up time, tear river height and eye redness grading.

f. Fundus blood flow perfusion: Use AngioVue OCTA blood flow imaging to quantitatively measure parameters of macula, optic disc and panretinal blood flow density, select superficial and deep blood vessel complexes, and calculate the proportion of the corresponding area occupied by the blood flow signal.

3.3.2 Data management

The purpose of this study is to explore the impact of different sunlight exposure modes on fundus blood flow perfusion in children. Eye examination data are collected through an online information system with a logical verification function, questionnaire results are independently entered into the O-Trial+ database for verification, and environmental monitoring data and outdoor light environment data are entered into and verified using an excel database. SAS (9.4) is used for data processing and analysis.

3.3.3 Statistical analysis

a. Baseline characteristic description

Compare the differences in baseline characteristics of the two groups of subjects, mainly including age, gender, refraction, axial length, etc. Continuous variable indicators are or approximately consistent with normal distribution are described as mean with standard deviation while those that do not conform to normal distribution are described as median and interquartile range. Categorical variables are described as frequency and relative frequency.

b. Main outcome analysis

The changes in fundus blood flow perfusion of the two groups of subjects will be counted and described using mean with standard deviation. Differences in changes in spherical equivalent between the groups are compared using T tests.

c. Secondary outcome analysis

Among secondary outcome indicators, basic assumptions of general linear regression models will be tested in the analysis, including linearity, normality of residuals, and homogeneity of variances. If these assumptions are not met, then data transformation or non-parametric analysis will be considered.

3.4 Monitoring

In order to ensure that this trial can be conducted in strict accordance with the clinical research plan, clinical researchers must strictly follow the requirements during the entire clinical trial process and ensure that the trial procedures are standardized, the data is accurate, and the research conclusions are reliable.

a. Research design stage

Clinical experts and statistical experts will be organized to discuss the scientific rationales and feasibility of the plan to ensure that the design can answer the research question.

b. Research implementation stage

All researchers will receive standardized training to ensure that the research is strictly implemented in accordance with the trial protocol. Keep complete records of clinical records and original medical records of subjects. Researchers responsible for quality control need to conduct random checks on each physician's examination data on a regular basis, and physicians with a high failure rate need to be retrained; the standards and equipment of each following test must be consistent with the baseline, and the examining physicians are as consistent as possible. An electronic data system was used to collect information. In order to ensure data security, irrelevant personnel cannot access or modify the data, and the data must be backed up. Any data changes require a consent form signed by the principal investigator, statistician and data administrator. After receiving the case report form, the data administrator will forward the questionable data to the researcher for

verification through the clinical coordinator, and the researcher should verify and return it as soon as possible. The data administrator establishes the database in a timely manner and performs secondary entry and verification of data. After the database is verified to be correct, the main researcher, data administrator, and statistician will lock the data.

c. Research and analysis stage

Perform statistical analysis strictly in accordance with the statistical analysis plan specifications in the project document. Two statisticians independently conduct statistical analysis and retain the statistical analysis procedures to ensure that the statistical analysis results are consistent and repeatable.

4. Ethics and dissemination

4.1 Research ethics approval

This research will be carried out after obtaining approval from the ethics committee of each center and strictly comply with the Declaration of Helsinki.

4.2 Consent or assent

Informed consent forms will be issued to children and adolescents participating in the study and their parents or guardians by the researchers after approval by the ethics committee. For children and adolescents and their parents or guardians who voluntarily agree to participate in this study, the parents or guardians must sign an informed consent form before conducting the baseline study. Before signing the informed consent form, the researcher must fully introduce the content of the study, the benefits and potential risks of the subjects, and confirm that children and adolescents and their parents or guardians fully understand it before voluntarily signing the informed consent form. When signing the informed consent form, parents or guardians are given ample time to consider and have the right to ask questions. For questions from parents or guardians, researchers need to provide adequate explanations for

their answers. For parents or guardians who agree to sign the informed consent form, after the researcher fully explains the informed consent form, the parents or guardians will sign the informed consent form and indicate their relationship with the children and adolescents being tested. The informed consent form is made in duplicate, with one copy kept by the parent or guardian and the other by the research institution.

4.3 Confidentiality

In order to protect the privacy of the children and adolescents tested, the subject's ID number or real name will be replaced by the subject's code or initials when research data are provided to other organizations, In addition, researchers and relevant staff involved in the research must keep the private information of the children and adolescents subject confidential.

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