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Research Article

A Randomized Double-Blind Evaluation of the Gastrointestinal, Body Composition, Stress Response and Cognitive Function Impacts of Creatine Supplementation in Healthy Adults

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Abstract

Creatine monohydrate is a popular ergogenic aid used by athletes, adolescents and older individuals. There are various forms of creatine supplements that are on the market, however, creatine monohydrate is the most popular. Creatine itself is considered as less stable in solution when left in solution over time. Advances in product development and science may allow for a more stable aqueous solution of creatine. One major concern of ready-to-drink creatine supplements is the potential adverse gastrointestinal effects. In this randomized, double-blind, placebo-controlled design, the potential gastrointestinal effects of stabilized creatine (CreaBev®) as compared to standard creatine monohydrate versus control was tested. Subjects were randomly assigned to receive the CreaBev® supplement, creatine monohydrate supplement or no supplement (control). Subjects were instructed to consume one serving of the supplement (delivering 5 gm creatine) on a daily basis for 28 days. Subjects underwent baseline testing and end of study testing. The Severity of Dyspepsia Analysis (SODA) questionnaire and National Institutes of Health (NIH) Cognitive Test Toolbox were used to evaluate GI effects and cognition. Additional testing included body composition analysis (including fluid balance), and exploratory measurement of the stress biomarkers, salivary alpha amylase and cortisol. Following the consumption of CreaBev, no adverse gastrointestinal side effects were reported. Cognition via the Dimension Change Test significantly improved (pre: 104 ± 14 to post: 116 ± 14; $p = 0.0017$) in the CreaBev group. There was no observed differences in total body fluid status over the 28 days between the groups ($p > 0.05$) No significant differences in levels of salivary alpha amylase, cortisol and anthropometrics were observed. The use of CreaBev did not cause any adverse GI effects and improved cognitive performance on the Dimension Change Test.

Keywords: *Creatine; Gastrointestinal; Bloating; Cognition; Dietary Supplement*

Abbreviations

Cr: Creatine; cm: Centimeter; gm: Gram; kg: Kilogram; CrM: Creatine Monohydrate

Introduction

Creatine is a compound synthesized in the body by a process involving arginine, glycine and methionine. This naturally occurring compound is primarily found in red meat and seafood [1]. The main function of creatine is to provide energy to the body via the creatine/

phosphorylcreatine system [1,2]. The highest levels of creatine are found in skeletal muscle and the heart [1] and much smaller amounts are stored in the brain [3,4]. Creatine containing products are among the more popular supplements used due to the potential ergogenic effects [2].

According to the International Society of Sports Nutrition's position paper, creatine is a safe and effective nutritional ergogenic aid [2]. The use of creatine supplementation is not limited to professional athletes. Recreational athletes, collegiate athletes and older adults supplement with creatine to improve performance and gain lean body mass [2]. Studies demonstrate that creatine improves performance on short-duration, muscular power, endurance and strength exercises [2,5]. The evidence suggests co-ingesting creatine and carbohydrate aids in recovery from intense exercise [2]. Numerous studies found that regular supplementation with creatine lowers the incidence of injury as well as aids in injury recovery [2,6,7]. Traditionally, a loading phase was recommended for creatine dosing; however, consuming 3 – 5 g/day of creatine can increase muscle creatine stores [2].

Creatine monohydrate, a commercially available form, is the most studied creatine supplement. Creatine monohydrate has a higher creatine content compared to other forms of creatine such as creatine malate or creatine citrate [2,8]. Powdered forms of creatine monohydrate are very stable and resistant to degradation [2,9,10]. In the solid state, creatine monohydrate is stable for as long as 2 years [9]. Conversely, creatine monohydrate has a rather low solubility in water. Unless the drink is consumed immediately, mixing creatine with a liquid is not ideal. Degradation occurs quickly and aqueous creatine can be broken down to the nonactive metabolite, creatinine [9,10]. Storing liquid creatine monohydrate solutions at a lower temperature is one way to lessen the breakdown [2,8]. However, this is not a feasible or cost-effective solution for some supplement companies. Altering the pH of a solution can influence the stability. Highly acidic solutions (< 2.5) or basic solutions decrease the likelihood of intramolecular cyclization, thus reducing the breakdown of creatine [2,8]. Developing a shelf-stable creatine ready to drink (RTD) beverage would be beneficial to athletes due to convenience and the potential to be easily absorbed into the blood stream.

