Caffeine, but Not Creatine, Improves Anaerobic Power Without Altering Anaerobic Capacity in Healthy Men During a Wingate Anaerobic Test

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There is a lack of evidence on the additional benefits of combining caffeine (CAF) and creatine (CRE) supplementation on anaerobic power and capacity. Thus, the aim of the present study was to test the effects of combined and isolated supplementation of CAF and CRE on anaerobic power and capacity. Twenty-four healthy men performed a baseline Wingate anaerobic test and were then allocated into a CRE (n = 12) or placebo (PLA; n = 12) group. The CRE group ingested 20 g/day of CRE for 8 days, while the PLA group ingested 20 g/day of maltodextrin for the same period. On the sixth and eighth days of the loading period, both groups performed a Wingate anaerobic test 1 hr after either CAF (5 mg/kg of body mass; CRE + CAF and PLA + CAF conditions) or PLA (5 mg/kg of body mass of cellulose; CRE + PLA and PLA + PLA conditions) ingestion. After the loading period, changes in body mass were greater (p < .05) in the CRE (+0.87 ± 0.23 kg) than in the PLA group (+0.13 ± 0.27 kg). In both groups, peak power was higher (p = .01) in the CAF (1,033.4 ± 209.3 W) than in the PLA trial (1,003.3 ± 204.4 W), but mean power was not different between PLA and CAF trials (p > .05). In conclusion, CAF, but not CRE ingestion, increases anaerobic power. Conversely, neither CRE nor CAF has an effect on anaerobic capacity.

Keywords: ergogenic aid, sports nutrition, peak power

Although many nutritional supplements are claimed to improve exercise performance, only some of them have reached an elevated level of evidence (Maughan et al., 2018). Among the substances that present an elevated level of evidence of an ergogenic effect, caffeine (CAF) and creatine (CRE) are the two most widely consumed (Aguilar-Navarro et al., 2019; Fraczek et al., 2016). Nevertheless, evidence supporting the combination of CAF and CRE instead of isolated administration is still under debate (Elosegui et al., 2022; Marinho et al., 2021).

CAF and CRE act on different physiological pathways. CAF acts on central and peripheral sites (for a detailed review, see Lima-Silva et al., 2021). In the central nervous system, CAF acts as an adenosine antagonist (Fredholm, 1995; Meeusen et al., 2013), increasing motor cortex and spinal excitability (Kalmar & Cafarelli, 2004; Walton et al., 2003). In peripheral sites, CAF might reduce extracellular potassium (K⁺) accumulation (Mohr et al., 2011) and increase muscle force contraction (Tarnopsky & Cupido, 2000), supposedly by increasing calcium release from sarcoplasmic reticulum (Allen & Westerblad, 1995; Weber & Herz, 1968). In turn, CRE increases phosphocreatine and free CRE content in skeletal muscles (Harris et al., 1992), the capacity to resynthesize adenosine triphosphate (Wynn & Kaddurah-Daouk, 2000), the muscle buffering capacity (Oliver et al., 2013), and the calcium reuptake into the sarcoplasmic reticulum, ultimately enhancing myofibrillar cross-bridge cycling and muscle force (Bazzucchi et al., 2009). Consequently, both CAF and CRE have been used in isolation to improve anaerobic power and/or anaerobic capacity (Grgic, 2018; Koçak; Karli, 2003; Zuniga et al., 2012). However, the lack of experimental studies combining CAF and CRE precludes the ascertainment of the potential interaction that a combination of CAF and CRE might have on anaerobic performance. From a mechanistic viewpoint, CAF and CRE might have potentially different effects on calcium handling, which may impair force production when used in combination (Trexler & Smith-Ryan, 2015). On the other hand, based on their other apparently independent mechanisms of action, their combined ingestion may potentialize gains in exercise performance (Elosegui et al., 2022; Marinho et al., 2021).

