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Creatine and Cognitive Functioning: What is the role of Exercise Frequency?

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Cover Page Footnote

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Creatine and Cognitive Functioning: What is the role of Exercise Frequency?

Creatine monohydrate is a popular nutritional aid for athletes, due to the beneficial effects on high intensity exercise performance (Cornish, Chilibeck, & Burke, 2006; Dawson, Vladich, & Blanksby, 2002), post-exercise recovery (Cooke et al., 2009), and increased tolerance to exercise in the heat (Kilduff et al., 2004). Although a good deal is known about the physiological effects of creatine supplementation, the impact on cognitive functioning is less certain. Because of its role in converting ADP to ATP (Schlattner et al., 2016), creatine provides an energy source to tissue with increased energy demands, including muscles and the brain (Persky & Brazeou, 2001), supplying creatine exogenously increases the energy supplied to neurons in health adults (Persky & Brazeou, 2001). Due to the role in supplying a reserve energy source, creatine may be most helpful in the brain or muscle during periods of greatest energy need. For example, mental training appears to increase naturally occurring creatine levels in the brain, presumably due to increases in cognitive demands during the training period (Valenzuela et al., 2003). Thus, it is possible that creatine supplementation should provide some benefit on more difficult cognitive tasks.

Evidence for beneficial effects of creatine on cognitive functioning has predominantly come from studies that examined the protective effects of creatine in the face of factors that are known to impair cognitive functioning. For example, McMorris and colleagues demonstrated that creatine supplementation has a protective effect on mood, working memory, and short-term memory following sleep deprivation (McMorris et al., 2006). Specifically, the declines in performance that are expected following sleep deprivation occurred in the control condition but were not as pronounced in the creatine condition. Creatine supplementation for five days decreases in mental fatigue and task-responsive oxygen delivery on repeated performance on a task requiring simple mathematical calculations (Watanabe et al., 2002). Beneficial effects of creatine on cognitive functioning have been found for children following a traumatic brain injury (Sakellaris et al., 2006) and in older healthy adults (McMorris, Mielcarz et al., 2007). Despite these findings, the effects of creatine on any area of cognitive functioning in otherwise healthy adults when they are not in an impaired state remains mixed.

In a recent review of the impact of creatine supplementation on cognitive functioning in healthy adults, Avgerinos and colleagues (2018) found that creatine supplementation may benefit performance on short term memory and reasoning tasks, but the evidence for any benefit on other cognitive functions including executive functioning or working memory tasks is conflicting. Ling, Kritikos, and

Tiplady (2009) demonstrated a greater improvement in performance in a creatine supplement condition compared to a placebo condition, on sustained attention task performance, response inhibition, as measured by the Flanker task performance, memory recall, and general intelligence. However, Rawson and colleagues (2008) did not find an effect of creatine supplementation on a series of cognitive tasks including simple reaction time measures, mathematical processing, running memory span, logical reasoning, code substitution, and Sternberg memory recall. It is possible that the Rawson and colleagues' findings differed from those of Ling and colleagues due to differences in task demands. Rawson and colleagues' tasks may have been less cognitively demanding both due to the duration and the difficulty of the task. Additionally, the sample size may have been insufficient to detect small effects.

The effects of creatine supplementation on cognitive functioning do appear to be greater in young healthy adults with a vegetarian diet. Creatine is naturally found in meat and fish and thus creatine levels are lower in vegetarians than omnivores (Delanghe et al., 1989). Benton and Donohoe (2011) found that following a five-day supplementation period, memory recall task performance was better in vegetarians in the creatine condition than meat eaters in the creatine condition, but no difference in memory task performance was observed in the placebo condition between meat-eaters and vegetarians. Further, reaction time variability on the most demanding trials of a reaction time task increased from pre-test to post-test in the placebo condition but not the creatine condition, for meat-eaters and vegetarians; suggesting that creatine offered a benefit on sustained attention performance when the demands are high, even among meat-eaters. These findings are in line with others showing a significant positive effect of creatine supplementation on working memory and intelligence in vegetarian young adults (Rae, Digney, McEwan, & Bates, 2003). Critically, both tasks in the Rae et al. (2003) study, the Ravens Advanced Progressive Matrices and Wechsler Auditory backward digit span task, are cognitively demanding tasks. Theoretically, creatine should have the largest effect on tasks that are the most cognitively demanding since these tasks would likely require greater energy demands (Scholey, Harper, & Kennedy, 2001).

In addition to the impact of creatine supplementation on cognitive functioning, creatine appears to also have an effect on mood and depression symptomatology. Dietary creatine intake appears to be related to depression risk, such that higher levels of dietary creatine is associated with reduced depression risk, but this relationship may be strongest for women (Bakian et al., 2020). Creatine supplementation protects mood during stressors, such as sleep deprivation (McMorris et al., 2006). The impact of creatine on cortisol is also of interest given the protective effects of creatine during psychological and physiological stressors. Short term creatine supplementation appears to reduce

exercise induced increases in cortisol following a high intensity swimming workout (Dobgenski, Santos, Campbell, & Krieder (2014) but does not alter cortisol levels following a 36-hour sleep deprivation period relative to a placebo group (McMorris, Harris, Howard, Langridge, Hall, Corbett, Dicks, & Hodgson, 2007). Further, creatine supplementation does not appear to alter resting cortisol levels in male strength power athletes (Hoffman, Ratamess, Kang, Mangine, Faigenbaum, & Stout, 2006). However, these effects may differ between individuals that exercise frequently and those that do not.

