**January 26, 2024**

**Title: Creatine Supplementation At Simulated Altitude**

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## Purpose

Military personnel are frequently exposed to extreme environments, including high altitude and hypoxic conditions, which severely limit exercise capacity and performance. For example, over 775,000 soldiers have been deployed to Afghanistan since 2001, many of which have been exposed to potentially life-threatening elevations above 4000 meters (13,123 ft) in locations such as the Tora Bora mountains (1,2). Surprisingly, very few methods have been developed to mitigate the effects of hypoxia on exercise performance (3), despite the Department of Defense prioritizing this area of research.

Previous studies have shown that creatine supplementation increases exercise performance at sea-level (4,5). Our team showed a 4% improvement in exercise performance with creatine supplementation (vs. Placebo) (4). However, no published study has examined whether creatine supplementation increases performance at altitude. The purpose of this study is to determine the effects of creatine supplementation on exercise performance at simulated-altitude.

## Methods and Protocol

### *Subjects*

Sixty healthy adults (30 men and 30 women) will be recruited and enrolled. Inclusion criteria includes: 1) 18-40 years old, 2) BMI 18.5-40.0 kg/m$^2$ 3) habitually active (>150 minutes per/wk of moderate-vigorous physical activity). Exclusion criteria includes: 1) pregnant or trying to become pregnant 2) recent use of creatine supplementation and 3) supplement allergies.

### *Subject Recruitment & Study location*

Recruitment efforts will be focused on California Polytechnic State University and the surrounding area. Participants will be recruited via flyers hat will be posted in physical locations around campus and the San Luis Obispo, CA area. These flyers will also be distributed electronically via campus lists and classes, and appropriate social media outlets. The flyer contains a QR code that can be used to access a contact information survey and preliminary eligiblity (See eligiblity script). If the participant is deemed eligible for the study, they will be asked to schedule a Zoom or in-person meeting with a member of the research staff to go over the study protocol in more detail and address any questions or concerns the participant may have.

If the participant is interested in taking part in the study, they will be provided with an informed consent form (electronic via Adobe Sign or paper copy). Subjects will give both verbal and written informed consent. Similar to our previous studies, subjects may sign the informed consent with ink signature or via electronic signature using RedCap. RedCap is a secure web application for building and managing online surveys and databases. All testing and procedures will occur in the Human Performance Laboratory in the Kinesiology building (43A) at California Polytechnic State University.

***Preliminary*** *testing*

Body weight via digital scale and height via stadiometer will be obtained. Subjects will also complete a physical activity questionnaire, health history questionnaire, and demographics questionnaire. Subjects will then complete a familiarization exercise performance test (repeated sprint test). Previous studies have shown a learning curve with the repeated sprint test (subjects get better at the test), and therefore will complete a familiarization test. The repeated sprint test involves six, 10-second sprints on a cycle ergometer (Racer-Mate Inc, Seattle, WA, USA) with 60 second recovery between each sprint [6]. Following a brief self-selected warm-up, the subject will build to their maximal cadence and then pedal maximally against a resistance of 0.075 kg/kg body weight for 10 seconds, followed by 60-seconds of recovery. The test will take approximately 10-15 minutes total time. We have previously used a similar protocol after creatine supplementation at sea-level (4), and the test is a validated measure of high-intensity exercise performance that mimics many physiological demands of military performance (6-8). Outcomes measures are peak power (watts), relative peak power (watts per kilogram), mean power (watts), relative mean power (watts per kilogram), and fatigue index (watts per second).

***Baseline*** *Testing*

At least 24 hours after the familiarization repeated sprint test, subjects will then return to the laboratory after an overnight fast. Subjects will complete a baseline Acute Mountain Sickness score (Lake Louise AMS score). Participants will then complete a baseline repeated sprint test, as described above. During the test, oxygen saturation and pulse will be collected on the index finger using a non-invasive pulse oximeter (ZacVrate, Inc.).

***Randomization and Supplementation***

After baseline testing, subjects will be randomized (by sex, age, ethnicity, and peak power), using a double-blinded parallel design, to placebo (calcium 500 mg) or creatine supplementation (20 grams) for 2 days. The supplements will be given to subjects in equal numbers of unidentifiable capsules. As we previously described (4), each total daily dose will be divided into 4 capsules to reduce gastrointestional stress. Subjects will be instructed to take 4 capsules a day, several hours apart, for two days straight (4,9,12,13).

