The Efficacy of Creatine Supplementation in Improving Cognitive Performance in Adolescents: A Phase-II, Triple-Blind, Randomized Study Protocol

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ABSTRACT:

Background/Aims: Creatine supplementation has demonstrated cognitive benefits in neurodegenerative conditions, having a protective effect in the brain's function in stressful situations, with excellent safety (Watanabe et al., 2002). However, any beneficial effects on the cognitive performance of healthy adolescents underperforming in school is unknown. Our objective is to assess whether creatine supplementation improves cognitive performance in 15- to 17-year-old students with an average school grade below the 50th percentile.

Methods: This will be a phase-II, triple-blinded, randomized, parallel-group, superiority, single-center trial. Students with grades below the 50th percentile in the prior semester will be enrolled and randomized to receive juice packages containing either creatine monohydrate supplementation (0.1 mg/kg/day), or placebo, for 12 weeks. The primary outcome will be the mean difference in change of Raven’s Standard Progressive Matrices (RSPM) scores from baseline to week 12 between groups. To achieve a 90% power for detecting a 3-point difference in change in the RSPM score, and accounting for drop-out, 116 participants will be included. Secondary outcomes will include the difference in processing speed (SpaceCode), working memory (SpaceMatrix), non-visual memory (backward digit span), percentage change in lean mass, and any safety events.
Conclusion: To our knowledge, this will be the most comprehensive study assessing creatine supplementation in adolescents. This is a low-risk intervention that has been shown to improve cognitive function in other populations. This study will potentially support the widespread use of creatine supplementation in adolescents with low school performance, while having a positive impact on this population.

Keywords: creatine; adolescents; cognitive function; raven's progressive matrices.

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Abbreviations:
Adenosine triphosphate (ATP)
Raven's Standard Progressive Matrices (RSPM)
Population Health Research Institute (PHRI)
Case Report Form (CRF)
High-Performance Liquid Chromatography (HPLC)
Backward Digit Span (BDS)
Principal Investigator (PI)
Standard Deviation (SD)
Interquartile Range (IQR)
Confidence Interval (CI)
Body Mass Index (BMI)

Introduction
Adolescence is a sensitive transition with significant growth and development of the brain. The brain uses almost 20% of the body's energy supply for proper functioning and changes in adenosine triphosphate (ATP) homeostasis have been related to deficient brain energy supply (Herting et al., 2017). Creatine is a naturally occurring amino acid derivative found to be relevant in energy production, increased in several brain regions after oral consumption of a supplement (Dechent et al, 1999), and crucial to many cognitive functions. These functions include reducing mental fatigue following mathematical calculation tasks while simultaneously increasing oxygen utilization in the brain (Dechent et al, 1999), and improving short-term memory, intelligence, and reasoning abilities (Avgerinos et al, 2018). Furthermore, creatine supplementation has also demonstrated benefits in certain conditions where neurodegeneration occurs, as well as in genetic creatine disorders where its depletion leads to neurological impairments (e.g., intellectual disability, seizure, developmental delay), with improvement after its supplementation (Joncquel-Chevalier Curt et al., 2015). In addition, a preventive supplementation of creatine could protect the brain's function in stressful situations such as sleep deprivation, cerebral hypoxia, and traumatic brain injury (Joncquel-Chevalier Curt et al., 2015).

Altogether, creatine supplementation has been shown to beneficially alter brain metabolism and function in adults. Of particular interest is the potential effect on improving cognitive development and the school performance in adolescents, as beneficial changes during this period may contribute to improve self-esteem and overall health, future career development, and educational policies. Creatine has been reported to be widely and safely used among high school students, and the cerebral metabolic alterations caused by oral creatine were shown to be reversible, as evidenced by control measurements at least 3 months after intervention (Dechent et al, 1999). However, the benefits of creatine on cognitive performance of adolescents have not been tested in a robust, randomized study.

The Creatine supplementation in Adolescent Volunteers (CREAtiVe) study aims to investigate if 12 weeks of daily creatine supplementation in high school students aged 15 to 17 years improves their cognitive performance, as measured by the Raven's Standard Progressive Matrices (RSPM) test and other measures, compared with placebo (Mayr et al., 2020).

Methods

Trial Design
This is a phase-II, randomized, triple-blind, parallel-group, superiority, single-center trial. We aim to assess the change in cognitive performance, as measured by the RSPM, at baseline and after 12 weeks of daily oral creatine monohydrate supplementation, or placebo, mixed in juices (Figure 1). Treatment allocation will be unknown to the research participants, the study personnel involved in administering the intervention, and the trial staff responsible for outcome assessment.

