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Original Research

Effect of Huperzine A on Cognitive Function and Perception of Effort during Exercise: A Randomized Double-Blind Crossover Trial

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ABSTRACT

International Journal of Exercise Science 14(2): 727-741, 2021. Huperzine A has shown the ability to acutely improve cognitive function in certain populations, and therefore is commonly added to pre-workout supplements. However, its effects have not been studied in exercise-trained individuals. OBJECTIVE: We hypothesized that acute consumption of huperzine A would improve cognitive function during exercise, which may be beneficial for exercise performance. METHODS: From January to April, 2018, 15 exercise-trained individuals (11 women [height 166 ± 2 cm, weight 60.5 ± 3.0 kg] and 4 men [height 173 ± 4 cm, weight 82.0 ± 11.0 kg], BMI 23.5 ± 1.4 kg/m^2, age 30.4 ± 3.6 years) were studied in a double blind randomized-sequence cross-over study, in which they underwent tests for cognitive function (digit span, verbal/word fluency, and Stroop), neuromuscular performance (sharpened Romberg and dart throwing), and exercise performance (estimated aerobic capacity, hand-grip strength, vertical jump, and push-up) after acute ingestion of huperzine A (200 mcg) or placebo. One week separated the two trials. RESULTS: No measures of cognitive function differed between placebo and huperzine A trials (all $p \geq 0.296$). Heart rates (157 ± 4 vs. 158 ± 4 bpm; $p = 0.518$) and ratings of perceived exertion (13.7 ± 0.56 vs. 13.9 ± 0.61; $p = 0.582$) did not differ between placebo and huperzine A trials, respectively. Ratings of subjective difficulty post-exercise (0-10 scale) were significantly higher (5.7 ± 0.38 vs. 6.8 ± 0.38; $p = 0.002$) in the huperzine A trial than the placebo trial. No differences were observed for neuromuscular or exercise performance measures between groups (all $p \geq 0.497$). CONCLUSIONS: Huperzine A does not enhance cognitive function during exercise despite it being marketed as a cognitive enhancer. Because of its inability to enhance cognitive function, its inclusion in pre-workout supplements warrants reconsideration. Other more practical and effective strategies should be considered.

KEY WORDS: Aerobic exercise, cognitive performance, sports nutrition, cardiorespiratory fitness, acetylcholinesterase inhibitors, ergogenic aid

INTRODUCTION

The use of supplements as ergogenic aids has become increasingly popular among exercise-trained individuals across many sports and competition levels (20). One of the most popular forms of these supplements is energy drinks, which were reported to be used by 73% of division

I collegiate athletes; one of the main reasons for consuming these drinks was to "provide energy during practice" (15). Many energy drinks and supplements contain multiple ingredients to which enhanced "energy" and ergogenic effect could be attributed. This makes it difficult to determine which ingredients are responsible any observed benefits. Although some ingredients in sports supplements and energy drinks, such as carbohydrate and caffeine, have been extensively studied for ergogenic effects, little or no scientific evidence exists for other common ingredients.

Huperzine A is an ingredient found in ~11% of multi-ingredient pre-workout supplements (1). It was originally isolated from the moss *Huperzia serrata* and has been used in Traditional Chinese Medicine to promote circulation, fever reduction, anti-inflammatory effects, and for analgesic purposes (17). Huperzine A is marketed in the United States as a cognitive enhancing supplement and is available over-the-counter. Some evidence suggests that huperzine A modestly improves cognitive function in patients with Alzheimer's disease and dementia (34), possibly through its potent inhibition of acetylcholinesterase (36). Huperzine A may also acutely enhance cognitive function in young, healthy adults (24, 35). Presumably based on these studies, huperzine A has been added to sports supplements to provide mental and physical "energy" for exercise training, which would be appealing to athletes and other exercise enthusiasts. Because exercise-induced mental fatigue affects physical performance (16), it is also conceivable that huperzine A could also have ergogenic effects. Despite these appealing possibilities, no studies have evaluated the effects of huperzine A on mental (cognitive) function during exercise or on exercise performance. In this context, the purpose of this study was to use a double blind, randomized-sequence, placebo-controlled crossover trial to evaluate the hypothesis that huperzine A enhances cognitive function during exercise and reduces perception of effort. A secondary aim was to evaluate the effects of huperzine A on several neuromotor tasks (e.g., balance) and on exercise performance.

METHODS

Participants

Exercise-trained men and women aged 18-60 (30.4 ± 3.6) years old from the greater Saint Louis area were recruited. Candidates were required to be exercise-trained, which was defined as moderate to vigorous intensity endurance exercise (i.e., brisk walking, running, cycling, etc.), at least three days per week, for at least 20-minutes per session, for at least six months prior to this study. Resistance training did not contribute to the definition of exercise-trained for the purposes of this study. Medical history, medication use, and diet history were used in conjunction with the American College of Sports Medicine's risk classification algorithms to identify and exclude those individuals that required medical clearance to participate in vigorous exercise (14). All of the participants provided informed written consent to participate in the study, which was reviewed and approved by the Saint Louis University Institutional Review Board. The trial was registered at ClinicalTrials.gov (identifier: NCT03445104). This research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (25).