It is possible that other factors alter the impact of creatine supplementation on cognitive functioning in younger adults. Chronic exercise appears to alter several cognitive functions, including attention (de Sousa et al., 2019), working memory (Padilla, Perez, & Andres, 2014), and cognitive control (Padilla, Perez, Andres, & Parmentier, 2013). Individuals that are active are better at suppressing cognitive interference when tasks have higher working memory demands compared to sedentary individuals (Padilla, Andres, & Bajo, 2018). Based on the impact of exercise on cognitive functioning, it is possible that any benefits from creatine supplementation are reduced among frequent exercisers. Specifically, it is important to understand whether the effects of creatine supplementation and exercise result in additive effects on cognitive functioning or if the effects of one of these manipulations (exercise or creatine) diminish the effects of the other.

In order to address this uncertainty, the current study examined the impact of a creatine supplementation on several key cognitive functions, including working memory, sustained attention, mind wandering, and speed of processing, in a young healthy adult population. Given the established impact of exercise on these cognitive functions, we were interested in determining whether the effects of creatine supplementation on cognitive functioning was moderated by exercise frequency. We hypothesized that creatine supplementation would improve sustained attention, working memory, speed of processing, and decrease mind wandering. Secondly, we hypothesized that creatine supplementation would result in lower levels of negative affect, state anxiety, and perceived stress but would not impact levels of trait anxiety. Due to the effect of exercise on sustained attention (de Sousa et al., 2019) and working memory (Padilla, Perez, & Andres, 2014), we hypothesized that the effects of creatine supplementation would be greater among individuals that did not report exercising frequently. Finally, we hypothesized that creatine supplementation would not alter physiological stress as measured via cortisol levels.

Methods

Participants

Forty-nine students at Nova Southeastern University participated in

the study in exchange for partial course credit. All participants were screened to ensure that they were not currently supplementing their diet with additional creatine. Participants were randomly assigned to either a creatine supplementation condition (n = 25) or control (n = 24). Participant testing was carried out in accordance with the Declaration of Helsinki and a study protocol approved by the Institutional Review Board at Nova Southeastern University. Students who chose not to participate in the study could participate in other studies or complete an alternative assignment to earn partial course credit.

The current study employed a cross over design, such that participants completed three sessions in which they completed the study measures. Between Time 1 and Time 2 half of the participants consumed 5g of creatine per day and the other half did not consume any creatine for a 6-week period. Between Time 2 and Time 3, condition was reversed, for an additional six-week period. However, data collection was halted due to the COVID-19 pandemic. As a result, we have an insufficient sample size to interpret the data from Time 2 to Time 3. Thus, we will examine the differences between the creatine and control groups between Time 1 and Time 2.

Seven participants failed to return for the second session resulting in a final sample of forty-two individuals (21 Female, *M* age= 20.67, *SD*= 4.51, creatine condition n = 20, control condition n = 22). No significant difference in frequency of males and females occurred across groups, χ^2 (1) = 0.33, p = .563.

Measures

Semantic Sustained Attention to Response Task (SEM-SART; McVay & Kane, 2012b). The SEM-SART is a go/no-go task that requires subjects to respond to frequent non-target stimuli (animal names; 89% of trials) while withholding a response to infrequent target stimuli (vegetable names, 11% of trials). Stimuli were presented on the screen for 300 ms, followed by a mask for 1500ms. A total of 675 trials were presented for a total of 600 non-target trials (composed of 40 unique animal names) and 75 target trials (composed of 5 unique vegetable names). The dependent measures from the SEM-SART were *d'* (i.e. a measure of sensitivity- hit rate to non-target trials to false alarm rate to target trials) and *SD* of RTs to non-target trials (RTSD). Prior work has shown that the SEM-SART has high levels of construct validity as demonstrated by its relationships with other measures of attention control (Kane, et al., 2016; McVay & Kane, 2012).

Thought Probes Thought probes were inserted into the SEM-SART at pseudorandom intervals appearing unpredictably during the task to measure mind wandering. Probes were inserted following 30 target trials (i.e. 4% of all SEM-SART trials). Each probe asked participants to respond to the prompt: "What were you just thinking about?". Participants responded by selecting one of the

following response options: (1). Task-related thoughts, (2). Task-related evaluative thoughts, (3). Task-unrelated thoughts, neutral content, (4). Task-unrelated thoughts, positive content, or (5). Task-unrelated thoughts, negative content. Task-related thoughts are thoughts that are directly about completing the task and task-related evaluative thoughts are thoughts about how the individual is performing on the task. Since we are interested in mind wandering rates, we will ignore the rate of both of these types of thoughts. Overall mind wandering percentages were calculated by summing the number of responses to the probe stage were not on task thoughts ("2", "3", "4", "5"), dividing by the total number of probes (30), and multiplying by 100. Internal consistency on thought probe measures appears to be moderate (α = .57– .75) with higher reliability estimates when probes are presented more frequently, as was done in the current study (Schubert, Frischkorn, & Rummel, 2020).

Symmetry Span Task (SYMSPAN; Redick et al., 2012). The SYMSPAN working memory task required participants to recall a series of consecutively presented stimuli while simultaneously engaging in a separate processing task. Participants were asked to verify the symmetry of an image (e.g. processing component), and then presented with a to-be remembered stimuli (e.g. a red-square presented in a 4x4 grid, for the storage component). The to-be-remembered stimulus was presented for 250 ms after the verification of the symmetry. After a set of two to five verification-square pairs a grid containing all 16 possible squares was presented. Participants were instructed to recall the squares from the set in the order they were presented. Each set length was presented three times for a total of 12 sets for the task. The dependent measure was the total number of red square's locations recalled in the correct serial position (as recommended by Conway et al., 2005), resulting in a maximum score of 42 for the task. The symmetry span has high test-retest reliability (r = .77) and high internal consistency (α = .76– .81; Redick et al., 2012).