Potential adverse reaction or side effects of supplements can be concerning for athletes. There are reports of gastrointestinal issues as following creatine ingestion [11-14]. The most common gastrointestinal issues are cramping, vomiting and diarrhea [11,14]. There is not sufficient evidence to conclusively confirm creatine-induced GI issues. Therefore, creatine is considered an effective supplement for performance with few adverse side effects [2,14]. The ergogenic effects of creatine have been well established. Research demonstrates the ability of creatine monohydrate to enhance performance, strength and training adaptations [2,11,15].

Additionally, creatine monohydrate supplementation boosts energy availability to neurons and increases creatine levels in the brain [4]. Rae., *et al.* showed that working memory and measures of intelligence improved following 6-weeks of oral creatine supplementation [16]. McMorris., *et al.* found that creatine monohydrate supplementation enhanced cognitive function in healthy elderly subjects [17]. In a second study by McMorris., *et al.* found that 20 g/day of creatine monohydrate improved executive functioning [18]. These studies demonstrate support the use of creatine monohydrate supplementation to aid in cognitive performance.

Purpose of the Study

The purpose of this study was to evaluate potential GI effects of stabilized creatine (CreaBev®) as compared to creatine monohydrate (CM) and effects of CreaBev on cognition. As a secondary outcome of interest, we also explored if creatine in general would have any effects on cognitive function in healthy adult subjects. Comparisons against a control group for the outcomes of interest were also conducted.

Materials and Methods

Using a randomized, double-blind, placebo-controlled design, this study explored if the daily use of a creatine monohydrate based dietary supplement would have differential impacts or any impacts on gastrointestinal wellness (possible gastrointestinal side effects), stress and cognitive function in healthy college-aged adults.

This study enrolled adults that were 18 to 50 years of age, and of the following description: Age 22 ± 7 years, Body Weight 77.6 ± 6.1 kg, Body Mass Index 24.8 ± 2.9 kg/m^2, Percent Body Fat 14.2 ± 4.16%, Total Training Years/Experience 9 ± 9 years, Hours per week of Weight training 5 ± 3 hours per week and Hours per week of Aerobic exercise 6 ± 3. This study evaluated a branded creatine (CreaBev®, Glanbia Nutritionals) as compared to creatine monohydrate (generic). Subjects were asked to maintain their standard diet throughout the study and to refrain from exercise in the 24 hours prior to any study visit. This study was reviewed and approved by the Institutional Review Board of Nova Southeastern University (NSU-IRB) as IRB Number: 2019-512-NSU and was approved on October 25, 2019.

Product testing

This study evaluated a branded creatine monohydrate-based product delivering 5 grams creatine per serving as CreaBev® (Glanbia Nutritionals, Richfield, Idaho USA) as compared to 5 grams of creatine monohydrate (Hunan Tiancheng Biochemical Technology, Co. Ltd. Hunan, China). This study also used a control group [three study groups, CreaBev®, creatine monohydrate (CM) and control (CON)]. The control group received no study product or intervention and was used for comparative purposes. The study products were blinded to study staff and participants. The study product was delivered in powder form and mixed with water (i.e., 240 - 300 ml water) and ingested per assignment on a daily basis.

Study visits overview

This study employed a Screening study visit, and for those who passed the screening visit and signed the Informed Consent, they were then placed for randomization at the baseline visit (Day 0) and testing with follow up evaluation at the end of study testing (Day 28 ± 1). Tests included at specific study visits included body composition analysis, measurement of salivary alpha amylase and cortisol, gastrointestinal wellness as by the Severity of Dyspepsia Analysis (SODA) questionnaire and cognitive function by the National Institutes of Health (NIH) Cognitive Test Toolbox. Details are shared within the manuscript.

Body composition testing

For baseline descriptive characteristics of the study participants, the InBody 270 bioelectric impedance was utilized (InBody USA, Cerritos, CA.). Aspects of body composition focused on evaluating potential changes in fat free mass as well as total body water (fluid status).