Two recently published systematic reviews suggested that high-intensity exercise performance can be optimized when CRE supplementation (5 days ingesting 20 g/day or 0.3 g·kg⁻¹·day⁻¹ of body mass) is followed by an acute CAF dose (5–6 mg/kg of body mass) ingested 1 hr prior to the exercise (Elosegui et al., 2022; Marinho et al., 2021). However, this conclusion may be premature because the three studies reporting synergic effects of CAF and CRE did not include a condition with CAF alone in their experimental design (Doherty et al., 2002; Lee et al., 2011, 2012). While these studies provided an important advance in our understanding of CRE and CAF...
combination, an experimental design including all possible combinations of CRE and CAF supplementation would have practical implications. By analyzing anaerobic outcomes from an experimental design with CRE and CAF in isolated and in combination, sports nutritionists could determine whether both supplements are necessary to improve anaerobic performance. In fact, potential interactions between concurrently used supplements are of high priority for scientific-based prescription (Burke et al., 2019). Further, as anaerobic performance can be reliably measured using the Wingate test, assessing the effect of combining CAF and CRE on peak (PP) and mean power (MP) during the Wingate test can provide important information in relation to the effects of CAF and CRE on anaerobic performance.

Therefore, the aim of the present study was to test the combined and isolated effects of CAF and CRE supplementation on anaerobic power and capacity. We hypothesized that combining CAF and CRE might improve anaerobic power and capacity to a larger extent than when CAF and CRE are administrated in isolation.

**Methods**

**Experimental Design**

The study was conducted using a matched-pair, controlled-trial, crossover design (Figure 1). Participants visited the laboratory five times, with a minimum interval of 72 hr and a maximum interval of 1 week between the visits.

In the first visit, chest, abdomen, and thigh skinfolds were measured and the body fat percentage was calculated using standardized equations (Jackson & Pollock, 1978; Siri, 1961). Participants also filled out a food and beverage frequency questionnaire to determine their level of habitual CAF consumption (Filip et al., 2020). Thereafter, a graded exercise test was performed to determine peak power output (PPO) and peak oxygen uptake ($\dot{V}O_2^{peak}$). In the second visit, participants performed a Wingate anaerobic test for familiarization with the experimental procedures. In the third visit, participants were weighed and performed a baseline Wingate anaerobic test. Participants were further paired according to their PP and MP during this baseline Wingate anaerobic test, with one participant of each pair randomly allocated to either the CRE or the placebo (PLA) group. The CRE group ingested 20 g/day of CRE for 8 days ($n = 12$), while the PLA group ingested 20 g/day of maltodextrin as a PLA for 8 days ($n = 12$).

In the fourth and fifth visits (sixth and eighth days of the loading period), participants were weighed and performed the Wingate anaerobic test 1 hr after the ingestion of a capsule containing 5 mg/kg body mass of CAF (CRE+CAF and PLA+CAF conditions) or 5 mg/kg body mass of cellulose as PLA (CRE+PLA and PLA+PLA conditions). These visits were performed using a randomized, crossover, and counterbalanced design through free available software (https://www.jerrydallal.com/random/permute.htm). Neither the investigator nor the participants were informed about the administered substances, with a

**Figure 1** — Schematic representation of the experimental design. ANT =anthropometry; GXT =graded exercise test; FFQ =food frequency questionnaire for determination of habitual caffeine consumption; FAM =familiarization; CAF =caffeine; PLA =placebo.
researcher from our laboratory who was not involved in the study being responsible for the blinding procedures and supplements administered.

Participants abstained from exhaustive exercise and food, beverages, or supplements containing CAF and alcohol 24 hr before trials. Food and beverage intake was registered during the 24 hr prior to the first trial, with food and beverage intake replicated in the 24 hr before subsequent trials. Participants consumed their last meal at least 2 hr before the trials. All trials were performed at the same time of the day to avoid the effect of circadian variation on anaerobic performance (Lopes-Silva et al., 2019).

