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EFFECT OF CREATINE AND CAFFEINE SUPPLEMENTATION TOGETHER AND SEPARATELY ON SERUM LACTATE LEVELES AND PERFORMANCE OF MALE WRESTLERS

Seyed Mohammad Seyedin Koojan[A]

Physical Activity and Health Promotion Course, Faculty of Medicine and Surgery, University of Rome Tor Vergata, Italy
ORCID: 0000-0001-6648-6873

Giuseppe Merra[A]

Department of Biomedicine and Prevention, Section of Clinical Nutrition and Nutrigenomics, Univeristy of Rome Tor Vergata, Italy
ORCID: 0000-0003-4753-3528 | e-mail:giuseppe.merra@uniroma2.it

Giulia Frank[A]

PhD School of Applied Medical-Surgical-Sciences, Univeristy of Rome Tor Vergata, Italy
ORCID: 0000-0003-4515-548X | e-mail: giulia.frank@ymail.com

Paola Gualtieri[C]

Department of Biomedicine and Prevention, Section of Clinical Nutrition and Nutrigenomics, Univeristy of Rome Tor Vergata, Italy
Physical Activity and Health Promotion Course, Faculty of Medicine and Surgery, University of Rome Tor Vergata, Italy
ORCID: 0000-0003-1533-4276

Mahsa Farahani[B]

Physical Activity and Health Promotion Course, Faculty of Medicine and Surgery, University of Rome Tor Vergata, Italy
ORCID: 0000-0001-8468-4283

Martin Hofmeister[A]

Department Food and Nutrition, Munich, Consumer Centre of the German Federal State of Bavaria, Germany
ORCID: 0000-0002-0693-7887

David Della Morte Canosci[D]

Physical Activity and Health Promotion Course, Faculty of Medicine and Surgery, University of Rome Tor Vergata, Italy
Department of System Medicine, Univeristy of Rome Tor Vergata, Italy
ORCID: 0000-0002-4054-5318

Fateme Shabkhiz[A]

Department of Sport Physiology, Faculty of Physical Education and Sports Sciences, University of Tehran, Tehran, Iran
ORCID: 0000-0003-1183-9517

Laura Di Renzo[D]

Department of Biomedicine and Prevention, Section of Clinical Nutrition and Nutrigenomics, Univeristy of Rome Tor Vergata, Italy
ORCID: 0000-0001-8875-6723

[A]Study Design; [B]Data Collection; [C]Statistical Analysis; [D]Manuscript Preparation

Abstract Supplementation is widely used by athletes to enhance physical capabilities, improve exercise performance, and achieve other ergogenic effects. These practices promote adaptability, recovery, and the ability to train and compete. The aim of this study was to investigate the effects of caffeine, creatine, and their combination on performance and blood lactate levels in semi-professional male wrestlers. A total of twenty wrestlers participated in a double-blind, crossover, randomized placebo-controlled study, which included five experimental treatments: creatine (Cr), caffeine (CAF), creatine + caffeine (Cr+CAF), control (Con), and placebo (PLA). The participants, with an average age of 19.43 ±1.75 and a body mass index of 23.11 ±3.34, were selected voluntarily. Creatine and caffeine powder were mixed in water and consumed 60 minutes prior to each evaluation session. Performance and blood lactate values were measured before and immediately after the Dummy throw test. The results showed that performance significantly improved following the Cr+CAF and Cr conditions ($p \leq 0.05$). Additionally, blood lactate values immediately after the Dummy throw test were significantly lower in the Cr condition ($P = 0.04$) compared to PLA and CON. However, although blood lactate values decreased in the Cr+CAF condition, the difference was not statistically significant ($P > 0.05$). In conclusion, male wrestlers may benefit from the ergogenic effects of creatine and caffeine when consumed separately or in combination.