Gastrointestinal wellness testing

In order to determine if the study product had any differential impacts on perceived gastrointestinal wellness (GI wellness), the validated Severity of Dyspepsia Assessment (SODA) was used [19-22]. The SODA measures gastrointestinal wellness (dyspepsia, bloating, gas, etc.) related to pain, non-pain symptoms and overall satisfaction.

Salivary stress hormone testing

In order to determine if the study product had any differential effects or impacts on stress, salivary alpha amylase (sAA) and salivary cortisol (cortisol) were obtained. Standard methods were utilized for the salivary hormone collection, with the processing and analysis per the validated testing kit and contract laboratory (Salimetrics, LLC. Carlsbad, CA) [23].

Cognitive function

A series of cognitive testing instruments from the National Institutes of Health (NIH) Cognition Toolbox [24-26] were utilized to offer a comprehensive assessment of cognitive processing. The assessments were run on an iPad app from the NIH Toolbox and Joggle. The tests have been designed and validated for use in clinical assessment and clinical trials. Each measure has excellent test-retest reliability (these are widely used instruments with reported Spearman's correlation range from 0.86 to 0.92). The individual assessments from the

NIH toolbox were measured independently with a T (normalized) score. The total testing time for the neurobehavioral assessments was approximately 20 min. The Flanker Inhibitory Control and Attention Test (Flanker) measured the participant's attention and inhibitory control. The Pattern Comparison Processing Speed Test (Processing Speed) assessed the participant's ability to quickly process information. The Dimensional Change Card Sort Test (DCCS) assessed executive function.

Statistical approach

For each outcome and demographic variable, summary statistics are provided by treatment (intervention) group and visit. For quantitative variables, the summary statistics are presented in standardized format. The study data presented is the Full Analysis Set following the intent to treat principle. This study targeted study enrollment of 24 subjects (eight subjects per CreaBev, CrM and control, respectively). Overall statistical significance was pre-set at $p \leq 0.05$ with 95 percent confidence intervals applied. The sample size for this study ($n = 24$) was based upon prior published studies with creatine and considered as such a convenience sample.

For the continuous dependent variables, paired t-test or Wilcoxon signed rank test was used to determine the significance of change from baseline within each group depending on whether the data was normally distributed. The analysis of variance (ANOVA) or Kruskal-Wallis test was applied to compare the change from baseline among the CreaBev group, CrM group, and control group depending on normality distribution and variance structures of three groups. The same analysis (ANOVA) was also applied to percent change from baseline.

For categorical dependent variables, chi-square test or Fisher's exact test was applied to compare the difference in proportion among CreaBev, CrM and Control study groups. The analysis of covariance (ANCOVA) model was applied to analyze the efficacy endpoint(s), with the change from baseline as the response variable, treatment as a fixed effect and the baseline score as a covariate. Based on the model, the least squares means (LS Means) for the CreaBev, CrM and Control groups were calculated with the 95% confidence interval. The differences in LS Means between the CreaBev, CrM and Control groups were also calculated together with a 95% confidence interval. For this study, a non-hierarchal statistical approach was utilized to treat each endpoint of interest separately as an independent endpoint of interest.

Results and Discussion

Anthropometric, body composition and training history

This study enrolled adults that were 18 to 50 years of age, and of the following characteristics: Age 22 ± 7 years, Body Weight 77.6 ± 6.1 kg, Body Mass Index 24.8 ± 2.9 kg/m^2, Percent Body Fat 14.2 ± 4.16%, Total Training Years/Experience 9 ± 9 years, Hours per week of Weight training 5 ± 3 hours per week and Hours per week of Aerobic exercise 6 ± 3. There were no differences amongst the study groups at baseline for any of the body composition variables or exercise related histories ($p > 0.05$).

When examining if the daily use of the assigned product (or placebo) had any differential effects on body composition, there were no differences observed in changes in body mass, fat mass, fat free mass, percent body fat, or in hydration, as total body water measurement for the CreaBev, CrM and Control groups, respectively ($p > 0.05$). See table 1.