Protocol

The study was conducted in a university setting and utilized a double blind, crossover design. An initial session was used to familiarize participants with the procedures and obtain baseline measures. The participants underwent two experimental trials in which they received huperzine A or placebo in randomized sequence. The randomization scheme was counter balanced with blocking (2 subjects per block; www.randomization.com) and allocation was performed by personnel not involved in data collection to maintain participant and investigator blinding (Figure 1).

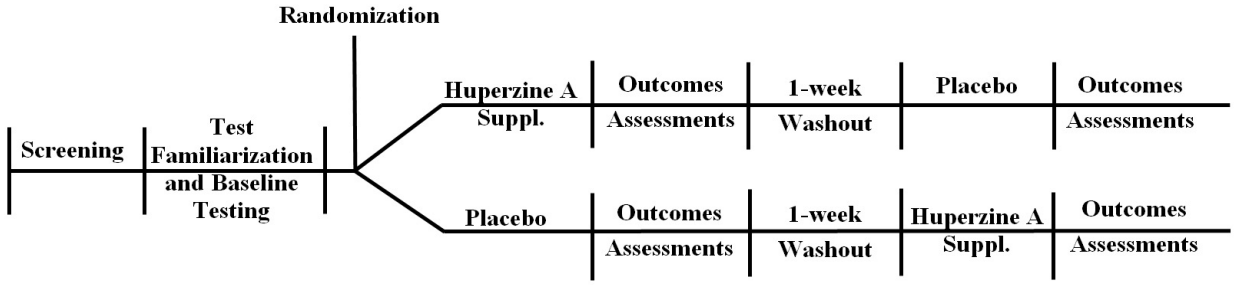

Figure 1. Study Design. The study was a randomized sequence cross-over trial in which participants underwent outcomes testing on one occasion after oral supplementation with Huperzine A and on another occasion after taking a placebo. The sequence for treatment administration (Huperzine A vs. placebo) was randomly assigned for each participant.

Huperzine A (200 mcg, Swanson®, Fargo, North Dakota) and placebo capsules were administered in a single oral dose. The dose of Huperzine A used is comparable to previous research (33). Placebo capsules consisted of rice flour and the capsules were identical in size and appearance. The participants ingested the capsules with 250 mL of water 30-45 minutes before exercise was initiated. Previous work has identified that orally ingested huperzine A appears in the blood within 15-minutes and reaches peak levels by 60-minutes (22).

Enrolled participants underwent an initial visit to be familiarized with diet, exercise, and sleep control procedures, exercise and cognitive function tests, exercise equipment, and supplementation procedures. Outcome tests were performed for familiarization purposes and for non-intervention baseline measures.

The participants returned within one week of the initial session for the first study trial. During this session, participants received either huperzine A or placebo and underwent testing. After one week, participants returned for the second study trial, and based on what they received during the first session, received either huperzine A or placebo before undergoing the same testing. Mood assessment was performed before and after exercise, RPE and HR were measured periodically throughout exercise, cognitive function tests were performed during the last 10-minutes of exercise. All remaining tests were performed following exercise. These outcome measures are described in further detail below.

During the first experimental session, the endurance exercise task consisted of a fatigue inducing protocol using a treadmill (PPS 55, Woodway, Inc., Waukesha, WI). The task involved an initial stepwise increase in work rate until 70% of heart rate reserve (HRR) was reached [70% HRR = 0.70 x (maximal heart rate – resting heart rate) + resting heart rate]. Age-predicted maximal HR was predicted via 208 – 0.7 x age in years (30). An additional 30 minutes of steady state exercise was then performed at this work rate. Initially, the treadmill was set to a speed of two miles per hour (MPH) at 0% grade. Speed was increased by 0.5 MPH every three minutes until the participant reached a self-defined "brisk" pace. Participants were permitted to walk or to run based on their perception of "brisk". From there on, percent grade was increased by 2% until 70% HRR was reached (Figure 2). The speed and grade at which 70% HRR was reached was used during the second experimental session (19). Total estimated time of this task was 35-45 minutes; exercise bouts of this duration have been shown to impair cognitive function in past studies (23, 31).