Key words: caffeine, creatine, sport performance, sports nutrition, wrestling

Introduction

Wrestling, an ancient combat sport with its origins traced back to the Ancient Greek Olympics, continues to hold a significant position as a discipline of great prominence within the contemporary Olympic Games (Barbas et al., 2011; Demirkan et al., 2015). It involves physical competition between two opponents of equal body mass, aiming to gain and maintain an advantage over the other. The sport incorporates various grappling techniques, including clinch combat, throws, takedowns, joint locks, pins, and other grappling positions, all performed on a matted area (Chaabene et al., 2017). Supplementation is widely utilized by athletes to enhance physical capabilities, improve exercise performance, and gain other ergogenic effects. These practices promote adaptability, recovery, and the ability to train and compete (Maughan et al., 2018). According to the consensus statement from the International Olympic Committee (Maughan et al., 2018), only a few supplements have sufficient evidence to support their efficacy in enhancing physical performance and athletic capabilities (Durkalec-Michalski et al., 2019). The ergogenic supplement widely recognized for its effectiveness in enhancing lean body mass and augmenting anaerobic performance is commonly acknowledged to be creatine monohydrate (Aedma et al., 2015). A commonly recommended protocol for creatine supplementation involves an initial loading phase of 20–25 grams per day for a duration of 2–7 days, followed by a maintenance phase of 2–5 grams per day (Cooper et al., 2012; Burke et al., 2006). A meta-analysis of 96 published studies, encompassing 1847 individuals, demonstrated that creatine supplementation had a greater impact on repetitive-bout exercises than on single-bout exercises and was more effective in upper-body performance tasks compared to lower-body performance activities (Branch, 2003). Furthermore, Koçak and Karli (2003) reported significant improvements in mean power and peak power

achieved during a 30-second Wingate test in professional male wrestlers following creatine supplementation. Oopik et al. (2002) discovered that administering creatine to well-trained wrestlers during a 17-hour recovery period from rapid body mass loss facilitated the restoration of physical functioning abilities as assessed in a performance test simulating a wrestling competition.

Caffeine (CAF) (1,3,7-trimethylxanthine) is a stimulant present in coffee, tea, energy drinks, chocolate, guarana, kola, and bissey nut supplements (Durkalec-Michalski et al., 2019). CAF is typically consumed orally and metabolized by the liver (Magkos & Kavouras, 2005). After intake, blood CAF concentrations start to rise within approximately 15–45 min and reach a peak around 60 min (with a range of 15–120 min) (López-González et al., 2018). CAF has a half-life of 2.5–10 hours when taken in dosages below 10 mg/kg (Magkos & Kavouras, 2005). The effects of caffeine (CAF) supplementation on sports performance have been attributed to multiple proposed mechanisms (van Duinen et al., 2005). These mechanisms include its role as an adenosine receptor antagonist (Fredholm et al., 1999; Spriet & Gibala, 2004), modulation of central nervous system (CNS) activity (Sökmen et al., 2008), influence on muscle excitation-contraction coupling (Tarnopolsky, 2008), and recruitment of motor units (Cornish et al., 2015). CAF also promotes glycolytic activity and enhances muscular energy generation by increasing blood catecholamine levels (Van Soeren et al., 1993). The International Society of Sports Nutrition recommends consuming 3–6 mg/kg of CAF approximately 15–30 min before physical activity. Doses exceeding 9 mg/kg may not provide additional benefits (Goldstein et al., 2010).

Both creatine and caffeine are recognized as useful ergogenic aids for improving aerobic and anaerobic performance (Aaserud et al., 1998; Ahmun et al., 2005; Bemben & Lamont, 2005; Graef et al., 2009; Schneiker et al., 2006). It is necessary to research the effects of combined supplementation so that active individuals can gain more confidence in their knowledge of the effects of the supplements they choose to consume for performance improvement. Specifically, considering the research on the effects of caffeine and creatine supplementation on wrestlers and the sport's popularity worldwide, it is important to study the effects of combining creatine and caffeine on wrestlers. Therefore, this research aims to determine the effect of creatine and caffeine supplementation, both separately and together, on serum lactate levels and the performance of male wrestlers. This research hypothesis suggests that when creatine and caffeine are combined, performance will be at a greater level than when used alone.

Methods

Ethics approval and consent to participate

This study was approved by the Human Ethics Research Committee of Sport Sciences Research Institute of Iran according to the compliance with Ethical Standards in Research of the Ministry of Science, Research and Technology, with the code SSRI.REC-2206-1708 (R1) (date of registration: 22/08/2022), as well as operating in accordance with the Declaration of Helsinki. Before participating in the study, wrestlers signed informed consent.