Pattern Comparison Task (Salthouse & Babcock, 1991). Participants completed the pattern comparison task as a measure of processing speed. Participants were presented with a paper and pencil test form with a column of pairs of symbols. They were asked to indicate if the two members of a pair are the same or different by marking S (for same) or D (for different) on a line between the two symbols. Participants were instructed to complete as many as possible within a 30 second time limit. This process was repeated on a second sheet for a total of 60 possible symbol comparisons in 60 seconds. The dependent measure was the total number of correctly completed comparisons made within the time limit. Test-retest reliability for the pattern comparison task is high (r = .85-.90; Salthouse, 1993).

Perceived Stress Scale (PSS; Cohen, Kamarck, & Mermelstein, 1983). The PSS consists of ten questions regarding the individual's thoughts and feelings

within the last month, such as "In the last month, how often have you felt nervous or "stressed"." Participants rated each question in terms of how often they have felt or thought a certain way on a 5-point Likert scale from 0 (never) to 4 (very often). The PSS was scored by reverse scoring the positively stated items, and then summing the scores of all ten questions, so that higher scores indicated more stress. Internal consistency for the PSS in the current sample was acceptable across both time points (Time 1 $\alpha = .87$; Time 2 $\alpha = .88$).

State Trait Anxiety Inventory (STAI; Spielberger, Gorsuch, Lushene, Vagg, Jacobs, 1983). The STAI is a 40-item self-report inventory that measures state (20 items) and trait anxiety (20 items). Participants rated items, such as "I feel calm" and "I feel satisfied with myself", on a 4-point Likert-type scale for state anxiety (1= "not very much", 4= "very much so") and trait anxiety (1= almost never, 4= almost always). Subscales for state (STAI-State) and trait (STAI-Trait) anxiety were computed by summing items in each subscale, with higher scores indicating greater anxiety. Internal consistency for the STAI was acceptable for both state (Time 1 $\alpha = .87$, Time 2 $\alpha = .86$) and trait scales (Time 1 $\alpha = .86$, Time 2 $\alpha = .83$) across both time points.

Daily Inventory of Stressful Events (DISE; Almeida, Wethington, & Kessler, 2002). The DISE is a seven-item scale measuring the number of daily stressors experienced. Participants indicated if they have experience one of the types of stressors, such as an argument or disagreement with someone, on the inventory during the last 24 hours. The DISE was scored by summing the number of items endorsed, resulting in a possible range from 0 to 7. Based on the nature of the scale, the internal consistency is lower as expected (Time 1 $\alpha = .52$; Time 2 $\alpha = .52$)

Demographics Questionnaire Participants completed a brief demographic questionnaire that assessed age, sex, ethnicity, exercise frequency, and exercise intensity.

Salivary Cortisol Assessment Saliva samples were collected via passive drool from each participant into polyethylene tubes during each of the sessions. Saliva samples were run in duplicate and quantified via a human cortisol enzyme immunoassay (EIA) kit (Salimetrics, LLC, Carlsbad, CA) which has a 0.91correlation with serum and a sensitivity < 0.007 ug/dL. The samples were immediately read in a BioTek ELx800 plate reader (BioTek Instruments, Inc., USA) at 450 nm with a correction at 630 nm. All samples were within the detection ranges indicated in the immunoassay kit, and the variations of sample readings were within the expected limits. The intra-assay and inter-assay coefficient of variation (CV) were within acceptable limits (U.S. Food and Drug Administration, 2018). The inter-assay CV was 11% and the intra-assay CV average was 13%. Final concentrations for the cortisol were generated by interpolation from the standard curve in µg/dL.

Procedure

During each session, participants completed the SYMSPAN, SART with thought probes, the pattern comparison task, DISE, PSS, and STAI. During the first session participants also completed a demographics questionnaire. Participants then supplied a saliva sample. Additionally, unrelated to the current manuscript, participants completed a body mass scan using a dual-energy X-ray absorptiometry (DXA) and InBody 270 Body Composition Analyzer to examine possible changes in body mass as a result of creatine supplementation. At the end of the first session, participants in the creatine condition were provided with a container of creatine monohydrate and given instructions to consume 5g per day for the 6 weeks between the two sessions. Participants were instructed to continue their regular diet and exercise schedule. The procedure was repeated for the second session.

Results

All measures were examined for skewness and kurtosis prior to analysis and no variables were outside of acceptable ranges for skewness or kurtosis. All data analyses were conducted using R (version 4.0.2). For all analyses, all available data will be used regardless of whether participants returned at Time 2.

A series of t-tests were conducted to determine if there were any differences between the two groups at Time 1, prior to any creatine supplementation. No differences were observed on any of the sustained attention task, d' or RTSD, SYMSPAN score, speed of processing, percentage of off-task thoughts, or on measures of state anxiety, trait anxiety, or perceived stress. A difference between the control and creatine condition was found on the DISE, $t(46) = 2.08$, $p = .043$, $d = .60$.