	Pre (Day 0)	Post (Day 28)	Change from baseline	P value (between group comparisons)
Body mass (kg)				
CreaBev	89.5 ± 5.1	90.5 ± 5.7	1.1 ± 1.1	P > 0.05
CrM	72.0 ± 5.7	72.2 ± 5.2	0.1 ± 0.9	P > 0.05
Control	71.3 ± 7.5	70.7 ± 8.2	-0.6 ± 1.1	P > 0.05
Fat mass (kg)				
CreaBev	13.9 ± 4.1	13.5 ± 5.2	-0.4 ± 1.8	P > 0.05
CrM	10.6 ± 3.6	9.4 ± 3.1	-1.2 ± 1.3	P > 0.05
Control	9.0 ± 2.4	8.3 ± 2.7	-0.7 ± 0.9	P > 0.05
Fat free mass (kg)				
CreaBev	75.6 ± 6.6	77.0 ± 8.6	1.4 ± 2.4	P > 0.05
CrM	61.5 ± 6.2	62.9 ± 5.6	1.4 ± 1.5	P > 0.05
Control	62.3 ± 6.7	62.3 ± 7.9	0.1 ± 1.6	P > 0.05
Percent Body fat				
CreaBev	15.5 ± 4.6	15.0 ± 6.0	-0.5 ± 2.0	P > 0.05

CrM	14.7 ± 5.0	13.0 ± 4.3	-1.7 ± 1.8	P > 0.05
Control	12.6 ± 2.9	11.7 ± 3.4	-0.8 ± 1.2	P > 0.05
Total body water - liters				
CreaBev	55.4 ± 4.8	56.4 ± 6.3	1.0 ± 1.8	P > 0.05
CrM	45.0 ± 4.5	46.0 ± 4.0	1.0 ± 1.1	P > 0.05
Control	45.7 ± 4.8	45.7 ± 5.8	0.1 ± 1.2	P > 0.05

Table 1: *Body composition.*
Data are expressed as the mean ± SD. There were no significant differences (p > 0.05) between groups nor were there significant differences in the delta (change) score.

Gastrointestinal wellness-severity of dyspepsia assessment: SODA

One aspect of the study was to determine if there were any within group or between group signals or direct data of a negative gastrointestinal impact of the study product(s). The study, using the validated SODA questionnaire was unable to detect any gastrointestinal side effects by the total scores of the SODA for each domain (dyspepsia, pain symptoms, non-pain symptoms and overall satisfaction), as well as for the sub-scores of the questionnaire. There were no within group changes in the SODA scores nor were there any between group differences for the change in SODA score over the course of the study.

	Pre	Post	P-value (between group differences)
Pain			
CreaBev	5.5 ± 6.0	7.4 ± 7.1	P > 0.05
CrM	7.0 ± 7.5	9.5 ± 9.9	P > 0.05
Control	2.0 ± 0.0	2.0 ± 0.0	P > 0.05
Non-Pain			
CreaBev	10.5 ± 3.9	10.9 ± 2.6	P > 0.05
CrM	9.0 ± 2.5	10.0 ± 3.3	P > 0.05
Control	7.9 ± 2.3	8.0 ± 2.6	P > 0.05
Satisfaction			
CreaBev	18.9 ± 5.1	20.6 ± 4.2	P > 0.05
CrM	21.4 ± 3.5	21.8 ± 3.5	P > 0.05
Control	23.0 ± 0.0	23.0 ± 0.0	P > 0.05

Table 2: *Data are expressed as the mean ± SD. There were no significant differences between the groups (p > 0.05) over the study period.*

Salivary stress biomarkers

As biomarkers of stress, salivary alpha amylase (sAA) and cortisol were obtained at baseline and over the duration of the study period. When examining within and between group changes from baseline, there were no significant differences observed (p > 0.05). See table 3.