Participants

The following parameters were inputted in G*Power software (version 3.1.9.7) to calculate the required sample size: (a) effect size of 0.28, as expected for the effect of CRE plus CAF versus CRE on exercise performance (Lee et al., 2011), (b) alpha of .05, (c) power (1-ß) of 0.80, (d) number of groups of 2, (e) number of measurements of 3, (f) correlation among repeated measures of .5, and (g) nonsphericity correlation of 1. The required sample size was estimated to be 24 participants; thus, 24 healthy men were recruited to participate in this study (Table 1).

The inclusion criteria were: (a) absence of risk factors associated with metabolic, pulmonary, or cardiovascular diseases; (b) no use of beta-alanine or CRE supplementation during the 6 months prior to the beginning of the study. We chose six rather than the conventional 2 months of CRE washout to guarantee that there would be no influence of previous CRE supplementation cycle on anaerobic performance. In addition, participants were all omnivorous. Participants were informed about the procedures, risks, and benefits of the study and signed a written informed consent form. The study was conducted according to the Declaration of Helsinki and was approved by the University Research Ethics Committee (number: 20698619.6.0000.5013).

Graded Exercise Test

The graded exercise test was performed on an electromagnetically braked cycle ergometer (Ergo-Fit). The test started with a warm-up, cycling at 50 W for 5 min, with subsequent increments of 25 W every minute until exhaustion. Participants maintained the pedal cadence at between 70 and 80 rpm, with task failure determined when participants were unable to maintain this target cadence. VO2 was monitored breath-by-breath throughout the test using an automatic metabolic cart (Quark Cardiopulmonary Exercise Testing, Cosmed). The metabolic cart was calibrated before each test using a 3-L syringe, ambient air, and one cylinder of known gas concentration (16% O2 and 5% CO2). PPO was considered as the power in the last completed stage; when the last stage was incomplete, PPO was determined by multiplying the fractional time supported in the last incomplete stage and increment rate. The VO2 peak was defined as VO2 mean of the last 20 s of the test.

The Wingate Anaerobic Test

A 30-s all-out Wingate anaerobic test was performed on a mechanically braked cycle ergometer (Biotec 2100, CEFISE), as previously described (Marinho et al., 2020). Briefly, participants arrived at the laboratory, rested for 5 min, and performed a 5-min warm-up, cycling against a resistance of 2.5 Kp at 90 rpm (~150 W), with 6-s all-out sprints at the second and fourth minutes. Thereafter, participants started a slow, unloaded cycle for 5 s, followed by a 30-s all-out effort against a resistance equivalent to 7.5% of body mass. Additionally, resistance was input based on the body mass on the day of the experimental session. Participants received standardized verbal feedback during the trials. The cycling speed during the trial was continually measured using sensors positioned on the wheel.

The external power output was calculated by multiplying cycle resistance by speed. PP was determined as the highest 1-s mechanical power and used as a marker of anaerobic power (Beneke et al., 2002). MP was calculated as the average power during the 30 s of the trial and used as a marker of anaerobic capacity (Minahan et al., 2007). All these parameters were determined using a custom code routine (MATLAB, version 6.0).

Supplementation

Participants received CRE monohydrate (5 g) or PLA (5 g of maltodextrin) in sachets of the same color, texture, shape, size, and weight. Participants were instructed to dilute the CRE or maltodextrin in 250 ml of water and to ingest four doses per day (4 × 5 g/day, total 20 g/day) along with meals (e.g., breakfast, lunch, afternoon snack, and dinner). The CRE supplement presented 98% of purity, in accordance with the certificate emitted by the manufacturer (NINGXIA BAOMA PHARM LIMITED COMPANY). The CAF (5 mg/kg) and PLA were delivered in gelatin capsules with the same color, texture, shape, size, and weight. The PLA and CAF capsules were provided by a reliable manufacturer that attested the purity of CAF via ultraviolet/visible spectrophotometry and high-performance liquid chromatography (Valdequimica).