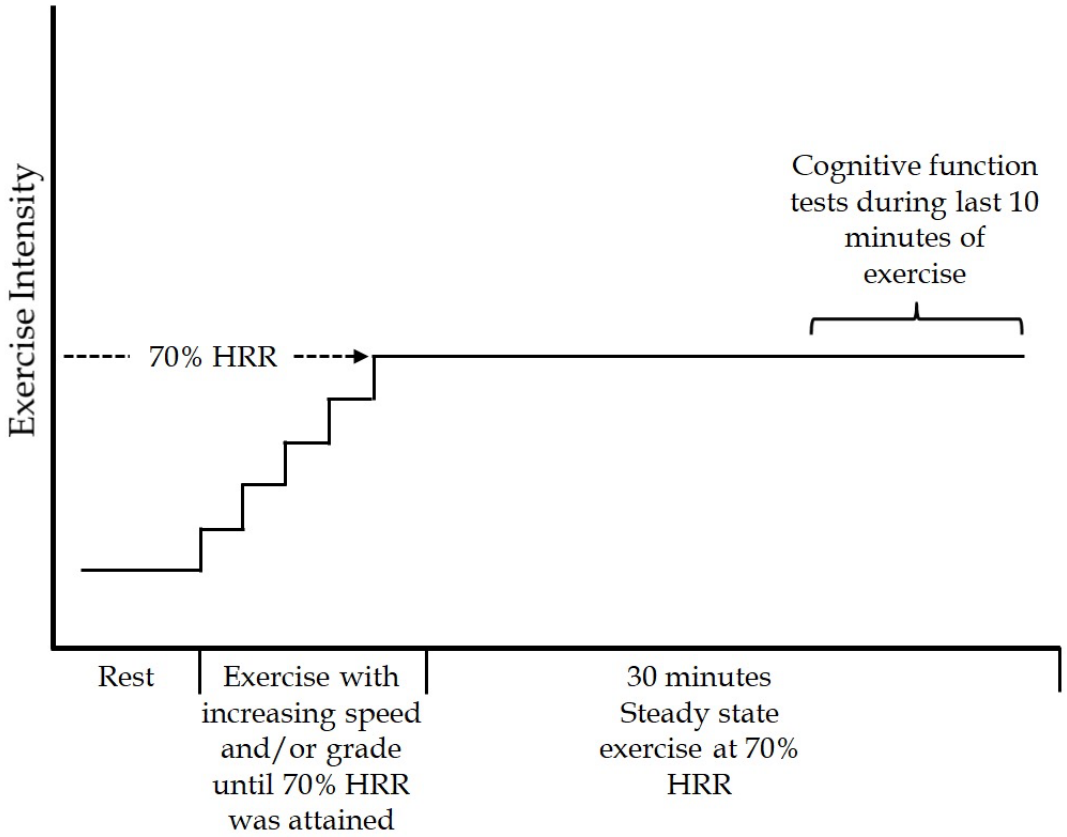

Figure 2. Treadmill exercise protocol. During each participant's first study trial, exercise was initiated at 2.0 mph and 0% grade for 3 minutes, followed by 0.5 mph speed increments every 3 minutes until the participant reached a self-described "brisk" pace; thereafter, grade was increased 2 percentage points every 3 minutes until 70% of heart rate reserve (HRR) was attained. The work rate that elicited 70% HRR was then performed for 30 minutes and cognitive function tests were performed during the last 10 minutes of this period. During each participant's second study trial, the speed and grade used during the first protocol were replicated exactly to ensure that the external work rate for the two study trials was matched.

Cognitive function tests that were performed include: digit span (forward), verbal fluency/word fluency, and the Stroop test. These tests have been utilized to assess cognitive

function through different cognitive domains during exercise in the past (4). During the study trials, these tests were implemented with 10 minutes remaining in the endurance-exercise task. By using a randomization process, the test sequence was homogenized to ensure that the test sequence differed every time a given participant was tested.

The digit span (forward) test was used as a measure of working memory (4). Variations of this test have been used to assess cognitive function in children and adults (21, 28). During a period of two minutes, the participants were presented with a series of digits at a rate of one digit per second and were required to repeat them verbatim. If they succeeded, they were presented with a longer series by one digit. The length of the series continued to increase by one digit until the participant failed to successfully repeat the digit span on two consecutive attempts or until the two-minute time limit was reached. The longest series the participant was able to recall was recorded as their digit span score.

Letter verbal fluency and category verbal fluency were used as measures of verbal ability and executive control (4). For letter verbal fluency, participants were instructed to generate words beginning with F, A, S, B, H, or R. Each participant was randomly assigned a different letter at each session. Participants were given one minute to name as many words as they could, beginning with the letter they were assigned. The total number of words named within one minute was recorded as the participant's letter fluency score. For category verbal fluency, participants were instructed to produce as many words as they could for a particular category (e.g., animals, items of clothing, boys' names, or girls' names) within one minute. Each participant was randomly assigned to a different category each session. The total number of words named within one minute was recorded as the participant's letter fluency score. A similar protocol was used by Nocera and colleagues to assess the impact of exercise on executive function in elderly adults (26).

The Stroop test was used as a measure of information processing (4). Participants were exposed to color names in a font color that did not match the color name. They were instructed to name the color of the font and to ignore the color name (e.g., if the word green was presented to them in red font, the goal was to say "red" and ignore the word green). The participants had two minutes to complete this task. The sum of correctly identified stimuli was recorded as their Stroop score. This test is a modified version of the Stroop effect task originally developed by Stroop (29).