	Pre	Post	Change from baseline	P value (between group comparisons)
Cortisol ug/dl				
CreaBev	0.20 ± 0.06	0.50 ± 0.44	0.29 ± 0.45	p > 0.05
CrM	0.10 ± 0.07	0.16 ± 0.09	0.06 ± 0.08	p > 0.05
Control	0.12 ± 0.06	0.22 ± 0.19	0.09 ± 0.17	p > 0.05
Alpha-amylase U/mL				
CreaBev	78.9 ± 57.8	53.7 ± 55.2	-25.2 ± 63.1	p > 0.05
CrM	111.3 ± 101.7	84.5 ± 56.8	-26.7 ± 81.7	p > 0.05
Control	57.5 ± 28.8	36.1 ± 22.3	-21.4 ± 27.5	p > 0.05

Table 3: *Data are expressed as the mean ± SD. There were no significant differences for the change within, or between groups.*

Cognitive function

Cognitive function was measured by the NIH Toolbox battery of select validated exams. The Flanker Inhibitory Control Test, the Dimensional Change Test and the Pattern Comparison Tests were employed. Over the course of the study, only the CreaBev group was observed to have experienced a significant impact on cognition via the Dimension Change Test (pre: 104 ± 14 to post: 116 ± 14; p = 0.0017). Changes on the Flanker Inhibitory Control Test and the Pattern Comparison Test were not different between the groups (p > 0.05). See table 4.

	Pre	Post	Change from baseline	P value (between group comparison)
Flanker Inhibitory Control Test				
CreaBev	93 ± 14	108 ± 18	15 ± 22	P > 0.05
CrM	113 ± 13	118 ± 16	5 ± 13	P > 0.05
Control	103 ± 21	121 ± 13	18 ± 20	P > 0.05
Dimensional Change Test				
CreaBev	104 ± 14	116 ± 14*	13 ± 14	P = 0.0017*
CrM	114 ± 21	116 ± 14	2 ± 18	P > 0.05
Control	118 ± 13	120 ± 8	2 ± 13	P > 0.05
Pattern Comparison Test				
CreaBev	112 ± 15	119 ± 7	7 ± 19	P > 0.05
CrM	122 ± 16	128 ± 11	6 ± 10	P > 0.05
Control	124 ± 13	133 ± 13	9 ± 14	P > 0.05

Table 4: *Data are expressed as the mean ± SD.*

Safety data

There were no reported adverse events in this study. There were no observed objective adverse events in this study. Comprehensive metabolic panel along with the complete blood count with platelets and differential were run at baseline and the end of the study as objective markers of safety. There were no significant changes in the blood safety biomarkers, with all also remaining within normal limits throughout the course of the study (no changes in glucose, liver function, renal function, electrolytes, hematological biomarkers, or in immune system biomarkers).

Conclusion

This study determined that daily supplementation of CreaBev® or creatine monohydrate as compared to each other, and placebo had no differential effects on impacting body composition. This is somewhat surprising given the fact that the majority of creatine related studies demonstrate a positive impact on body mass (weight) and the ratio in fat free mass to fat mass. The reason for the discrepancy is that the studies where creatine is demonstrated to positively impact body weight and body composition status, have been training studies (exercise intervention included). The current study was not an exercise training or intervention study and was undertaken to more learn about potential gastrointestinal side effects, potential cognitive impacts amongst other detailed outcomes, and thus within the context of the study design, the results should be treated without surprise [27-29]. Within the confines of this research study and design, we also note that popular press has stated, and professionals have wrongly commented that creatine supplementation will induce a person to retain water ("hold fluid"), we found that a daily five-gram dosage of CreaBev® or creatine monohydrate had no within group detectable

effects or between group effects for influencing total body water (fluid) status of the participant. Said differently, our findings demonstrate that creatine supplementation does not impact overall body fluid status over the duration examined in this study.

Over the course of the study period, daily supplementation of five grams creatine through either CreaBev® or the creatine monohydrate comparator and as also compared to the control group, there were no observed differences between the groups on gastrointestinal side effects as measured by the SODA questionnaire. This study found that the investigated product alone (within group) and comparisons (to creatine monohydrate and the control group; between groups) revelated no signals, nor markers of gastrointestinal side effects as detected by the SODA questionnaire or by adverse events monitoring. This finding is meritorious and of conversation because of earlier data from elite athletes indicated possible gastrointestinal side effects of creatine supplementation [14]. Our data for lack of a gastrointestinal negative side effect is in line with earlier studies published by Cancela [30], Antonio [27], de Guigand and others [31]. Within the confines of this study design, a daily five-gram creatine dosage of CreaBev® and creatine monohydrate are not associated with gastrointestinal side effects any different than taking nothing (control group).