Table 1 Main Demographic Characteristics of the Participants

<table>
<thead>
<tr>
<th></th>
<th>PLA group (n = 12)</th>
<th>CRE group (n = 12)</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>24.6 ± 4.2</td>
<td>25.1 ± 3.1</td>
<td>.77</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>175.0 ± 6.4</td>
<td>174.8 ± 5.9</td>
<td>.94</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>77.0 ± 8.3</td>
<td>73.5 ± 11.5</td>
<td>.39</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>12.3 ± 4.2</td>
<td>11.0 ± 4.6</td>
<td>.45</td>
</tr>
<tr>
<td>VO2peak (ml·kg⁻¹·min⁻¹)</td>
<td>46.9 ± 7.8</td>
<td>48.6 ± 6.3</td>
<td>.60</td>
</tr>
<tr>
<td>PPO (W)</td>
<td>237.5 ± 31.1</td>
<td>254.2 ± 48.7</td>
<td>.32</td>
</tr>
<tr>
<td>Habitual caffeine consumption (mg·kg⁻¹·day⁻¹)</td>
<td>3.2 ± 4.4</td>
<td>5.2 ± 3.4</td>
<td>.22</td>
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Note: VO2peak = peak oxygen uptake; PPO = peak power output achieved in the maximal graded exercise test; PLA = placebo; CRE = creatine.
One hour after capsule ingestion, participants were asked which substance they thought they had ingested (CAF or PLA).

### Statistical Analyses

The Shapiro–Wilk test was used to check data normality. The Student’s t test for independent samples was used to compare age, height, body mass, body fat, VO2peak, and PPO between control and CRE groups. As data of habitual CAF consumption were not normally distributed, the Mann–Whitney U test was used for comparisons between control and CRE groups. To check reliability in PP and MP measurements, the Student’s t test for paired samples, intraclass correlation coefficient, technical error of measurement, and coefficient of variation were calculated to compare the familiarization and baseline trials (Hopkins, 2000).

A Student’s t test for independent samples was used to compare PP and MP in the baseline trial between control and CRE groups. Two-way mixed analysis of variance (ANOVA), with group (PLA and CRE groups) as the independent factor and moment (PLA and CAF trial) as the dependent factor was used to compare PP and MP. Two-way mixed ANOVA was also used to compare absolute changes in PLA and CAF trials in relation to the baseline trial. To compare body mass changes during the loading period between control and CRE groups, delta changes between baseline and experimental sessions (i.e., fourth and fifth visits) were calculated and two-way mixed ANOVA, with group (control and CRE groups) as the independent factor and moment (fourth and fifth visits) as the dependent factor, used in the comparisons. The Mauchly sphericity test was used to check sphericity; when the sphericity was violated, the Greenhouse–Geisser correction was applied. The partial eta squared (ηp²) for ANOVA analysis was calculated, where ηp² < .059 indicates a small effect, .059 ≤ ηp² ≤ .138 a moderate effect, and .138 < ηp² a large effect (Cohen, 2013). When ANOVA detected a main effect, the Bonferroni correction for multiple comparisons was used to locate differences. Data are presented as mean ± SD, significance level was set at p ≤ .05. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 25.0).

### Results

#### Demographic Characteristics, Test Reliability, and Baseline Comparisons

Participants of the control and CRE groups did not differ in relation to the main demographic characteristics (Table 1). There were no differences for PP and MP between the familiarization and baseline trials in either group (p > .05). In general, the technical error of measurement was low and the intraclass correlation coefficient moderate to high, indicating an elevated degree of reliability in PP and MP determination (Table 2). In addition, there was no difference for PP and MP in the baseline trial between control and CRE groups (p > .05). Based on the habitual CAF consumption, participants were classified as either low–moderate (62.5%) or high consumers (37.5%).

#### Anaerobic Power and Capacity

There was only a main effect of moment (F1, 22 = 6.64; p = .01; ηp² = .23), without a main effect of group (F1, 22 = 0.53; p = .47; ηp² = .02) or group–moment interaction (F1, 22 = 0.53; p = .47; ηp² = .02) for PP (Table 3). Regardless of group, PP was higher in the CAF than in the PLA trial (p = .01). Similarly, there was a main effect of moment for the absolute changes in PP (F1, 22 = 6.64; p = .01; ηp² = .23), without main effect group (F1, 22 = 0.39; p = .53; ηp² = .01), or group–moment interaction (F2, 44 = 0.53; p = .47; ηp² = .02). In this case, CAF presented greater gains in PP than PLA (p = .01, Figure 2A). In addition, seven participants of the CRE group and seven participants of the PLA group increased PP with CAF ingestion.