Particpants

Sixteen male wrestlers completed the entire research procedure and were included in the analyses (Figure 1, Table 1). The participants consisted of members from the Ebrahim Hadi wrestling gym, including top wrestlers who had participated in league events. To qualify for participation in the study, athletes were required to meet several eligibility criteria, including being in excellent health, presenting a valid and current medical certificate certifying their capability to engage in sports activities, possessing a minimum of three years of training experience, and

actively participating in four weekly exercise sessions that specifically focused on combat sports. Any individuals with current injuries, health-related contraindications, a reported overall feeling of unwellness, or those unwilling to comply with the research protocol were excluded (Figure 1). The research was conducted during the five-week preparatory period of the annual training program. During this transition phase, the wrestlers trained five times per week, which included five wrestling-specific sessions incorporating resistance, endurance, and power training.

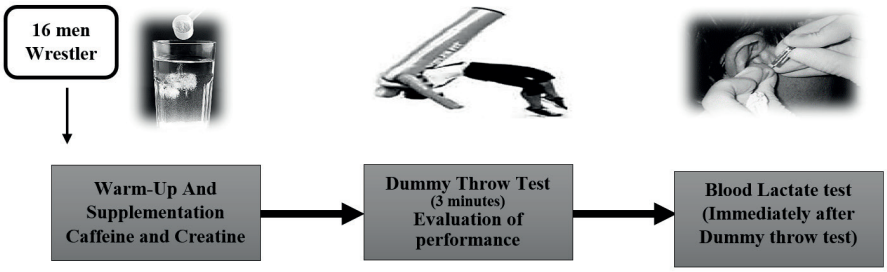

Figure 1. Schematic of research plan.

Table 1. Descriptive statistics of performance and Blood lactate variables in five supplementation conditions (Mean ± SD).

Condition	Performance (Repetition)	Shapiro-Wilk (P value)	Blood Lactate ($mmol.L^{-1}$)	Shapiro-Wilk (P value)
CAF+PLA	13.93 ±2.01	0.69	12.14 ±2.66	0.74
Cr+PLA	14.18 ±1.86	0.35	9.46 ±2.02	0.13
Cr+ CAF	16.18 ±1.86	0.18	10.51 ±1.78	0.15
PLA+PLA	12.75 ±2.01	0.14	11.42 ±1.40	0.93
CON	12.18 ±1.79	0.14	11.35 ±1.29	0.68

* CAF; Caffeine, Cr; Creatine, PLA; Placebo, CON; Control.

Experimental design

The present study was designed as a randomized, double-blind, crossover, placebo-controlled experiment. To ensure blinding for both researchers and participants, an independent pharmacist produced and provided all the supplements. Anthropometric measurements were taken during the preliminary visit. After two familiarization sessions with the training protocol, participants were randomly assigned to one of five conditions: Creatine (Cr), Caffeine (CAF), a combination of Cr and CAF, placebo (PLA), and control (CON). To account for potential training effects, each participant randomly determined the sequence of these conditions. The data from the CON session were used as a baseline for comparative analysis. A washout period of seven days was implemented between sessions.

Supplementation protocol

The supplements and PLA (Maltodextrin as a placebo for creatine monohydrate and Cellulose as a placebo for caffeine anhydrous) were packed in identical Gelatin Capsules (China Gelatin Capsule Co., China), ensuring that participants could not discern the contents of the capsules. Supplementation was administered on evaluation days as follows: Creatine monohydrate powder (0.1 g/Kg-1 .d-1 [Creapure AlzChem Trostberg GmbH, Germany] + 3 mg/Kg-1 .d-1 caffeine placebo), Caffeine anhydrous (3 mg/Kg-1 .d-1 [CSPC Innovation Pharmaceutical, China] + 0.1 g/Kg-1 .d-1 Creatine monohydrate placebo), Creatine monohydrate + Caffeine anhydrous (0.1 g/Kg-1 .d-1 + 3 mg/Kg-1 .d-1), or PLA (0.1 g/Kg-1 .d-1 Maltodextrin + 3 mg/Kg-1 .d-1 Cellulose). Participants mixed their supplement powder with water on evaluation days and consumed the solution 60 min before exercising. The duration of 60 min was selected based on the approximate time to reach peak plasma caffeine concentrations after caffeine consumption (Graham, 2001), and pre-exercise creatine supplementation enhances muscle growth and performance. In order to specifically evaluate the effects of pre-exercise creatine and caffeine supplementation, participants in the study were instructed to refrain from supplementing on non-training days. Additionally, they were advised to avoid consuming other sources of caffeine for a period of three hours prior to taking their supplement. This precaution was implemented to ensure an accurate assessment of the impact of caffeine supplementation on muscle.

