DOI 10.26773/smj.231002

SportMont

ORIGINAL SCIENTIFIC PAPER

Effects of Acute Caffeine Administration on Pituitary–Testicular Hormonal Responses and Muscle Damage Biomarkers following Muscular Endurance in Well Resistance–Trained Males

Mohammad Fayiz AbuMoh'd

Yarmouk University, Department of Sports/Movement Sciences, Faculty of Physical Education, Irbid, Jordan

Abstract

The purpose of this study was to investigate the effects of acute caffeine administration on pituitary-testicular hormonal (prolactin (PRL), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and total testosterone (TT)) responses and muscle damage biomarkers (creatine kinase (CK), creatine kinase-MB, myoglobin, and troponin) following muscular endurance test. In addition, total repetitions to failure were determined. Eleven well resistance-trained males (29.11±3.21 years) performed two consecutive trials (7 days apart). Using a double-blind, placebo-controlled, randomized crossover design, participants administered either 5 mg/kg of caffeine or a placebo 1 hour prior to the test. The test consisted of 3 sets × repetitions to failure at 70% of 1-RM, with 90 s recovery interval, in each bench press, biceps curl, shoulder press, leg press, back squat, and leg extension. Repetitions were counted in each set in each exercise. Blood samples were collected 1 h after the end of each trial from each participant to measure the aforementioned study parameters. Results indicated that caffeine had positively effect on FSH (p=.046) and TT (p=.049) but had negatively effect on CK (p=.012) compared to the placebo. However, no significant (p>0.05) differences were found in PRL, LH, CK-MB, myoglobin, and troponin between trials. Total repetitions to failure were significantly (p=.013) greater in caffeine trial (334.38±9.70) than in the placebo (309.07±9.43). In conclusion: 5 mg/kg of caffeine administered 1 h prior to muscular endurance test performed by multiple resistance exercises had beneficial effect on FSH and TT probably due to affecting androgen receptors. However, the values of biomarkers of muscle damage were similar in both trials. Hence, male strength athletes may consider using this dose pre-training as an effective ergogenic aid during muscle endurance training.

Keywords: *adenosine receptors, androgen receptors, catecholamines, hypothalamus, prolactin*

Introduction

Resistance exercise enhances muscle function (Heavens et al., 2014) by increasing myofibrillar recruitment (Fragala et al., 2011; Machado et al., 2012) as a result of mechanical stress, metabolic demands, and endocrine activity (Heavens et al., 2014; Raastad et al., 2000). Mechanical stress represents a primary stimulus for hormonal response and, in turn, acclimatization to resistance training (Glover et al., 2022). Contraction cycle requires accelerated physiological demands during resistance exercise to restore protein breakdown. Of relevance, hormonal activity plays a crucial role in gene expression (Machado et al., 2012; Heavens et al., 2014) and remodeling process of muscle proteins (Raastad et al., 2000; Wu & Lin, 2010).

Testosterone, a potent antagonistic catabolic hormone, is secreted from testes by two main hormones include follicle-stimulating hormone (FSH) that stimulates spermatogen-

Correspondence:

Mohammad Fayiz AbuMoh'd
Yarmouk University, Department of Sports/Movement Sciences, Faculty of Physical Education, Shafiq Irshidat st., Zip code: 21163, Irbid, Jordan
Email: famohammad@yu.edu.jo

esis and luteinizing hormone (LH) which synthesizes testosterone (Willoughby et al., 2014; Glover et al., 2022). These hormones are secreted from pituitary and regulated by hypothalamus through release of gonadotropin-releasing hormone (GnRH) (Cadegiani et al., 2019), making hypothalamic-pituitary-gonadal (HPG) axis is a pivotal factor in the process of protein synthesis. Increased blood testosterone level strengthens muscular strength (Spiering et al., 2009; Stokes et al., 2013; Willoughby et al., 2014). However, GnRH elicits excretion of prolactin (PRL) (Willoughby et al., 2014), and high abnormal blood PRL level can affect androgen receptors (Spiering et al., 2009), contributing to protein degradation. All of these hormones maintain homeostasis of muscle function in resistance training, especially when myofibrils predispose to damage resulted from repetitive contractions (Heavens et al., 2014).