The RPE scale is used to allow the exerciser to subjectively rate their effort during exercise. The scale can also be used to indicate impending fatigue. Participants were asked to report their RPE on a scale of 6 to 20 (6 being no exertion at all, 20 being maximal exertion) at 5-minute intervals during the endurance exercise task (13). Immediately prior to the endurance exercise task, participants were asked to rate their confidence regarding the task on a scale from 0-10. Immediately following the endurance exercise task, participants were asked about the ease/difficulty of the task.

Following endurance exercise, participants' hand-grip strength and vertical jump height were used as measures of muscular strength. Grip strength was measured using a hydraulic hand grip dynamometer (27) (model J00105, Lafayette Instrument, Inc., Lafayette, IN). Both arms were used for testing while bent at a right angle with the elbow at the participants' side. Participants were instructed to squeeze the dynamometer handle with maximal effort for five-seconds three separate times, with the best of the three results being recorded. The vertical jump test was conducted by instructing participants to stand side onto wall and to jump from a standing position using their arms and legs to propel themselves upward. Their fingertips were covered in chalk, which they used to mark the wall at the highest point of the jump. The highest of three jumps was recorded (5).

A push-up test standardized through the ACSM was used as a measure of muscular endurance following handgrip and vertical jump protocol. To begin, men started in the standard "down" position, while women started in the modified "knee push-up" position. Hands were pointed forward and under the shoulder with the back straight and head up. The participants were then instructed to raise their body by straightening their elbows before returning to the down position. For this test, the back must remain straight at all times, the participant must continue downward until their chin touches the floor or a mat, and the participant must fully straighten their arms during each repetition. The test was stopped when the participant strained forcibly or was unable to maintain proper form. The maximum number of pushups without form breakdown was recorded (13).

Aerobic capacity was calculated via standard techniques (37) and was used to predict maximal oxygen uptake (VO2max) during treadmill running. A regression equation was generated for each participant based on their resting HR, submaximal exercise HR, and predicted oxygen uptake (VO2). Their resting and exercise HRs were measured as described above, while their resting VO2 was assumed to be 3.5 mL/kg/minute. VO2 at age-predicted maximal HR was calculated using the subsequently generated regression equation, where age-predicted maximal HR = 208 – 0.7 x age in years. This was considered VO2max (30).

Dart throwing was used as a measure of hand-eye coordination following the muscular endurance task. Modelled after previous dart throwing procedures used in previous research, the dartboard consisted of ten concentric circles. The board was positioned in accordance with international standards; the bull's-eye was 1.73 m from the floor while the throwing line was 2.37 meters away from the board. The score ranged on a scale from 0-10 (0 being off of the board, 10 being a bull's-eye). Participants completed three rounds of three throws. The total score from all throws was the recorded score (37).

The Sharpened Romberg test (SR) was used as a measure of balance performance. The participants were asked to remove their shoes and stand with their feet in tandem position (heel to toe), their arms crossed over their chest, and the palms of their hand on the opposite shoulder. They were first asked to stand quietly with their eyes open and then with their eyes closed. They were instructed to attempt to maintain this position for 60-seconds. If they were unable to do so, they were given up to three additional attempts. The sum of each trial was recorded. If a score

of 60-seconds was reached at any attempt the subsequent attempts were forgone and given the score of 60. Study personnel were nearby to ensure safety of the participant should they have fallen (18).

During an initial meeting the participants were instructed regarding keeping a 24-hour food log before both testing days. The 24-hour food log prior to the first session served as a guide to replicate dietary intake prior to the second session. Participants were instructed to continue their current training regimens but were instructed to avoid strenuous activity 24 hours prior to each testing session. Additionally, participants were asked to keep a record of their sleep patterns prior to testing (i.e. bed time, wake up time, and any naps). If participants were unable to duplicate dietary, exercise, or sleep patterns from their first visit for their second visit, the principal investigator used subjective clinical judgement to determine if the severity of difference warranted rescheduling of the participant.

Resting heart rate was measured via manual palpation of the radial artery for one minute. A wristwatch-type heart rate monitor (Polar RS200, Polar Electro Oy, Kempele, Finland) was used to monitor exercise heart rate, which was recorded at intervals of 5-minutes during endurance exercise.

Statistical Analysis

A statistical power analysis was performed (G*Power software, version 3.1.5, University of Kiel, University of Dusseldorf, and University of Mannheim, 157 Germany) based on the following inputs: two-tailed paired t-test, alpha = 0.05, desired power = 0.80, sample size = 15 subjects. The results indicated that a standardized effect size of 0.78 (i.e., large effect) would be detectable. 15 subjects were recruited and enrolled in the study.