Diet

To evaluate changes in total energy intake and macronutrient composition between groups over the course of the supplementation and evaluation period, participants' dietary intake was documented during the initial and final weeks. A 3-day food booklet was utilized by participants to record their food consumption for two weekdays, which could be consecutive or non-consecutive, as well as one weekend day. The food records were analyzed using MyFitnessPal (Under Armour, Inc.), a reliable tool for dietary analysis (Teixeira et al., 2018).

Anthropometric measurements

Anthropometric measurements were obtained during the initial visit to the laboratory in the early hours of the day, with the subjects in a fasted state. Body mass and height were measured using a stadiometer and a professional medical scale (WPT 60/150 OW, RADWAG®, Radom, Poland). The stadiometer provided height measurements with an accuracy of 0.1 cm, while the medical scale provided body mass measurements with an accuracy of 0.1 kg.

Training protocol and Performance evaluation

The capacity for wrestling-specific performance was assessed using a modified dummy throw test designed to simulate wrestling conflict (Anioł-Strzyżewska & Starosta, 2012). The test was conducted in two modes:

- slow mode—four compulsory suplex throws in 30 s
- quick mode—as many suplex throws as possible in 15 s

For three minutes, the modes were alternated in one round. Each round consisted of four slow mode parts and four quick mode parts. The score for the test was determined by the number of correctly completed throws made during the quick mode parts. Athletes were given the necessary instructions before starting the test. The size of the dummy was adjusted to match the athlete's body weight and height.

Blood samples Analysis

The blood lactate (BL) samples were obtained from wrestlers' earlobe before warm-up and immediately after the training protocol. The concentration of BL (mmol/L) was measured using the photometric method with a portable analyzer (Lactate Scout+ analyzer, SensLab GmbH, Germany).

Statical Analysis

The statistical analysis was performed using a statistical software package (IBM SPSS Statistics 23.0, Armonk, NY: IBM Corp.), and the results are reported as mean and standard deviation (SD). After ensuring normality (i.e., Shapiro-Wilk) and variance (i.e., Levene), a one-way repeated-measures analysis of variance (ANOVA) was performed to examine the impact of various supplements on performance and blood lactate (BL) after each training regimen. When significant differences across conditions were discovered, a Bonferroni posthoc analysis was used to determine the differences.

Results

Performance (Dummy throw test)

The results showed a significant effect of supplementation on performance in male wrestlers during the Dummy throw test (Table 1), $F [4, 75] = 10.41$, $P = 0.001$, $\eta\_P^2 = 0.35$. Pairwise comparisons revealed that the number of repetitions during the 3-minute Dummy throw test was significantly greater in the Cr+CAF condition (16.18 ±1.86, $P < 0.01$) and the Cr condition (14.18 ±1.86, $P = 0.04$) compared to the PLA condition (12.75 ±2.01) and the CON condition (12.18 ±1.79). However, a non-significant effect of CAF supplementation alone (13.93 ±2.01, $P = 0.11$) was observed on performance compared to the PLA condition (12.75 ±2.01) and the CON condition (12.18 ±1.79). Furthermore, a significant difference was observed between CAF and Cr+CAF ($P = 0.01$), indicating a greater effect of the combination of CAF and Cr on performance during the Dummy throw test in male wrestlers.

Blood Lactate

A significant difference was also observed in BL levels following the Dummy throw test, $F [1, 75] = 4.69$, $P = 0.002$, $\eta\_P^2 = 0.2$. Pairwise comparisons revealed that BL levels immediately after the Dummy throw test were significantly lower in the Cr condition (9.46 ±2.02, $P = 0.04$) compared to the PLA condition (11.42 ±1.40) and the CON condition (11.41 ±1.29). However, no significant differences were observed between the CAF condition (12.14 ±2.66, $P \geq 0.05$) and the Cr+CAF condition (10.51 ±1.78, $P \geq 0.05$) compared to the PLA condition (11.42 ±1.40) and the CON condition (11.41 ±1.29).