To test the first hypothesis, that creatine supplementation will increase sustained attention, working memory, speed of processing, and decrease mind wandering and reaction time variability on the SART, we conducted a series of linear mixed model regression analysis using the LME4 package (Bates, Mächler, Bolker, & Walker, 2015) in R (version 4.0.2), with condition, time, and the condition by time interaction entered as predictors. We did not find significant effects of condition, time, or a time by condition interaction predicting *d*', SYMSPAN scores, or percentage of off-task thoughts, all *p*'s > .05. We did observe a significant effect of time on the speed of processing scores, b = 2.49, $t = 2.77$, $p = .008$, such that scores increased from Time 1 ($M = 44.9$, $SD = 5.42$) to Time 2 ($M = 47.1$, $SD = 5.64$). However, there was no effect of condition or a time by condition interaction, *p's* > .05.

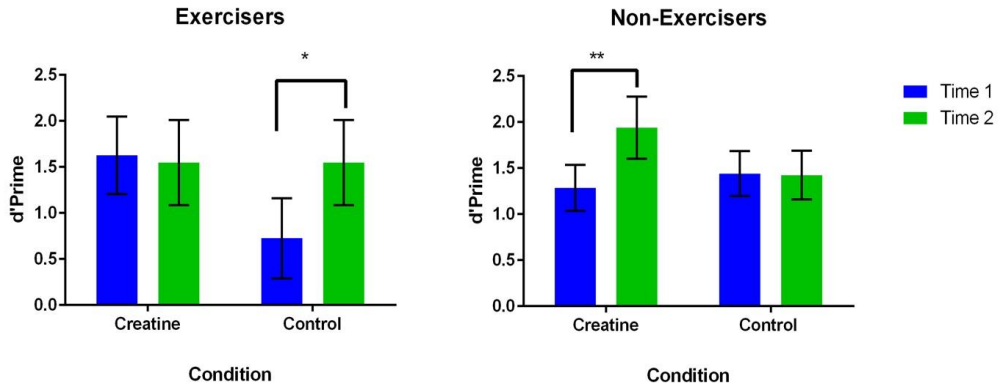
To test the second hypothesis, that creatine supplementation would alter stress and anxiety, we conducted a second set of series of linear mixed model regression analysis. We found no effects of time, condition, or a time by condition

interactions on the self-report measures of stress and anxiety, the DISE, STAI-State, STAI-Trait, or PSS, all *p*'s > .05.

To test the third hypothesis, that exercise frequency would moderate the impact of creatine supplementation on cognitive functioning, we split the sample based on reported exercise frequency. We classified individuals that reported exercising 2 or fewer times per week as infrequent exercisers (n = 32) and individuals that reported exercising more than 3 times per week as frequent exercisers (n = 16). The distribution of frequent and infrequent exercisers did not differ by creatine condition, $\chi^2 = 0.094$, p =.759, (infrequent exercisers: control n = 17 and creatine n = 15; frequent exercisers: control n = 7 and creatine n = 9). Exercise group was then dummy coded (0 = infrequent exercisers, 1= frequent exercisers). To determine if there were any differences between frequent and infrequent exercisers at the baseline Time 1 session, we conducted a series of t-tests on all cognitive measures and self-report stress measures. No differences were observed between frequent and infrequent exercisers on *d*', reaction time variability, speed of processing, or mind wandering at Time 1, all *p*'s > .05. Frequent exercisers did have higher working memory task performance (M = 21.7, SD = 3.50) than infrequent exercisers (M = 16.6, SD = 5.05), t (44) = 3.46, p = .0012, d = 1.11. Exercise group differences in state and trait anxiety approached but were not statistically significant with the frequent exercisers reporting numerically lower state anxiety (M = 32.9, SD = 7.09) than the infrequent exercisers (M = 37.6, SD = 8.78), t (45) = 1.88, p = .067, d = 0.58, and trait anxiety being numerically lower in frequent exercisers (M = 40.8, SD = 10.2) than the infrequent exercisers (M = 46.1, SD = 9.46), t (46) = 1.77, p = .084, d = 0.54. No differences were observed between frequent and infrequent exercisers on perceived stress or DISE.

The exercise group dummy variable was then entered in the prior linear mixed model analyses with condition, time, exercise group and their interactions entered as predictors. In the first model predicting *d*', with the inclusion of exercise group, we found a significant interaction between time and condition, b = 0.73, t = 2.64, p = .012; however, this interaction was qualified by a three-way interaction between time, condition, and exercise group, b = -1.09, t = 2.40, p = .021. To decompose this interaction, we conducted contrast analyses examining change from Time 1 to Time 2 separately for each condition and exercise group, using an FDR method adjusting for multiple comparisons. A significant change was observed from Time 1 to Time 2 in the infrequent exercisers in the creatine condition, t (10) = 2.62, p = .029, d = 0.57, but not for frequent exercisers in the creatine condition or either exercise group in the control condition. As seen in Figure 1, infrequent exercisers in the creatine condition evidenced an improvement in *d*' from Time 1 (M = 1.28, SD = 0.968) to Time 2 (M = 1.94, SD = 1.30).

