Astaxanthin Supplementation Improves Cognitive Performance Following Mental Fatigue in Recreationally Active Females

NCT06460181

02/11/2025

Materials and Methods

Experimental Approach to the Problem

The present study utilized a double-blind, 2-arm parallel study design (AX or placebo (PLA)) to examine the effect of AX on a battery of cognitive tests via touch screen tablet pre-post MF. Before subject recruitment, the required sample size was calculated *a priori* using an effect size in the psychomotor vigilance task (PVT-B) from previous research (Cohen's $d = 0.3$), which demonstrated a significant improvement in reaction time (ms) following consumption of a high-caffeinated energy drink (Antonio et al., 2019). Thus, using G*Power (version 3.1) for a within-between repeated measures design using an effect size of 0.40, expected power of 0.80 (1-β), and an α of $p \leq 0.05$, our power analysis indicated that a sample size of 16 would be sufficient to demonstrate significant differences for our chosen battery of cognitive tasks, if differences indeed exist. Anticipating a 1:3 dropout rate, we recruited 25 subjects for this study. Subjects reported to the laboratory for testing on a total of four occasions: visit 1) a familiarization with the cognitive software and battery of cognitive tests (BCT; described further below), visit 2 and 3) completion of the BCT after either a MF protocol or time-matched control, and visit 4) completion of the BCT, post-MF following 4-weeks of supplementation. Following visits 1-3, subjects were randomly assigned by order of recruitment, either AX or PLA to supplement daily for 4-weeks.

Pre-Trial

All trials took place at the same time of day (±30 min) for each subject and were each separated by 48 h. Upon arrival for trial 1, a physical activity readiness questionnaire, an exercise history questionnaire, and a menstrual cycle questionnaire were completed. Each subject then had their body mass (Tanita Corporation, Tokyo, Japan), height (Deteco, Webb City, MO, USA),

and body fat assessed using bioelectrical impedance analysis (SECA mBCA 514, SECA North America, Chino, CA, USA).

Subjects were given a list of dietary supplements (e.g., multivitamins, creatine, antioxidants, energy drinks, fruit powders, etc.) to refrain from for at least two weeks before testing. A list of AX-rich foods (e.g., salmon, crustaceans, etc.) was also provided to avoid during the 4-week supplementation period. Subjects also completed weekly dietary and physical activity logs (using a fitness and nutrition tracking app on their smartphone or computer), refrained from alcohol and caffeine for 24 h, avoided strenuous physical activity for 48 h, and were asked to arrive well-hydrated and well-rested (i.e., ±30 min of their usual sleep and wake times) before each experimental trial. Subjects verbally confirmed each criterion prior to completing the battery of cognitive tasks described below. To standardize habitual diet and exercise volume, subjects maintained dietary and physical activity logs before testing and throughout the 4-week intervention. Logs were submitted weekly to the lead investigator. While training was not controlled, exercise volume was monitored, and subjects were instructed to maintain their usual diet and exercise routines during supplementation.

Familiarization and Mental Fatigue

The BCT (Soma Technologies, Lucerne, Switzerland) was adopted from two previous studies our research team conducted examining the effect of a dietary supplement on markers of cognitive performance following a MF protocol in females (Waldman et al., 2023a, 2023b). The test was administered via a touchscreen iPad tablet (10th generation, Apple) and lasted 16 min. Each cognitive task was chosen to measure low- and high-level cognitive processing domains based on proposed definitions (Chang et al., 2012). During visit 1, the investigator briefed all subjects on each task by verbally explaining and demonstrating each task to them. After each

subject verbally acknowledged the purpose and execution of each respective task, the entire BCT was completed for a total of three times.

During visits 2 and 3, subjects were counterbalanced to complete either the control or MF protocol. During the control trial, subjects watched a 20-min video titled “World Class Trains—The Venice Simplon Orient Express” (Pegasus-Eagle Rock Entertainment, 2004). This documentary was chosen due to its utilization in prior MF investigations (Marcora et al., 2009; Martin et al., 2015; Waldman et al., 2023a) and has been shown to maintain a neutral mood and stable heart rate during viewing (Silvestrini & Gendolla, 2007).

The MF protocol was a 20-min time load dual back (TLDB) task which has shown to be an effective protocol for inducing MF in prior investigations (Mortimer, et al., 2024; O’Keeffe, et al., 2019; Staiano, et al., 2024). During the TLDB, subjects were presented with a series of numbers and letters with three labels at the bottom of the screen (the word “Left”, the number “1” or the number “2”). When the letter that was displayed was the same as the previously displayed letter, the subject would press the left button at the bottom of the screen. When an odd number was presented, subjects were to tap the button label “1” and when an even number was presented, subjects were to tap the button label “2” as fast as possible. The task operated in an adaptive mode whereby task difficulty varied with performance to maintain a heightened cognitive load. Upon an incorrect or no response, an audible adverse buzzer would emit from the tablet.