Discussion

The present study was the first to examine the effects of creatine and caffeine supplementation on male wrestlers. It was hypothesized that the co-ingestion of creatine and caffeine would have a superior effect compared to creatine and caffeine supplementation alone in improving performance and reducing blood lactate levels in male wrestlers. The results demonstrated that the combination of creatine and caffeine supplementation, as well as creatine alone, significantly affected performance during the Dummy throwing test in male wrestlers. Additionally, the creatine alone condition resulted in significantly lower blood lactate values.

Creatine and caffeine are two of the most commonly used nutritional ergogenic aids, and numerous studies have demonstrated that both supplements offer ergogenic advantages in terms of strength and sprint performance. Furthermore, both ingredients are generally regarded as safe for consumption. (Nawrot et al., 2003; Persky & Rawson,

2007), they have independent mechanisms of action on performance, and there are no apparent pharmacokinetic interactions when they are taken together (Vanakoski et al., 1998). As a result, they are an attractive choice for combined supplementation among athletes involved in high-intensity exercise.

Individuals supplemented with creatine alone experienced an improvement in performance during the Dummy throw test. Harris et al. (1992) were the first to show that creatine supplementation with creatine monohydrate enhanced muscle creatine concentrations by 20%. Creatine supplementation has been shown to improve lean body mass, strength, power, and performance in high-intensity, short-duration workouts (Greydanus & Patel, 2010). These ergogenic benefits have been extensively studied in the gym and the laboratory, with limited research conducted in actual gameplay situations. In contrast to our findings, Hespel et al. (2002) conducted a study in which they discovered that the inclusion of caffeine at a dosage of 5 mg/kg, administered during a three-day period of creatine supplementation at a dosage of 20 g/day, led to a reduction in muscular relaxation time compared to individuals who solely underwent creatine supplementation. This finding was observed in a group of young, healthy males. Additionally, Vandenberghe et al. (1996) conducted research that revealed the consumption of caffeine at a dosage of 5 mg/kg for three days during a creatine loading phase (0.5 mg/kg for six days) prevented young adults from experiencing an increase in muscle torque subsequent to creatine supplementation. The contradictions in the results are likely due to differences in supplement dosage and supplementation protocols. However, Gonzalez et al. (2011) and Ratamess et al. (2007) demonstrated that acute supplementation of creatine and caffeine together, 10 to 20 min before exercise, improved power, training volume, and endurance. Vanakoski et al. (1998) discovered that although creatine did not impair the ergogenic effects of caffeine, it did not enhance aerobic or anaerobic performance when combined with caffeine.

Furthermore, a recent study by Pakulak et al. (2021) showed a non-significant effect of combining caffeine and creatine on resistance training performance. This discrepancy may be attributed to differences in the type of training protocol employed. Consistent with the present study's findings, multiple investigations have demonstrated that multi-ingredient supplements incorporating both creatine and caffeine have the capacity to enhance strength, power, and lean mass. Notably, these beneficial effects have been observed under both acute and chronic consumption conditions (Fukuda et al., 2010; Hoffman et al., 2008; Kedia et al., 2014; Kendall et al., 2014; Kraemer et al., 2007; Lowery et al., 2013; Ormsbee et al., 2012; Shelmadine et al., 2009; Smith et al., 2010; Spillane et al., 2011; Spradley et al., 2012).

The combination of creatine and caffeine did not significantly alter blood lactate values compared to the placebo and control conditions in male wrestlers. However, the creatine alone condition resulted in a significant decrease in blood lactate values. Consistent with our findings, previous studies by Balsom et al. (1993, 1995) using sprints of 10 seconds or longer also reported lower lactate levels following creatine supplementation, which may be associated with increased AMPK activation in vitro (Ceddia & Sweeney, 2004). During intense exercise such as running, glycolysis is initiated, and within 6–10 s, it becomes the primary source of ATP production (Bogdanis et al., 1996). For instance, during a 10-second sprint, glycolysis contributes to approximately 44% of total ATP synthesis (Bangsbo, 2003). The absence of changes or reductions in blood lactate levels suggests that the increased muscle phosphocreatine (PCr) in the creatine group contributed to higher ATP generation during the final sprints of the session in our study. Additionally, caffeine had a significant impact on blood lactate during incremental exercise, independent of exercise intensity (Glaister et al., 2016). However, caffeine does not affect blood lactate at rest, indicating that some mechanical stress is required to elicit this response. Furthermore, the lack of caffeine's effect