There was no main effect of moment (F1, 22 = 0.65; p = .42; ηp² = .02), main effect of group (F1, 22 = 0.01; p = .97; ηp² = .01), or group–moment interaction (F2, 44 = 0.01; p = .97; ηp² = .01) for MP. There was no main effect of group (F1, 22 = 0.28; p = .86; ηp² = .01), moment (F1, 22 = 0.65; p = .42; ηp² = .29), or group–moment interaction (F2, 44 = 0.01; p = .97; ηp² = .01) for the absolute changes in MP (Figure 2B).

#### Body Mass

There was a main effect of group for changes in body mass (F1, 22 = 4.96; p = .03; ηp² = .18), with the CRE group presenting greater (p = .03) gains in body mass (0.87 ± 0.23 kg) than the control group (0.13 ± 0.27 kg).

#### Blinding Assessment

Eighteen out of the 24 participants (75%) incorrectly reported that they had ingested CAF in the PLA trial. Fourteen out of the 24 participants (59%) incorrectly reported that they had ingested PLA in the CAF trial.

### Discussion

While previous systematic reviews have pointed out the potential of combining CRE and CAF supplementation to promote gains in...
peripheral mechanisms. CAF is a powerful antagonist of the probable that CAF increased PP by a combination of central and present study was not designed to provide mechanism, it is larger than between-day reliability calculated in the present study. In addition, this 3% of improvement in PP with CAF ingestion can be attributed to a genuine effect of CAF rather than a potential between-day variability in PP, since the effect of CAF on PP was larger than between-day reliability calculated in the present study (coefficient of variation = 1.9%). While the present study was not designed to provide mechanism, it is probable that CAF increased PP by a combination of central and peripheral mechanisms. CAF is a powerful antagonist of the CAF might have likely improved PP via a combination of these neuromuscular mechanisms. On the other hand, the effects of CRE are confined to the skeletal muscle, which may not have been sufficient to improve MP. Thus, our findings suggest that CAF, but not CRE, improves anaerobic power during a Wingate anaerobic test.

### Anaerobic Capacity

Different from PP, MP was not altered by CRE or CAF supplementation. This is in disagreement with previous reports showing a higher MP after CAF ingestion (Grgic, 2018) or CRE loading (Zuniga et al., 2012). While it is difficult to explain these contrary findings, an elevated degree of intramuscular metabolite perturbation during a Wingate anaerobic test carries to a considerable amount of peripheral fatigue (Bogdanis et al., 1995; Takei et al., 2021). Thus, a potential ergogenic effect of combined or isolated intake of CRE and CAF may not have been sufficiently strong to overcome the deleterious effect of the accelerated process of muscle fatigue affecting MP. Our findings suggest that neither CAF nor CRE, in isolation or in combination, seem to improve anaerobic capacity during the Wingate anaerobic test.

### Strengths, Practical Applications, and Limitations of the Study

From a methodological standpoint, testing the combination of two supplements demands a complex experimental design. By combining matched-pair, controlled-trial, and crossover designs, we were able to test the combined and isolated effects of CRE and CAF supplementation. A challenge is to guarantee that the CRE loading protocol was effective. Attesting to the effectivity of the CRE loading protocol in the present study, body mass gain was higher in the CRE than in the control group. A gain in body weight is a well-recognized effect of CRE supplementation (Kreider et al., 2017; Mujika et al., 2000). Another aspect is the successful blinding when investigating the ergogenic effects of CAF (Shabir et al., 2018, 2019). In this regard, most of the participants in the present study incorrectly identified which substance they had ingested, indicating success in the blinding process. Finally, the lack of difference in PP and MP between the