Out of curiosity, we also measured salivary alpha amylase (sAA) and cortisol as biomarkers of stress. The study population was not specifically a stressed population (by exercise or by inclusion/exclusion criteria); however, as the current study was run during the SARS-CoV2-19 (coronoavirus-19) pandemic, we thought it worthy of testing. Over the 28-day period of the study, there were no significant within group or between group changes in sAA or cortisol. The lack of an impact on the stress hormones tested is in line with earlier published research examining similar parameters [18,32]. One study did find that providing 20 grams of creatine monohydrate daily along with exercise may have an impact on cortisol levels, however, the current study used a dose that was 25% that of the tested dose [33]. Consistent with prior published research, creatine monohydrate supplementation, at least at a dose of five grams daily for 28 days has no known impacts on biomarkers of stress (sAA or cortisol).

As creatine is used by the phosphagen energy system, and the brain is one of the areas of the body which has higher creatine usage (for ATP synthesis), we thought it worthwhile to explore if there is any association with cognitive function in a young college-aged cohort (study group). Within the current study, cognitive function was measured by select tests from the National Institutes of Health Cognitive Toolbox (NIH-Toolbox) [34]. To measure attention and executive function, the Flanker Inhibitory Control and Attention test and the Dimensional Change Card Sort test were employed. To measure brain processing speed, the Pattern Comparison test was utilized. Within the confines of this study design, only those receiving the CreaBev experienced a significant improvement in cognitive executive functioning as measured by the Dimensional Change Card Sort test (baseline: 104 ± 14 to end of study: 116 ± 14; $p = 0.0017$). This improvement in executive function with the CreaBev® was not observed in the creatine monohydrate or control groups. In general, some research has found a cognitive supporting role and impact of creatine monohydrate supplementation (five grams per day, when sleep deprived); however, the effect was observed in a sleep-deprivation study [18] which utilized a daily dosage of 20 grams per day (four-times as much as the current study). The current study found cognitive support for CreaBev® for executive function in college aged adults who were classified as healthy subjects. Our data differs from Rawson., *et al.* who did not find a cognitive benefit over a six-week supplementation period through the variety of tests they employed [35]. More research regarding the potential role of creatine to support cognitive functioning is most certainly warranted and appears it may have utility in a variety of ages for such.

In summary, this study found that there were no gastrointestinal side effects (no non-pain or pain or other related GI symptoms) of five grams per day of creatine supplementation when delivered as CreaBev® or as a generic creatine monohydrate ingredient or as compared to a control group. We also found that there were no reported adverse effects, nor objective adverse events, leading us to further state the safety of CreaBev® and creatine monohydrate within the confines of this study design. The data also indicates that daily creatine supplementation (as CreaBev® or creatine monohydrate) has no impact on overall hydration status as measured by total body water status over a 28-day period. Both the CreaBev® creatine and generic creatine monohydrate were found to be as safe as the control over the course of this study. Interestingly enough, and deserving of follow-up, is that there was a significant improvement in executive function, cognitive functioning in the CreaBev® group over the course of the study. In conclusion, creatine delivered as CreaBev® was found to be safe, have no

gastrointestinal side effects and appears to improve executive function, cognitive performance in a college-aged population.

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Conflict of Interest

Douglas Kalman (DSK) reports that he is an ad-hoc consultant to Atheneum Partners and the Round Table Group.

Corbin Hohl (CH) reports that he is the Bioactive Team leader for Glanbia Nutritionals.

Bret Petersen (BP) reports that he is the Senior Director of Ingredients for Glanbia Nutritionals.

Sarah Flynn (SF) reports that she is the Product Strategy and Technology Manager for Glanbia Nutritionals.

Cassandra Evans (CE) has no real or perceived conflicts of interest to declare.

Jose Antonio (JA) is the CEO of the ISSN, an academic non-profit. The ISSN receives funding from companies the manufacturer, sell, and brand creatine and creatine-containing products.

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