A paired-sample t-test with alpha set to $p \leq 0.05$ for statistical significance was used to analyze the data. Outcomes measured during the first and second experimental sessions were compared to each other. The participants were compared to themselves during previous sessions, so a paired sample t-test was used to calculate the difference between the sessions. Data was presented as means ± SE. Data analyses was performed with Microsoft Excel and Cohen's effect size analyses were performed using an online calculator (http://www.campbellcollaboration.org/escalc/html/EffectSizeCalculator-Home.php).

RESULTS

Fifteen women (n = 11) and men (n = 4) were enrolled and completed the entire study (Table 1). Age of the participants was in the lower end of the targeted range. Thirteen of the participants were in the normal range for BMI, while two were in the overweight category, and one was in the obese category. All participants were exercise trained, defined as participating in moderate to vigorous intensity endurance exercise ≥ 3 days/week, for ≥ 20-minutes/session, for ≥ 6 months. On average, participants self-reported participating in moderate exertion endurance exercise 5 days/week for 67 min/session (Table 1).

Table 1. Subject Characteristics

Gender	
Men	4 (27%)
Women	11 (73%)
Age, years	30.4 ± 3.6
Height, cm	
Men	173 ± 4
Women	166 ± 2
Body mass, kg	
Men	82.1 ± 11.0
Women	60.5 ± 3.0
BMI, kg/m^2	23.5 ± 1.4
Habitual exercise modes	
Endurance exercise	15 (100%)
Endurance & strength exercise	10 (67%)
Habitual exercise frequency, sessions/week	5 ± 0
Habitual exercise duration, min (all modes combined)	67 ± 7
Habitual exercise intensity, sessions/week	
Light exertion	1 (7%)
Moderate exertion	15 (100%)
Hard exertion	5 (33%)
Baseline cognitive function tests	
Digit span	6.7 ± 0.3
Letter fluency	17.3 ± 1.1
Category fluency	24.3 ± 1.7
Stroop	83.5 ± 3.5
Predicted VO_{2max}, mL/kg/min	
Men	53.7 ± 2.6
Women	45.4 ± 3.0

Values are counts (%) or means ± SE. Estimates of exercise frequency, duration, and intensity include all modes of exercise.

Digit span did not change from rest to exercise in either the placebo trial (5.22 ± 5.43%, p = 0.510) or huperzine A trial (5.04 ± 4.69%, p = 0.458). Furthermore, there was no difference (p = 0.96) in percent change (Figure 3) observed between placebo (5.22 ± 5.43%) and huperzine A (5.04 ± 4.69%) trials for digit span. Letter fluency tended to improve from rest to exercise in the placebo trial (22.93 ± 10.02%, p = 0.076) and improved significantly in the huperzine A trial (28.46 ± 10.68%, p = 0.018). However, there was no significant difference (p = 0.49) in percent change observed between placebo (22.93 ± 10.02%) and huperzine A (28.46 ± 10.68%) trials for letter fluency. Category fluency did not improve from rest to exercise in either the placebo (8.86 ± 9.80%, p = 0.756) or huperzine A trial (0.53 ± 8.00%, p = 0.609). Further, there was no significant difference (p = 0.39) in percent change observed between placebo (8.86 ± 9.80%) and huperzine A (0.53 ± 8.00%) trials for category fluency. Stroop tended to improve from rest to exercise in the

placebo trial (9.91 ± 4.30%, p = 0.067) and improved significantly in the huperzine A trial (11.95 ± 4.32%, p = 0.022). However, there was no significant difference (p = 0.59) in percent change observed between the placebo (9.91 ± 4.30%) and huperzine A (11.96 ± 4.32%) trials for the Stroop test.