Repetitive eccentric and concentric contractions until momentary muscle failure (muscular endurance) can induce muscle damage. Its signs include sarcolemmas tears, myofibrillars disruption (Heavens et a., 2014), Z-discs mutilation, and increased permeability of muscle proteins into blood (Owens et al., 2019), such as creatine kinase (CK), myoglobin, and troponin (Machado et al., 2012). In this context, Heavens et al. (2014) found greater prolonged muscle damage markers included CK and myoglobin following descending pyramid (starting at 10 repetitions and descending 1 repetition as fast as possible) for squat, deadlift, and bench press, but in trained men compared in women. On the other hand, short period of recovery between sets can induce muscle damage and change hormonal response (Heavens et al., 2014; Smilios et al., 2013). For instance, high volume (3-5 sets × more than 10 repetitions) with short recovery (60-90 s) elevates muscle damage biomarkers and blood testosterone level (Smilios et al., 2013) probably to restore protein degradation. Further, submaximal intensity has more efficacy at triggering hormonal response (McCaulley et al., 2009). In this sense, Raastad et al. (2000) revealed that acute response of testosterone was higher in submaximal (70% of one repetition maximum (1-RM)) than in maximal (100% of 1-RM) intensity after resistance exercises. They also found no statistical differences in FSH and LH between both intensities. Stokes et al. (2013) demonstrated significant increased blood testosterone, but without response on PRL, between pre- and post-resistance exercises. These prior studies, however, have not used any ergogenic intervention, such as caffeine.

Caffeine is ingested before resistance exercise session to increase athletic performance and enhance more repetitions to failure (Grgic & Mikulic, 2021; Ruiz-Fernández et al., 2023) by reducing pain sensation and rating of perceived exertion (RPE) (Wu & Lin, 2010; Polito et al., 2019; Ferreira et al., 2021). Some prior studies (Green et al., 2007; Polito et al., 2019) have found significantly increased the number of repetitions to failure following caffeine ingestion compared to placebo. Conversely, other studies (Astorino et al., 2008; Duncan et al., 2013; Grgic & Mikulic, 2021) have failed to find the same caffeine's effects. Further, Ferreira et al. (2020) reported by review a contradiction in caffeine's effects regarding muscular endurance, especially lower extremities. A common alert on resistance exercise is that "Further research is merited on the topic of caffeine and muscular endurance". Most of these studies investigated a repetition to failure tests have consisted of 2-4 resistance exercises. To the best of knowledge, however, only one study conducted by Davis et al. (2012) has been investigated a muscular endurance with high volume (4 sets per exercise) of six exercises, but in healthy men. Surprisingly, they reported a significantly higher repetition to failure in only one (bench press) exercise.

On the other hand, caffeine plays an important role in hormonal changes, such as increased testosterone levels (Glover et al., 2022). In a study conducted on resistance-trained men who performed 8 exercises (3 sets of 10 repetitions at 75% of 1-RM), results showed no statistical difference in testosterone between caffeine and control (Wu & Lin, 2010). For muscle damage, a few studies (Machado et al., 2008; Soleimani et al., 2018) have demonstrated that caffeine had no effect on signs of muscle damage after resistance session. However, other studies reported that caffeine decreased oxidative stress but in brain rat (Vieira et al., 2017), and attenuated delayed-onset muscle soreness (DOMS) (Hurley et al., 2013). Due to the paucity of data in the literature regarding caffeine's effects on combination of hormonal response and muscle damage biomarkers following muscular endurance, a practical applicability needs further research.

Consequently, the primary purpose of the current study was, for the first time, to investigate the acute effects of caffeine administration on pituitary-testicular hormonal (PRL, FSH, LH, and TT) responses and muscle damage biomarkers (CK, CK-MB, myoglobin, and troponin) following muscular endurance test performed by multiple exercises in well resistance-trained males. The second aim was to determine the sum of repetitions to failure in each exercise and the total repetitions after caffeine and placebo administration. The hypothesis of the study was that caffeine may enhance performance by attenuation of pain sensation during performance.

Methods

Participants

Eleven well resistance-trained males (age: 29.11±3.21 years, height: 180.64±4.41 cm, body mass: 92.55±3.80 kg, BMI: 28.18±2.76 kg/m2, training experience: 7.82±1.25 years) volunteered to participate in the study. The inclusion criteria were considered as follows: regular resistance training (5 times per week) for at least 5 years prior to the study; familiar to perform successful all exercises used in the study; low habitual coffee (1-2 cup per day, roughly ≤100 mg per day); and aged between 25-35 years. Participants who had consumed anabolic steroids (one month prior to the study), ergogenic aids (one week prior to the study), and smokers were not allowed to engage. Each participant was informed about the protocol of the study as well as a possible problem resulting from ingestion of caffeine such as numbness, and thus, provided written informed consent to participate. The study was approved by the Al-Ahliyya Amman University Ethics Committee (Code: FES-18G-280-2023). All the study procedures were in accordance with the latest version of the Declaration of Helsinki.