***Simulated Altitude Testing*** After the 2 day supplementation treatment, subjects will arrive in the laboratory after an overnight fast and weight will be obtained. Subjects will rest for 30 minutes breathing a hypoxic gas mixture containing 12.8% oxygen, equivalent to 4000 meters elevation (13,123 feet elevation). The hypoxic gas will be emitted from a hypoxic generator (Hypoxico Hypoxic Generator, New York, NY) that lowers the oxygen content of room air and administers it to subjects through a HEPA filter and sealed facemask. This is a validated method commonly used to simulate altitude in research (10). Subjects will then complete the Acute Mountain Sickness score, and then perform the same repeated sprint test as described above. Oxygen saturation and pulse will be collected on the index finger using a non-invasive pulse oximeter (ZacVrate, Inc.).

After completion of the study, a researcher will go over the subjects' repeated exercise test results with them in person.

***Description of Risks and Benefits***

***General Risk of Exercise*** Despite the well-documented benefits, there are minimal risks associated with exercise (11). Participants may experience fatigue and muscle soreness due to an increase in physical activity from previous sedentary states. These health risks are small in people with no prior history of cardiovascular, respiratory, or musculoskeletal disease or injury. Any ordinary fatigue or muscle soreness is temporary and usually lasts 24-96 hours (about 4 days). With any exercise, there is a slightly increased risk of acute cardiovascular complications. The American College of Sports Medicine notes that although the relative risk of unfavorable outcomes slightly increases with exercise, the absolute risk of a cardiac event is still very low (1 per 133,000 men and 1 per 769,000 women). To ensure the safety of subjects during all exercise tests, we will follow American College of Sports Medicine guidelines. During the test, if the subject experiences any problems (light-headed, nauseous, excessive redness in the face, or heart palpitations) the exercise test will be immediately stopped. Additionally, we will be monitoring the participant's oxygen saturation and provide additional caution if their oxygen saturation levels drop below 80% (which is not expected). After the exercise test, subjects will cool-down for a 3-minute period or until heart rate reaches close to resting values. There will always be at least 2 CPR certified persons in the room during testing, one who can perform CPR and one who can call 911**.** The lead investigator and/or trained students have administered numerous exercise tests with no adverse events.

***Supplementation Risks***

Creatine supplementation has minimal side effects, espically short-term supplementation. There is a slight risk of acute water retention [7]. Creatine supplementation has been deemed safe in the dosages prescribed in this study in individuals free of gastrointestinal, metabolic, renal, liver, or cardiorespiratory diseases [8,9]. Individuals with these conditions will be screened and excluded from participation using the previously described screening methods.

***Simulated-Altitude***
There are minimal risk associated with simulated altitude for 45 minutes. Subjects may transiently feel lightheaded, dizzy, or fatigued, and may stop at any time.

***Questionnaires***
Subjects may omit any questions on the surveys (physical activity, health history, demographics, Acute Mountain Sickness score).

***Benefits***
There are no direct benefits for participating in this study, and subjects will not be compensated.

***Data Collection***
***Statistics and Sample-size Calculation*** A repeated measures ANOVA will be used to determine differences on all variables of the repeated sprint test, adjusting for age, sex, ethnicity, and peak power A $\alpha < 0.05$ will be considered significant, and Tukey's HSD tests will be used to correct for multiple comparisons.

***Data Storage, Sharing, and Destruction*** All data will be collected by trained student researchers under the authorization of the lead investigators. Data will be examined in a confidential fashion to preserve the privacy of each participant. All members of the research staff will have completed the required CITI and Cal Poly training modules. The data collected in this study will be accessible only to the researchers involved. All data will be stored on a password-protected computer and transferred to a password-protected cloud-based storage drive. All data will be de-identified using both a unique study and participant ID. The file linking the participant's identifiable information to their ID number and confidential data will be kept in a separate password-protected location. After 7 years following the completion of the study, hard copies of the data will be destroyed. Data specific to the aim of this study will be shared with scientific journals for publication purposes only, when appropriate.

**Works Cited**


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