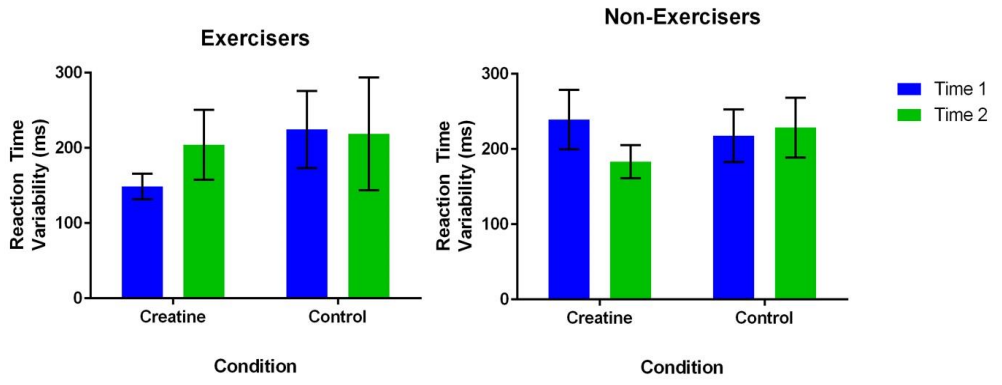
Figure 1
Dprime by Time and Condition



Note: * p <.05, ** p < .01, Error bars represent SEM

In addition to the observed effects on *d*', we also observed a significant interaction between condition, time, and exercise group on reaction time variability, b = 109.41, t = 2.05, p = .047. Contrast analyses examining change from Time 1 to Time 2 for each condition and exercise group were conducted using an FDR method to adjust for multiple comparisons. Consistent with the *d*' findings, we observed a decrease in reaction time variability in the infrequent exercisers in the creatine condition from Time 1 (M = 239.0, SD = 154.0) to Time 2 (M = 183.0, SD = 84.7), t (10)= 2.17, p = .040, d = 0.73, as seen in Figure 2. However, this effect did not withstand a correction for multiple comparisons, adjusted p = .127.

Figure 2
Reaction Time Variability by Time and Condition

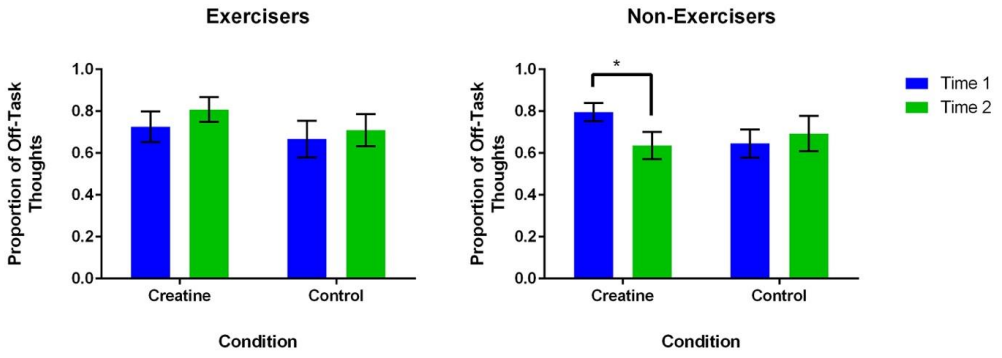


Note: Error bars represent SEM

In a model examining the impact of creatine on mind wandering, we observed a significant time by condition interaction, b = -0.22, t = 2.54, p = .015. However, when we attempted to examine this interaction using contrast analysis controlling for multiple comparisons, no significant effects emerged. Since we did not find a significant interaction between time and condition prior to the entry of exercise group and the subsequent interactions, we conducted mixed model linear regressions separately for the frequent exercisers and infrequent exercisers. No significant effect was observed in the frequent exercisers, but a significant interaction between time and condition was observed in the infrequent exercisers, b = -0.22, t = 2.43, p =.022. As seen in Figure 3, contrast analyses revealed a significant decrease in off-task thoughts from Time 1 (M = 0.796, SD = 0.170) to Time 2 (M = 0.636, SD = 0.251) in the creatine condition, t (10) = 2.43, p = .022, d = 0.63, but not in the control condition.

Figure 3

Mind Wandering by Time and Condition



Note: * p <.05, Error bars represent SEM

Consistent data previously reported regarding exercise group differences at Time 1, a significant effect of exercise group was observed on the SYMSPAN task, b = 5.97, t = 2.49, p = .003, such that SYMSPAN performance was higher among frequent exercisers (M = 17.2, SD = 5.66) than among infrequent exercisers (M = 21.8, SD = 3.58), but there was not interaction between exercise condition and time or condition.

A significant effect of Time was observed on the pattern comparison task, b = 3.78, t = 3.62, p < .001, and an interaction between session and exercise group, b = -4.07, t = 2.18, p = .035. Participants' speed increased from Time 1 (M = 44.9, SD = 5.42) to Time 2 (M = 47.1, SD = 5.64). However, the increase was only significant among the infrequent exercisers (T1: M= 44.6, SD = 5.08; T2: M = 47.3, SD = 5.29), t (10)= 2.83, p = .007, d = 0.28; but not among the frequent

exercisers (T1: *M*= 45.8, *SD* = 6.21; T2: *M* = 46.7, *SD* = 6.55), $p > .05$. Of interest to the current study, we found no effect of creatine or interaction between creatine and exercise group on change in the pattern comparison task.

No effects of condition, exercise group, time, or their interactions were observed for the self-report measures including PSS, STAI-ST, STAI-TR, and DISE, all *p*'s > .05. Additionally, no effect of condition, exercise group, time, or their interactions were observed on cortisol levels, all *p*'s > .05. At Time 1, individuals that were frequent exercisers had lower levels of cortisol (*M* = 0.13, *SD* = 0.09) than individuals that did not exercise frequently (*M* = 0.18, *SD* = 0.07) but this difference did not reach statistical significance, $t(46) = 1.90$, $p = .063$, $d = 0.583$.