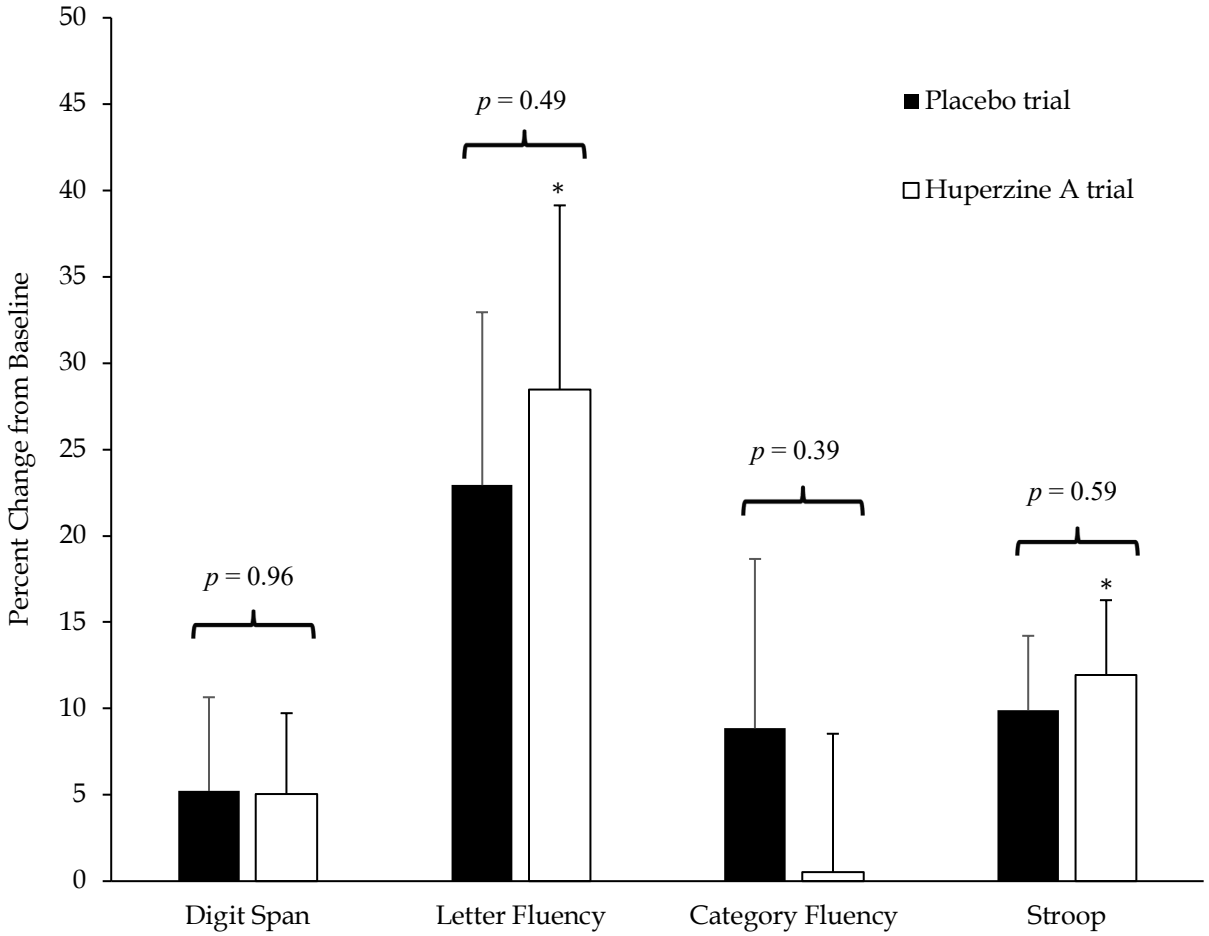

Figure 3. Average percent change of cognitive function task performance from the resting baseline to exercise during the study trials.

The study results were also analyzed by using standardized effect sizes (Cohen's d) to gain insights about the effects of huperzine A on cognitive function. Results from these analyses were mixed, suggesting no treatment effects on digit span performance (d = 0.03), small to moderate beneficial effects of huperzine A on letter fluency (d = 0.37) and Stroop tests (d = 0.29), and small to moderate detrimental effects of huperzine A on category fluency (d = 0.46).

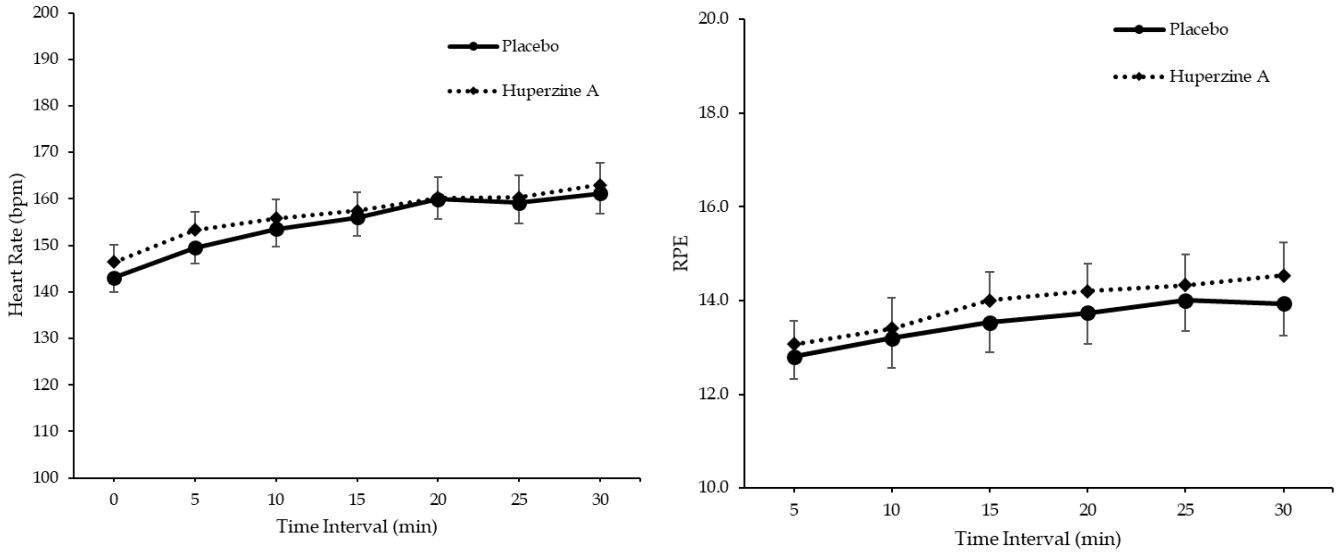

Figure 4. Average heart rate and rating of perceived exertion during 5-minute intervals during a 30-minute steady state treadmill exercise.