Study Setting
Data will be collected from one high school, given the estimated sample size, in a middle-income area in the United States, to reduce risk of participant
malnutrition or rigid dietary regimens. The supplementation packages, in the form of juices, will be delivered on-site.

**Randomization**

The participants will be randomly assigned to either placebo or creatine supplementation group with a 1:1 allocation as per a computer-generated randomization schedule using RedPill (Sealed envelope, 2021) in permuted blocks of 4-6. Participants who fulfill inclusion criteria, provide assent for participation, and whose parental or legal guardians provide informed, written consent, will be randomized. A group of designated trial staff will be responsible for recruitment, clinical interviews, and insertion of participant data into RedPill. A unique randomization number will be generated and concealed to all trial staff and participants to assure allocation concealment. Only pharmaceutical staff responsible for preparing the supplement packages with either creatine supplementation, or placebo will have access to the randomization list. The randomization list will remain within RedPill for the duration of the study. If RedPill has technical issues, a physical list will be printed and sealed in the possession of an independent research team member, with no direct involvement in the conduction of the trial, chosen by the Principal Investigator (PI), and responsible for emergency unblinding procedures, if necessary.

**Blinding**

This is a triple-blind study where participants, parents or legal guardians, high school staff, data collectors, staff responsible for recruitment, monitors, analysts, statisticians, and outcome assessors will be masked from allocation.

Packages delivered to the intervention and control groups will look identical in shape, flavour, taste, smell, and color. They will be administered at the same frequency and time of day. Data will be collected and
entered after coding participants. Only pharmaceutical staff and one independent research team member will be aware of the allocation by having access to the randomization list in case of emergency unblinding.

**Emergency Unblinding**

Code breaks will be exceptionally performed if adverse reactions occur, such as allergic reactions, anaphylaxis, or situations where the actual intervention is crucial for treatment of the participants. This will be decided by an on-call independent researcher. The research coordinator will use the system of emergency unblinding through the Population Health Research Institute (PHRI) toll-free helpline as the main system or through the local emergency number as the back-up system. Any case of emergency unblinding will be accurately documented in a detailed case report form (CRF) including all event-related details. The participant will no longer be provided the intervention or placebo, and their data will be analyzed as per intention-to-treat analysis. Allocation will not be disclosed to other study personnel. There will be no written or verbal disclosure of the code in any of the corresponding patient documents. Unblinding of other participants could be considered in situations of package contamination or adverse events indicating that other participants of the study might also be at risk. Body composition alterations that could suggest accidental unblinding are not expected during the short study period.

**Eligibility Criteria**

Adolescents aged 15 to 17 years who achieved an overall grade below the 50th percentile in the prior school semester are eligible. Exclusion criteria includes participants with previous diagnoses or comorbidities that affect creatine metabolism and/or absorption. Participants with renal diseases or who regularly take medications that decrease renal function will be excluded (Davani-Davari et al., 2018), (Whittaker et al., 2018). *(Appendix 1)*

**Recruitment Strategy**

Adolescents will be recruited using a variety of methods to maintain privacy. The school's administrators will be contacted to obtain information on the low performing students. All participants' personal information will be protected. All interested students will be screened, but only students with below the 50th percentile will be enrolled. To avoid any potential ostracization or psychological repercussions, grades or rankings will not be publicly shared, and the study will be advertised as testing video game performance, a secondary outcome, with creatine supplementation.

At the beginning of the study, a USD$5 prepaid gift card will be given to participants willing to fill out initial paperwork. To encourage adherence, incentives will be offered throughout the study. Participants who request further information will receive an educational pamphlet explaining the specifics of the trial and a phone call a few days after initial contact to further increase recruitment (Trewick et al., 2018).

**Interventions**

Coded juice bottles (supplement packages) containing either creatine or placebo will be delivered in identical packaging to the participating high school weekly. Research staff will observe consumption during weekdays, and the packages will be given to students on Fridays to consume on weekends. Parents of participants who do not attend school certain days will be able to collect the appropriate packages for the week. Administration to students on-site will be according to the assigned student code by a designated research team member unaware of allocation.

Packages will contain either creatine monohydrate 0.1g/kg mixed with juice, or juice alone (placebo), for 12 consecutive weeks following baseline assessment. To verify the purity of the creatine used, monthly random samples will be analyzed by high-performance liquid chromatography (HPLC) to verify a purity level >99%. Supplements will be prepared by pharmaceutical staff members off-site, who are aware of the treatment allocation barcode and the participant’s weight to prepare packages. Supplements will be dissolved in juice to mask the low solubility of creatine and prepared according to preference for fruit juice. Pharmaceutical staff members will not have any participation in data acquisition, analyses, or interpretation.