To determine if the changes in cognitive functioning in the infrequent exercise group from Time 1 to Time 2 were due to changes in mood or anxiety, we examined correlations between change in the cognitive measures— *d*', NTSD, Mind wandering rates, and speed of processing —and change in the self-report measures of perceived stress, state anxiety and change in cortisol. Change in cortisol and change in perceived stress were not related to change in any of the cognitive measures. Change in state anxiety was correlated with change in speed of processing, $r(23) = 0.51$, $p = .010$. Change in mind wandering in the infrequent exercise group was related to change in *d*', $r(25) = -0.47$, $p = .010$.

Discussion

The purpose of the current study was to examine the impact of creatine supplementation on cognitive functioning, specifically sustained attention, working memory, speed of processing, and mind wandering, and on state anxiety, perceived stress, and cortisol. Further, we were interested in determining the role of exercise frequency in moderating the effects of creatine. We did not find a main effect of creatine supplementation on either cognitive functioning, stress or anxiety. However, we did find a significant effect of creatine supplementation on sustained attention and mind wandering in individuals that did not exercise frequently (i.e., twice or less per week) but not in those that reported exercising frequently (i.e., three or more times per week).

Although prior work has demonstrated that diet (e.g. vegetarian compared to omnivorous) moderates the effects of creatine on cognitive functioning (Benton & Donohoe, 2011; Rae et al. 2003), the current study is the first, to the authors knowledge, to identify exercise frequency as a significant moderator for the impact of creatine on cognitive functioning. Acute and chronic exercise have been linked to a variety cognitive functions including improved attention in healthy populations (de Sousa et al., 2019), improved attention in individuals with attention deficit hyperactivity disorder (Rassovsky & Alfassi, 2019), and protecting against age related cognitive declines (Churchill et al., 2002). One

reason for the lack of an effect of creatine on cognitive functioning among frequent exercisers may be that any boost the frequent exercisers would have received from the creatine was reduced because a possible effect of exercise on cognitive functioning.

One possible mechanism for the impact of exercise on cognitive functioning is a "cardiovascular hypothesis" which suggests that increases in cardiovascular fitness are associated with changes in cerebral blood flow (Endres et al., 2003) and brain-derived neurotrophic factor (BDNF, Vaynman et al. 2003; 2004). In line with this hypothesis, exercise appears to have a greater influence on more demanding executive control functions, including working memory, than on less demanding control or visuospatial processes (Colcombe & Kramer, 2003). In agreement, we observed differences between frequent and infrequent exercisers on the working memory task but not on the sustained attention or speed of processing task. The effect of creatine on cognitive functioning appears to be due to the impact on providing increased energy when task demands are higher (Persky & Brazeou, 2001). Accordingly, it should be expected that creatine supplementation will have a larger effect on more demanding cognitive tasks, such as the working memory task, than on moderately demanding tasks, such as the SART. However, we observed an effect of self-reported exercise frequency on the more demanding working memory task, but creatine did not impact working memory task performance. It is possible that while frequent exercise may benefit performance on more demanding tasks in younger healthy adults, the benefits of creatine supplementation in younger health adults are limited to moderately demanding tasks in individuals who do not exercise frequently. Future work should examine the impact of an exercise intervention and creatine supplementation in a sedentary sample of young adults to determine if the effects observed following a creatine supplementation are masked by effects of an exercise intervention.

Alternatively, it is possible that creatine did not impact cognitive functioning among frequent exercisers, because of their diet. It is possible that the frequent exercisers were consuming a greater amount of protein to support their workouts, which in turn may reduce the benefit of creatine in a younger healthy population. However, we did not collect data regarding the participants' diet and therefore cannot confirm this possible explanation. Future work should examine the impact of exercise frequency on creatine supplementation in vegetarians and omnivores.

Finally, consistent with prior work in athletes (Hoffman, Ratamess, Kang, Mangine, Faigenbaum, & Stout, 2006), creatine supplementation did not alter resting cortisol in either frequent or infrequent exercisers. Creatine supplementation did not alter mood or perceived stress. Together, these suggest that the cognitive effects observed in the current sample are due to changes in

cognitive functioning and not due to changes in mood or stress. The change in state anxiety was related to changes in speed of processing, but not to any other cognitive measure and changes in perceived stress were unrelated to change in any measure of cognition.

There are two important limitations worth discussing in the current study. The design of the study had to be altered as a result of the COVID-19 pandemic. The intended cross-over design would have resulted in a more robust test of our hypothesis, however the lack of a difference between the control and creatine conditions on all measures, except the DISE, allowed for a successful test of the current hypotheses. Secondly, the lack of a placebo for individuals in the control condition could have resulted in placebo effects. However, we believe that the pattern of our findings would argue against placebo effects since the benefits to creatine consumption occurred among infrequent exercisers and not frequent exercisers. There is no reason to believe that one of these groups would be more likely to experience a placebo effect than the other.

This is the first study to our knowledge to examine the impact of exercise frequency on creatine supplementation in young healthy adults. Consistent with prior work suggesting that creatine supplementation in young adults may have little to no effect overall, but that it may be helpful for some groups including vegetarians (Benton & Donohoe, 2011; Rae et al., 2003), and older adults (McMorris, Mielcarz et al., 2007). The current study adds infrequent exercisers to this list. The impact of creatine among infrequent exercisers is important as it may provide a method to boost cognitive functioning for individuals who cannot boost cognitive functioning though frequent exercise.