on blood lactate clearance rate after recovery suggests that the increase in blood lactate was likely due to enhanced lactate efflux from the active muscles, supporting the caffeine-induced increase in glycolysis (Glaister et al., 2016). While these findings contradict the results of Graham et al. (2000), it is important to note that Graham et al.'s methodologies may have lacked the sensitivity to detect the magnitude of alterations reported in our study, despite being substantial. Additionally, studies by Tarnopolsky et al. (1989) and Wiles et al. (1992) reported a higher mean blood lactate response with caffeine, indicating discrepancies in exercise intensity, blood lactate measurement methodologies, dosage, and supplementation protocols among previous studies. Our findings also revealed that blood lactate in the creatine plus caffeine (Cr+CAF) condition was lower than in the placebo (PLA) and control (CON) conditions but higher than in the creatine alone (Cr) group. This can be attributed to increased blood lactate resulting from caffeine supplementation and decreased blood lactate due to the mechanism of creatine supplementation described earlier.

This study has several limitations: Firstly, we were unable to measure blood lactate levels more accurately by obtaining blood samples and conducting laboratory measurements. Secondly, due to limited laboratory equipment and the absence of ergometer wheels at the test site, we were unable to measure other functional factors. Additionally, the limited time available for subjects to participate in the test further restricted our ability to gather additional data.

In conclusion, this study examined the impact of acute creatine and caffeine supplementation, both individually and in combination, on performance during the Dummy throwing test and blood lactate levels in male wrestlers. This study is the first to investigate the effects of combining creatine and caffeine on the performance and blood lactate response of male wrestlers. The findings revealed that both creatine and creatine plus caffeine supplementation improved performance during the Dummy throwing test, while creatine supplementation alone significantly decreased blood lactate levels. These results suggest that male wrestlers can benefit from the ergogenic effects of creatine and caffeine when consumed separately or in combination.

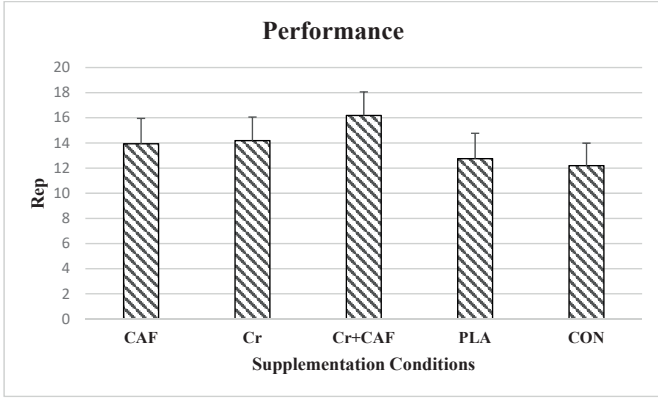

Figure 2. A comparison of performance scores between supplementations conditions, PLA and CON during Dummy throw test.

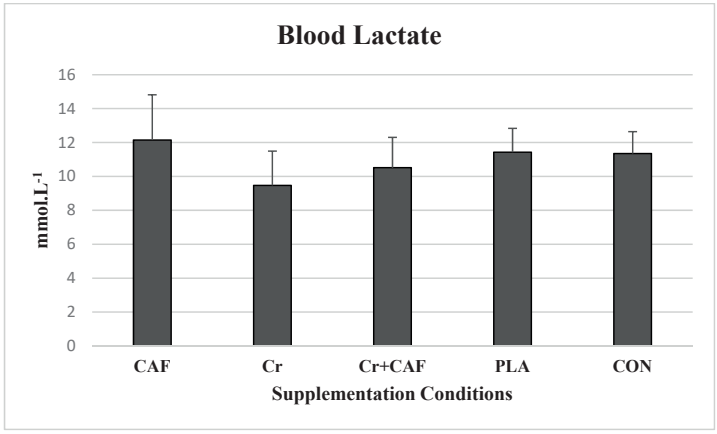

Figure 3. A comparison of performance scores between supplementations conditions, PLA and CON during Dummy throw test.

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