**Adherence**

Participants will receive an informative talk about the trial and proper use of our application software (“App”) designed for this trial (Kosse et al., 2019). Adherence with study protocol will be monitored Mondays through Fridays by observing participants consuming the supplement inside the school. Participants will log each consumed juice drink in the App. On weekends, adherence will be monitored on the App with reminders to participants to register if the supplement was properly consumed. Additionally, the App will collect data to monitor adverse effects, difficulties to drink the supplement and reasons why participants would keep drinking the supplement. To encourage adherence, a USD$5 gift card will be given at screening for all participants, a USD$10 gift will be given to all
participants who remain in the trial at week 6, and a USD$20 gift card will be given to all participants who continued for the entire 12-week trial.

Phone calls will occur every two weeks to directly ask participants how else the research team can help participants improve or handle the intervention better, as well as monitor for important adverse reactions (Korelitz et al., 1976). Empty packages coded for creatinine at the beginning of creatine supplementation as “generally recognized as safe”, without clinically relevant adverse effects (Jagim et al., 2021). Therefore, we expect the discontinuation of creatine supplementation in this study to be rare, though may consist of (i) gastrointestinal intolerance, (ii) allergic reaction, or (iii) withdrawal of participant’s or parents’ consent. If this occurs, the participant will no longer receive the intervention and outcomes will be assessed by intention-to-treat.

Modification/ Discontinuation

Multiple prior reports have indicated that creatine supplementation is safe in healthy individuals. In March 2020, the United States Food and Drug Administration designated creatinine supplementation as “generally recognized as safe”, without clinically relevant adverse effects (Jagim et al., 2021). Therefore, we expect the discontinuation of creatine supplementation in this study to be rare, though may consist of (i) gastrointestinal intolerance, (ii) allergic reaction, or (iii) withdrawal of participant’s or parents’ consent. If this occurs, the participant will no longer receive the intervention and outcomes will be assessed by intention-to-treat.

Outcomes

The primary outcome of the study is the change in RSPM score from baseline to 12 weeks. The RSPM measures non-verbal and abstract reasoning and fluid reasoning ability (Anderson et al., 2019). This single assessment will reduce the burden of multiple cognitive tests in adolescents, due to its ease of application and interpretability and validity as a measure of abstract memory and fluid intelligence (Assessment-Training.com, 2021). (Appendix 2).

Secondary outcomes include additional cognitive measures of processing speed, working memory, and non-visual memory, as well as body composition and safety. All cognitive tasks will be measured as the change in scores from baseline to week 12.

Processing speed will be measured with SpaceCode (Myszkowski et al., 2018), a computer game where participants are presented with a number at the bottom of the screen and must select the corresponding number in a central 3x3 grid. If done correctly, an enemy spaceship is destroyed. The total number of correct responses in 2 minutes is measured (McPherson et al., 2008). (Appendix 2).

Working memory will be measured with SpaceMatrix. This game combines SpaceCode while also monitoring a separate 5x5 grid where participants see the location of the enemy spaceships. After destroying 2-4 enemy ships, participants must fill another empty 5x5 grid with the correct locations of the destroyed ships. The number correctly reported is measured. The duration of this test is 4.5 minutes (McPherson et al., 2008). (Appendix 2).

Non-visual working memory will be assessed by the backward digit span (BDS). The BDS is a subcomponent of the Wechsler Adult Intelligence Scales (Kaufman et al.,1983) where participants will be provided a sequence of 10 digits, 1 second apart, and need to repeat them backwards, which has been shown in previous studies to assess short-term storage and verbal working memory, which complements the visual-dominated fluid memory test of the RSPM and SpaceCode and SpaceMatrix games (Rae et al., 2003).

Creatine supplementation has previously been shown to increase lean mass and decrease body fat, which is incompletely captured by body-mass index (BMI) but measurable with percentage lean body mass (Branch et al., 2003). The percentage in lean mass changes in the intervention and control groups after 12 weeks by several measurements will be investigated. Another objective is to determine if the dose provided shows changes in both cognitive performance as well as physical composition. These values will be measured through the physical examination associated with Bioelectrical impedance analysis.

Finally, though no adverse events are expected given prior studies, serum creatinine at the beginning and at 12 weeks will be measured to evaluate kidney function for safety (Branch et al., 2003).

Data Collection and Management

Data will be collected and managed by research assistants and will be filled in electronic records using RedCap software. Only research personnel and the PI will have access to the participants’ data that will be collected throughout the study, as it will be encrypted.

Documentation of all the study processes and the administration of the intervention will recorded in Case Report Forms (CRFs) (McPherson et al., 2008). Data collected in CRFs of this study by research personnel will include subject’s ID, biographical data (sex, date of birth, and ethnicity), medical history, medication lists, body composition, cognitive performance scores, eligibility, adherence to intervention, and prior grade percentile. All CRFs will be locked.