References

Almeida DM, Wethington E, & Kessler RC. (2002). The daily inventory of stressful events: An interview-based approach for measuring daily stressors. *Assessment*, *9*(1), 41-55.

Avgerinos, K. I., Spyrou, N., Bougioukas, K. I., & Kapogiannis, D. (2018). Effects of creatine supplementation on cognitive function of healthy individuals: A systematic review of randomized controlled trials. *Experimental Gerontology*, *108*, 166–173.

Bakian, A.V., Huber, R.S., Scholl, L., Renshaw, P. F., & Kondo, D. (2020) Dietary creatine intake and depression risk among U.S. adults. *Translational Psychiatry* 10, 52.

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, *67*, 1–48.

Benton, D., & Donohoe, R. (2011). The influence of creatine supplementation on the cognitive functioning of vegetarians and omnivores. *British Journal of Nutrition*, *105*(7), 1100-1105.

Churchill, J. D., Galvez, R., Colcombe, S., Swain, R. A., Kramer, A. F., & Greenough, W. T. (2002). Exercise, experience and the aging brain. *Neurobiology of Aging*, *23*, 941-955.

Cohen, S., Kamarck, T., & Mermelstein, R. (1983). A global measure of perceived stress. *Journal of Health and Social Behavior*, *24*(4), 385-396.

Colcombe, S., & Kramer, A. F. (2003). Fitness effects on the cognitive function of older adults: A meta-analytic study. *Psychological science*, *14*(2), 125-130.

Conway, A. R., Kane, M. J., Bunting, M. F., Hambrick, D. Z., Wilhelm, O., & Engle, R. W. (2005). Working memory span tasks: A methodological review and user's guide. *Psychonomic Bulletin & Review*, *12*(5), 769-786.

Cooke, M.B., Rybalka, E., Williams, A.D., Cribb, P.J., & Hayes, A. (2009). Creatine supplementation enhances muscle force recovery after eccentrically-induced muscle damage in healthy individuals. *Journal of*

*the International Society of Sports Nutrition, 6***(**13), 1-11. doi: 10.1186/1550-2783-6-13

Cornish, S.M., Chilibeck, P.D., & Burke, D.G. (2006) The effect of creatine monohydrate supplementation on sprint skating in ice-hockey players. *The Journal of Sports Medicine and Physical Fitness, 46,* 90-98.

Dawson B, Vladich T, & Blanksby BA. (2002) Effects of 4 weeks of creatine supplementation in junior swimmers on freestyle sprint and swim bench performance. *Journal of Strength and Conditioning Research*, *16*(4), 485-490.

Delanghe, J., De Slypere, J. P., De Buyzere, M., Robbrecht, J., Wieme, R. & Vermeulen, A. (1989). Normal reference values for creatine, creatinine and carnitine are lower in vegetarians. *Clinical Chemistry, 35*, 1802–1803.

de Sousa, A. F. M., Medeiros, A. R., Del Rosso, S., Stults-Kolehmainen, M., & Boullosa, D. A. (2019) The influence of exercise and physical fitness status on attention: a systematic review, *International Review of Sport and Exercise Psychology, 12*, 202-234.

Dobgenski, V., Santos, M. G., Campbell, B., & Krieder R. (2014). Short-term creatine supplementation suppresses the cortisol response to a high-intensity swim-spring workout. *Journal of Nutrition and Health Science, 1*(2), 204.

Endres, M., Gertz, K., Lindauer, U., Katchanov, J., Schultze, J., Schrock, H., Nickenig, G., Kuschinsky, W., Dirnagl, U., & Laufs, U. (2003). Mechanisms of stroke protection by physical activity. *Annals of Neurology*, *54*(5), 582–590.

U.S. Food and Drug Administration (2018). *Bioanalytic method validation guidance for industry.*

Hoffman, J., Ratamess, N., Kang, J., Mangine, G., Faigenbaum, A., & Stout, J. (2006). Effect of creatine and ß-Alanine supplementation on performance and endocrine responses in strength/power athletes. *International Journal of Sport Nutrition and Exercise Metabolism, 16, 430-446.*

Kane, M. J., Meier, M. E., Smeekens, B. A., Gross, G. M., Chun, C. A., Silvia, P. J., & Kwapil, T. R. (2016). Individual differences in the executive control

of attention, memory, and thought, and their associations with schizotypy. *Journal of Experimental Psychology: General, 145,* 1017-1048.

Kilduff, L.P., Georgiades, E., James, N., Minnion, R. H., Mitchell, M., Kingsmore, D., Hadjicharlambous, M., & Pitsiladis, Y. P. (2004). The effects of creatine supplementation on cardiovascular, metabolic, and thermoregulatory responses during exercise in the heat in endurance-trained humans. *International Journal of Sport Nutrition & Exercise Metabolism, 14*(4), 443-460.

Ling, J., Kritikos, M., & Tiplady, B. (2009). Cognitive effects of creatine ethyl ester supplementation. *Behavioural Pharmacology*, *20*(8), 673-679.

McMorris, T., Mielcarz, G., Harris, R. C., Swain, J. P., & Howard, A. (2007). Creatine Supplementation and Cognitive Performance in Elderly Individuals. *Aging Neuropsychology and Cognition*, *14*(5), 517-528.

McMorris, T., Harris, R. C., Howard, A. N., Langridge, G., Hall, B., Corbett, J., Dicks, M., & Hodgson, C. (2007). Creatine supplementation, sleep deprivation, cortisol, melatonin and behavior. *Physiology and Behavior, 90,* 21-28.