The purpose of visits 2 and 3 were to confirm that the MF protocol did indeed impair cognition in our female subjects compared to a control. Following these visits and prior to group randomization, a paired *t*-test was performed on reaction time (ms) in the PVT-B following either the control or MF protocol. Results indicated that MF resulted in a significantly slower

reaction time (439.3 ± 42.4 ms) compared to control (432.7 ± 37.1 ms)(p = 0.02). Following confirmation of MF, all variables of interest (reaction time, speed, and responses correct per second) collected during the MF visit then served as a baseline (BL) for comparing post-supplementation values (POST) during later analysis.

Cognitive Test Battery

The battery of cognitive tasks was completed in the same order for each trial. The tablet was placed ~1 m away from each subject on a table and subjects were instructed to place their dominant index finger on a legend ~15 cm from the screen and return it after each response. Subjects were placed in a quiet and dark room while being monitored by an investigator through a double-sided window during testing. Three metrics were collected from the battery of cognitive tasks to be used for statistical analysis: (1) reaction time (ms; RT), (2) speed, defined as (Mean $_{\text{step N1}}$/response time) and also known as reciprocal response time, (3) and responses correct per second (RCS).

The first cognitive task completed was a 5-min psychomotor vigilance test (PVT-B). The PVT-B measures sustained low-level cognitive processing by assessing the subject's attention and consistently responding to a visual stimulus. The PVT-B provided the subjects with a visual stimulus in the form of a circle that appeared in the middle of the screen. The stimulus was presented intermittently for 500 ms or until a response was given.

Following the PVT-B, subjects completed a 3-min task-switching (TS) test. This task measures the subject's ability to switch between different tasks and represents high-level cognitive processing (also known as cognitive flexibility). Numbers 0-10 were shown to the subjects in either white or red colored font. For white numbers 0-5 and red odd numbers, subjects were instructed to tap a left-facing arrow displayed at the bottom left-hand corner. For white

numbers 6-10 and red even numbers, subjects were instructed to tap a right-facing arrow displayed at the bottom right-hand corner. If subjects responded incorrectly or failed to respond in the allotted time, an audible adverse buzzer would emit from the tablet.

The third task was a 3-min incongruent flaker (IF) design to test inhibition. This task assessed the subject's ability to ignore task-irrelevant information and represented high-level cognitive processing. Subjects were presented with a series of five arrows, with four of the five arrows pointing in either the left or the right direction. Subjects were instructed to focus on the middle arrow when it appeared and ignore the two arrows immediately to the left and right of it. Whichever direction the middle arrow was pointing, subjects were to tap at the bottom left- or right-hand corner of the screen, the arrow that corresponded with the middle arrow's respective direction. When incorrect responses occurred, or a response was not given, an audible adverse buzzer would emit from the tablet.

Supplementation Schedule

Following visit 3, subjects were assigned either AX or a matched PLA and supplemented for 4-weeks at a dose of 12 mg/day. The AX (AX combined with sunflower oil) and PLA (sunflower oil only) capsules were provided by AstaReal (AstaReal, Inc., Moses Lake, WA, USA) and were matched for shape, size, color, and odor. An independent investigator labeled the pill bottles "A" or "B", counted them, and distributed them to the subjects. Each capsule contained 12 mg of either AX or PLA, and subjects were instructed to ingest one capsule daily with a meal containing a dietary fat source. If a capsule was missed, the subject ingested the missed capsule as soon as possible. Each subject was provided with an undisclosed but previously counted number of capsules in each bottle. Compliance ([capsules ingested]/$N \times 100$) was evaluated once the remaining capsules were returned following the conclusion of all testing.

A compliance of less than 90% was considered unacceptable, resulting in the subject's removal. However, all subjects had acceptable compliance; therefore, no subjects were removed from the study prior to statistical analysis.

Statistical Analyses

Data are presented as the mean ± SD with an a priori alpha level of $\alpha \leq 0.05$ used to determine significance in all analyses. Provided the primary interest was whether AX supplementation mitigated MF following supplementation (BL vs. POST) and not whether between group differences existed, we chose to analyze all primary variables (RT, speed, RCS) for each cognitive task (PVT, IF, TS) with a paired *t*-test for BL to POST changes within groups. Effect sizes were calculated and reported as Cohen's *d* (*d*: trivial = 0–0.19, small effect = 0.20–0.49, moderate effect = 0.50–0.79, and large effect = ≥0.80) in instances where significance occurred. IBM SPSS Statistics v. 27 was used for all statistical analyses.