Other forms such as deviation forms, withdrawal forms, or any protocol changes such as changes of doses or intervention details, will be documented in the subject’s file along with the CRF. Study records including all participants documents, SOPs, and
regulatory binders should be retained 3 years post study in a clinical master file in electronic format.

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

**Interim Analysis**

Given the expected safety of creatinine supplementation, and short duration of the study, no interim analysis will be performed (Davani-Davari et al., 2018), (Anderson et al., 2019).

**Sample Size Calculation**

The sample size was calculated using STATA/BE 17.0 by performing the t-test for mean difference in change in RSPM score between the two groups. No prior studies reported the standard deviation (SD) for RSPM in our study population; therefore, we estimated a SD of 4.46 by assessing the percentile differences from a published age-stratified cohort (Assessment-Training.com, 2021) (Appendix 1). The minimum detectable difference was determined to be a change from the 50th percentile to the 75th (change in RSPM score of 3) (Myszkowski et al., 2018). To attain an alpha of 5% and 90% power, as well offset an expected drop-out rate of 17% based on prior studies using supplements in adolescents (Myszkowski et al., 2018), our sample size was calculated to be 116 participants, 58 per group. Given the number of students in an average middle-income area high school, one high school is expected to have sufficient students below the 50th percentile.

**Statistical Analysis**

Baseline characteristics will be presented as frequencies with percentages for categorical data, means with SD for data with normal distribution, or medians with interquartile range (IQR) for data with non-normal distribution.

For each continuous outcome (RSPM score, SpaceCode score, SpaceMatrix score, BDS score, percentage lean body mass, and serum creatinine), we will calculate the change from baseline to 12-week value for each participant. We will compare groups by reporting the mean differences with 95% confidence interval (CI) and P-value using the t-test if normally distributed or median difference with IQR and P-value using the Mann-Whitney U test if non-normally distributed. Any adverse safety events will be tabulated between groups and reported as counts with percentages, and compared between groups using the Fisher exact test, as rates of events are expected to be low. A P-value less than 0.05 will be deemed significant.

**Missing Data**

Intention-to-treat analysis will be used. A multiple imputation approach will be used to replace the missing data to preserve randomization using age, sex, baseline BMI, baseline RSPM, and baseline grade percentile to generate the outcome score. A sensitivity analysis with complete case data will also be performed.

**Discussion**

The CREATiVe study is a 12-week randomized controlled trial testing creatine supplementation versus placebo in adolescents with grades below the 50th percentile in the prior semester to investigate for a change in cognitive performance. This is a first of its kind study investigating a safe supplement in adolescents for a change in cognitive performance through multiple measurements. If the study results are positive, it would support the use of creatine supplementation to improve cognitive function of adolescents who are under-performing the median of their grade. If the dropout rate is also low, it would further support that daily creatine supplementation is tolerable and safe in adolescents. If study results are negative, then it would imply that, in high school students below the 50th percentile, intensive supplementation with creatine for 12 weeks is not significantly beneficial for cognitive function as compared with placebo. Though creatine has been proven safe, since this is an adolescent population, we will monitor if any unexpected safety concerns arise with respect to creatinine (kidney function), frequency of side effects, or higher rates of dropout than expected by capturing rates through the App and at end of study to further establish the safety of creatine supplementation in adolescents.

This study’s main strengths are that this randomized trial would provide robust evidence of creatine supplementation’s potential benefit in this adolescent population. Furthermore, assessing cognitive performance through several established cognitive tests will provide a more accurate assessment of potential changes that may occur with this intervention. Finally, we have estimated a sample size given a paucity of prior data in this population and were conservative in the potential dropout rate and required power leading a higher sample size still manageable for a single high school.

There are also limitations in this study. Though choosing a school from a middle-income population area was intentional to reduce confounders such as
malignant malnourishment or rigid dietary patterns, further generalizability may require a larger multicenter subsequent study. We have chosen our population under the assumption that participants who score below the 50th percentile may have a greater magnitude of improvement in their cognitive performance measures. Thus, they would be more likely to show an improvement after 12 weeks. If this occurs, repeating this study in adolescents with above average grades may be needed.

Improving cognitive function in adolescents who are underperforming in school is a challenging and important topic worthy of study. Creatine supplementation is a low-risk potential solution that has been shown to improve cognitive function in other populations but has not been specifically studied in healthy adolescents with below-average school performance. The results of this study would potentially support the widespread use of creatine supplementation in adolescents beyond physical health and can be further studied in populations with above-average academic performance or potentially performance in specific academic areas.

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References