McMorris, T., Harris, R.C., Swain, J. Corbett, J., Collard, K., Dyson, R.J., Dye, L., Hodgson, C., & Draper, N. (2006). Effect of creatine supplementation and sleep deprivation, with mild exercise, on cognitive and psychomotor performance, mood state, and plasma concentrations of catecholamines and cortisol. *Psychopharmacology, 185***,** 93–103.

McVay, J. C., & Kane, M. J. (2012). Why does working memory capacity predict variation in reading comprehension? On the influence of mind wandering and executive attention. *Journal of Experimental Psychology: General, 141*, 302–320.

Padilla, C., Andres, P., & Bajo, T. (2018). Improving memory inhibition: A study of retrieval induced forgetting, executive control, and chronic aerobic exercise. *Frontiers in Behavioral Neuroscience, 12*:*318.*

Padilla, C., Pérez, L., & Andrés, P. (2014). Chronic exercise keeps working memory and inhibitory capacities fit. *Frontiers in Behavioral Neuroscience, 8:49*. doi: 10.3389/fnbeh.2014.00049

Padilla, C., Pérez, L., Andrés, P., & Parmentier, F. B. R. (2013). Exercise improves cognitive control: Evidence from the stop signal task. *Applied Cognitive Psychology,27,* 505-511.

Persky, A.M., & Brazeau, G.A. (2001). Clinical Pharmacology of Dietary Supplement Creatine Monohydrate. *Pharmacology Reviews, 53*(2), 161-176.

R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rae, C., Digney, A. L., McEwan, S. R., & Bates, T. C. (2003). Oral creatine monohydrate supplementation improves brain performance: A double-blind, placebo-controlled, cross-over trial. *Proceedings: Biological Sciences*, *270*(1529), 2147-2150.

Rassovsky, Y., & Alfassi, T. (2019). Attention improves during physical exercise in individuals with ADHD. *Frontiers in Psychology*, *9*(2747), 1-5.

Rawson, E.S, Lieberman, H.R., Walsh, T.M., Zuber, S.M., Harhart, J.M., & Matthews, T.C. (2008). Creatine supplemenration does not improve cognitive function in young adults. *Physiology & Behavior, 95*(1-2), 130-134.

Redick, T.S., Broadway, J.M., Meier, M.E., Kuriakose, P.S., Unsworth, N., Kane, M.J., & Engle, R.W. (2012). Measuring working memory capacity with automated complex span tasks. *European Journal of Psychological Assessment, 28*, 164-171.

Sakellaris, G., Kotsiou, M., Tamiolaki, M., Kalostos, G., Tsapaki, E., Spanaki, M., Spilioti, M., Charissis, G., & Evangeliou, A. (2006). Prevention of complications related to traumatic brain injury in children and adolescents with creatine administration: An open label randomized pilot study. *Journal of Trauma-Injury Infection and Critical Care, 61*(2), 322–329.

Salimetrics, L. L. C., & SalivaBio, L. L. C. (2011). Saliva collection and handling advice. *State College, PA: Salimetrics LLC, SalivaBio LLC*, 1-14.

Salthouse, T. A. (1993). Speed mediation of adult age differences in cognition. *Developmental Psychology, 29*, 722-738.

Salthouse, T. A., & Babcock, R. L. (1991). Decomposing adult age differences in working memory. *Developmental Psychology, 27*, 763-776.

Schlattner, U., Klaus, A., Ramirez Rios, S., Guzan, R., Kay, L., & Tokarska-Schlattner, M. (2016). Cellular compartmentation of energy metabolism: creatine kinase microcompartments and recruitment of B-type creatine kinase to specific subcellular sites. *Amino Acids, 48***,** 1751–1774.

Scholey, A. B., Harper, S., & Kennedy, D. O. (2001). Cognitive demand and blood glucose. *Physiology and Behavior, 73*, 585-592.

Schubert, A-L., Frischkorn, G. T., & Rummel, J. (2020). The validity of the online thought-probe spacing procedure of mind wandering is not threatened by variations of probe rate and probe framing. *Psychological Research, 84*, 1846-1856.

Spielberger, C.D., Gorsuch, R.L., Lushene, R., Vagg, P.R., & Jacobs, G.A. (1983). *Manual for the State-Trait Anxiety Inventory (form Y).* Palo Alto, CA: Mind Garden.

Valenzuela, M.J, Jones, M., Caroline, R., Wen, W., Scott, G., Shnier, R., & Sachdev, P. (2003). Memory training alters hippocampal neurochemistry in healthy elderly, *NeuroReport*, *14*(10), 1333-1337.

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2004). Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. *European Journal of Neuroscience*, *20*(10), 2580-2590.

Vaynman, S., Ying, Z., & Gomez-Pinilla, F. (2003). Interplay between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. *Neuroscience, 122*(3), 647-657.

Watanabe, A., Kato, N., & Kato, T. (2002). Effects of creatine on mental fatigue and cerebral hemoglobin oxygenation. *Neuroscience Research, 42*(4), 279-285.

Supplementary Table 1. Descriptive Statistics by Participant Sex

	Males *Mean (SD)*		Females *Mean (SD)*		*t*	*p*	*d*
Height (inches)	71.02	(4.43)	64.08	(3.35)	6.15	< .001	1.78
Weight (pounds)	167.14	(33.40)	135.58	(30.29)	3.40	.001	0.99
Age (years)	20.17	(6.89)	22.00	(6.89)	1.10	.275	0.32