Average RPE did not differ (p = 0.582) between the placebo (13.7 ± 0.56) and huperzine A (13.9 ± 0.61) trials. RPE did not different significantly at any 5-minute interval during the treadmill exercise (all $p \geq 0.12$; Figure 4). Average ratings of pre-exercise confidence (0-10 scale) did not differ (p = 0.546) between the placebo (8.2 ± 0.45) and huperzine A (8.3 ± 0.39) trials. Average ratings of subjective difficulty post-exercise (0-10 scale) differed significantly (p = 0.002) between the placebo (5.7 ± 0.38) and huperzine A (6.8 ± 0.38) trials (Figure 5).

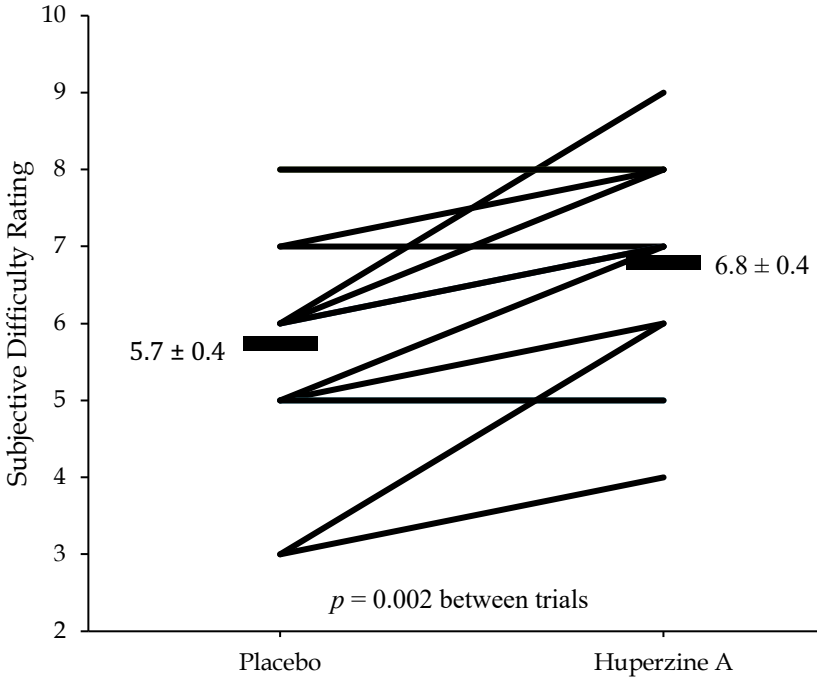

Figure 5. Mean and subject-level post-exercise subjective difficulty ratings following the 30-minute treadmill exercise.

Measures of neuromuscular performance included the sharpened Romberg balance test and a dart-throwing test. Average balance time did not differ (p = 0.629) significantly between placebo (113 ± 16.4 min) and huperzine A (121 ± 17.1 min) trials. Also, average dart-throwing scores did not differ (p = 0.676) between placebo (49.4 ± 3.2) and huperzine A (50.0 ± 2.7) trials.

Table 2. Cognitive Function & Exercise Performance in response to Placebo and Huperzine A

	Placebo (n = 15)	Huperzine A (n = 15)	p-value
Cognitive Function			
Digit span score	6.9 ± 0.3	6.9 ± 0.2	1.000
Letter fluency score	20.1 ± 1.0	20.9 ± 0.9	0.498
Category fluency score	24.9 ± 1.4	23.3 ± 1.4	0.296
Stroop score	92.1 ± 3.7	94.9 ± 4.6	0.345
Treadmill Exercise			
Aerobic Capacity, mL/kg/min	47.6 ± 2.5	47.4 ± 2.0	0.856
Heart Rate, bpm	157 ± 4	158 ± 4	0.518
RPE	13.7 ± 0.56	13.9 ± 0.61	0.582
Confidence & Mood			
Confidence Pre-Exercise (0-10)	8.2 ± 0.45	8.3 ± 0.39	0.546
Difficulty Post-Exercise (0-10)	5.7 ± 0.38	6.8 ± 0.38	0.002
Neuromuscular Performance			
Balance, min	113 ± 16.4	121 ± 17.1	0.629
Darts score	49.3 ± 3.2	50.0 ± 2.7	0.676
Muscular Strength & Endurance			
Handgrip Strength, kg	61.5 ± 4.6	61.3 ± 4.3	0.895
Vertical Jump Test, inches	16.4 ± 1.1	16.6 ± 1.1	0.497
Push-Ups	38.2 ± 5.0	37.7 ± 4.0	0.795

Values are means ± SE. p-values are from paired t-tests. $p < 0.05$ was considered significant.