### Table 3  PP and MP During a Wingate Anaerobic Test After PLA and Caffeine Ingestion in the Control and Creatine Groups

<table>
<thead>
<tr>
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<th>PLA group (n = 12)</th>
<th>CRE group (n = 12)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>PLA + PLA trial</td>
<td>PLA + CAF trial</td>
</tr>
<tr>
<td></td>
<td>CRE + PLA trial</td>
<td>CRE + CAF trial</td>
</tr>
<tr>
<td>PP (W)</td>
<td>1,020.2 ± 207.0</td>
<td>1,058.9 ± 201.7*</td>
</tr>
<tr>
<td></td>
<td>986.4 ± 209.4</td>
<td>1,007.9 ± 222.5*</td>
</tr>
<tr>
<td>MP (W)</td>
<td>577.7 ± 103.5</td>
<td>572.7 ± 100.3</td>
</tr>
<tr>
<td></td>
<td>589.6 ± 108.2</td>
<td>585.06 ± 105.9</td>
</tr>
</tbody>
</table>

*Significantly higher than PLA (p < .05).

**Note.** PLA = placebo; CRE = creatine; PP = peak power; MP = mean power; CAF = caffeine.

#### Figure 2  — Absolute changes from baseline trial for peak power (A) and mean power (B). PLA = placebo; CRE = creatine. Data are described as mean (SEM). *Significantly higher than placebo regardless group (p < .05).
familiarized and control trials confirms that participants were well familiarized with the Wingate anaerobic test and the observed effects were not due to a learning effect. Thus, a strength of the present study is its experimental design, which enabled us to effectively test the effect of CRE and CAF in isolation and in combination.

Practical applications can be obtained from the findings of the present study. CRE and CAF are frequently present in commercial preworkout supplements claiming to enhance exercise performance, mainly performance during high-intensity exercises. Although the use of CRE and CAF in combination is mostly based on practitioners’ beliefs, the present study does not provide support for these beliefs and suggests that CAF alone is sufficient to improve anaerobic power, and that neither CAF or CRE, in isolation or in combination, improves anaerobic capacity.

Some limitations of the present study must be acknowledged. First, the lack of blood and muscle samples preclude to determine the main mechanism by which CAF but not CRE increases PP without any effect on MP, as well any potential influence of individual response associated with genetic variations (e.g., CAF-associated polymorphisms, muscle fiber type distribution, and baseline intramuscular levels of CRE). Second, intramuscular CRE content was not measured; thus, we cannot affirm that CRE loading increased the intramuscular CRE content. Nevertheless, we adopted a classical and well-established CRE loading protocol that increases intramuscular CRE and phosphocreatine levels (Harris et al., 1992; Hultman et al., 1996; Kreider et al., 2017). Third, plasma CAF concentration was not measured in the present study. However, it has been shown that CAF doses from 1 to 6 mg/kg increase plasma CAF concentration (Matsumura et al., 2023). Fourth, only men participated in the present study. While previous evidence suggests that women experience similar ergogenic effects of CAF (Skinner et al., 2019) and CRE (Antonio et al., 2021) to men, future studies should test the effect of combining CRE and CAF in women. Fifth, although we ran an a priori sample size calculation, we cannot fully disregard an underpowered sample size. Nevertheless, the effect size for nonsignificant outcomes (i.e., the effect of CRE supplementation on PP and MP, and CAF supplementation on MP) was all small (ηp2 ≤ .02), indicating that larger sample size would have not returned to significant differences. Sixth, we were unable to independently confirm the content of CRE and CAF supplements, although manufacturers have provided certificate attesting content and purity. Finally, we tested the effect of CRE and CAF combination on a single Wingate anaerobic test; thus, the effect of CRE and CAF combination on multiple Wingate anaerobic test deserves further investigation.

Conclusions

The findings of the present study indicate that CAF, but not CRE ingestion, increases anaerobic power during a Wingate anaerobic test. However, CAF and CRE have no effect on anaerobic capacity during a Wingate anaerobic test.

Acknowledgments

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