During the treadmill exercise, estimated aerobic capacity did not differ (p = 0.856) between placebo (47.6 ± 2.5 mL/kg/min) and huperzine A (47.4 ± 2.0 mL/kg/min) trials. Also, average HR did not differ (p = 0.518) between placebo (157 ± 4 bpm) and huperzine A (158 ± 4 bpm) trials (Figure 4). Performance on handgrip strength (p = 0.895), vertical jump (p = 0.497), and push-up (p = 0.795) tests did not differ between trials (Table 2).

DISCUSSION

Huperzine A is included as an ingredient in many pre-workout nutritional supplements that are advertised to "increase energy levels" during exercise. While the justification for including huperzine A in these supplements is not clear, it may be linked with studies that showed enhanced cognitive function, even though these were studies of patients with Alzheimer's disease and dementia (34). Therefore, we proposed that huperzine A may acutely augment cognitive function during a bout of endurance exercise. The results of this study do not support

this hypothesis as evidenced by no significant differences in cognitive function between study trials.

Further analysis reveals the perceived difficulty of the endurance exercise was rated an average of 1.1 points (0-10 scale) more difficult during the huperzine A trial than the placebo trial (Figure 5). This coupled with RPE and HR data (Figure 4) suggest huperzine may have made exercise more difficult. To our knowledge this is the first study to investigate huperzine A's ability to enhancing cognitive function during exercise in any population, despite its fairly common inclusion in pre-workout supplements (1). Additionally, measures of neuromuscular and exercise performance did not differ between the two study trials.

Huperzine A is a competitive inhibitor of AChE with various properties that imply its usefulness as a cognitive enhancer (34, 36). Huperzine A forms two hydrogen bonds within the gorge of AChE and has a longer dissociation time than what is typical of most therapeutic AChEIs (6, 32). Orally ingested huperzine A appears in the blood within 15-minutes and reaches peak levels by 60-minutes while remaining in the system for at least 12-hours (22). These properties seem to warrant huperzine A's use as a cognitive enhancer. Other AChEIs have been shown to attenuate transient decline of cognitive function associated with sleep deprivation (7, 35). Therefore, we speculated that it would provide beneficial effects on cognitive function during exercise.

It is unclear why evidence for improved cognitive function was not observed in this study, but there are possibilities that warrant discussion. First, there was no decline in cognitive function observed from baseline to exercise. Other AChEIs have demonstrated the ability to attenuate transient cognitive decline associated with sleep deprivation (7, 11). Had the participants experienced transient cognitive decline during exercise, it is possible huperzine A could have attenuated such decline. Second, this study used an acute dose of huperzine A. Previous research showing improvement of cognitive function with AChEIs used chronic dosing (2, 3). Perhaps chronic dosing of huperzine A may have offered a benefit in terms of cognitive function. Third, it is likely that the mechanism for cognitive decline in Alzheimer's disease and dementia is different than that of any cognitive decline that would be associated with exercise.

The double-blind, placebo controlled, cross-over design study design is clearly a strength of this study. However, the small sample size (n = 15) might be viewed as a limitation, contributing to low statistical power. Therefore, in addition to more conventional statistical analyses, the results were also evaluated by calculating Cohen's effect size estimates. Results from these analyses were mixed, with one cognitive function outcome showing no treatment effect (letter fluency test), others showing small to moderate beneficial effects of huperzine A (letter fluency and Stroop tests), and another showing detrimental effects of huperzine A (category fluency test). In aggregate these supplemental analyses do not provide definitive information about the effects of huperzine A but do suggest that future trial with larger sample sizes may be warranted.

This study also had other limitations. Because nutritional supplements are not subject to the same regulations as therapeutic medications, and because we did not analyze the content of the supplements used in our study, we cannot confirm the potency and purity of the supplement

used in this study. However, we did select a brand that underwent voluntary third-party certification to mitigate this limitation. In the present study, the authors did not perform any analyses regarding the effects of the supplement relative to body size or body mass; this may be a potential avenue for researchers to pursue in the future. Further, this exercise protocol did not produce fatigue sufficient enough to cause transient cognitive function decline. Past research utilizing protocols of higher intensity and longer duration have produced fatigue sufficient to cause cognitive decline during exercise (8, 9, 12, 23). It may be speculated, had the protocol in the present study produced cognitive function decline, huperzine A may have been able to ameliorate the decline.

In conclusion, the results from this study indicate that huperzine A does not have clear effects on cognitive function during exercise in exercise trained individuals. However, small effects (positive or negative) cannot be definitively ruled out and larger trials may be warranted. Huperzine A is a common component of pre-workout supplements, presumably for enhancing mental function during exercise; however, based on our data, it is not likely to provide such benefits. A practical implication of these findings is that athletes and other exercisers should be advised to seek other approaches for maintaining or enhancing cognitive function during exercise.

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