Experimental design

Intervention order (caffeine or placebo) was randomly assigned to participate. The study used a double-blind crossover design, which neither examiners nor participants alert of intervention order. All participants were instructed not to engage strenuous exercise 48 hours prior to trials, maintain their dietary pattern during the days between trials, not to consume coffee after 8:00 PM the night of the trial, and not to ingest breakfast meal on the day of the trial. Roughly 500 ml of water

were drunk by participants during each trial to avoid throat dehydration, which may affect the performance. The study trials were performed in at 20-22°C and 46-49% relative humidity.

Experimental protocol

Data collection occurred over 4 days with different interval periods (started on 2/7/2023). On the first day, participants were asked to measure their demographic characteristics and subjected to a 1-RM test for bench press, biceps curl, shoulder press, leg press, back squat, and leg extension. After 5 days, blood samples were collected from each participant as baseline measurements (Table 1). After 3 days, participants were randomly assigned to either caffeine intake (CAF trial) or placebo (7 days apart). The caffeine and placebo were administered 1 h prior to muscular endurance test (Polito et al., 2019). During this time, participants were seated in the Gym room (talking or reviewing social media by mobile). After that, participants performed a prior warm-up (10 repetitions at 50% of own 1-RM) in each exercise. Then, participants were ordered to perform the test. The muscular endurance test consisted of 3 sets × repetitions to failure in bench press, biceps curl, shoulder press, leg press, back squat, and leg extension at 70% of 1-RM, with a short rest interval (90 s) and 1 s cadence of each concentric and eccentric contraction (Polito et al., 2019). Momentary muscle failure was considered when the participant could not preserve the success extension and flexion. All participants were given verbal encouragement throughout the test to complete as many repetitions as possible. At the end of each trial, blood samples were collected from each participant to measure the study parameters.

Table 1. Baseline measurements (Mean ± SD).

	Baseline values	Reference range
PRL (ng/ml)	11.94±1.81	4.04–15.2
FSH (IU/L)	7.05±2.82	1.5–12.4
LH (IU/L)	5.36±2.67	1.7–8.6
TT (ng/ml)	6.51±3.56	2.49–8.82
CK (U/L)	166.82±4.53	38–197
CK-MB (U/L)	14.95±2.24	Up to 25.0
Myoglobin (µg/L)	51.88±3.82	<90
Troponin (ng/ml)	0.10±0.11	0.001–0.16

PRL: Prolactin; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; TT: Total testosterone; CK: Creatine kinase; CK-MB: Creatine kinase-MB

One repetition maximum

At the beginning, participants subjected a warm-up with a single set (10 repetitions) in aforementioned exercises at 50% of estimated 1-RM, with 30 s rest interval. Subsequently, a 1-RM test for each exercise was started. Determined 1-RM ensued according to the methods of Willoughby et al. (2014). 1-RM constituted the maximum weight lifted once with proper technique and success repetition. Pilot test showed no difference in 1-RM bench press (t=1.71, p=.211), biceps curl (t=0.44, p=.610), shoulder press (t=0.36, p=.492), leg press (t=-0.46, p=.693), back squat (t=0.43, p=.236), and leg extension (t=0.76, p=0.812).

Intervention administration

5 mg/kg of caffeine (Florida Supplement Caffeine Capsule, Nutrix Research, USA) that swallowed by 250 ml of water (18°C) or placebo (dextrose) was provided to participants in an identical capsule 1 h prior to muscular endurance test. These capsules were coded and prepared by a nutritionist who was not involved in the study. The selected caffeine dose was chosen due to its role in elevation of plasma caffeine concentration (Graham, 2001). One week later, participants repeated the same procedure with other intervention.

Blood samples

Blood samples (6 ml) were collected from each participant 1 hour following the completion of each trial to measure the study parameters (pituitary-testicular hormones and muscle damage biomarkers). Serum samples were used for all parameters analysis and centrifuged at 3500-4000 rpm for 10 min. PRL, FSH, LH, were analyzed by (Cobas6000-REI, Switzerland), CK and TT were analyzed using (Snibe- immunoassay, Shenzhen, China), CK-MB was analyzes using (S1/CobasC311-REI, Switzerland), and the analysis of myoglobin and troponin were done by (Immunolyte, 210, USA). The reference ranges of parameters were found in Table 1.

Statistical analysis

The Shapiro-Wilk test was applied to verify the distribution of the data. All the analysis variables were normally distributed (p>0.05). Two-way ANOVA (CAF/PLA × sets) with repeated measurements was used to analyze differences between the sets in each exercise within-group and between-group. One-way ANOVA with repeated measurements was used to test differences between the sum of repetitions within-group and between-group. Bonferroni post-hoc was used to determine a significant difference. A Paired sample t test was used to test between-trials differences in the total number of repetitions to failure and to test between-trials differences in the study parameters. SPSS version 23.0 (Statesoft, Tulsa, USA) was used for all analyses. Descriptive statistics are presented as Mean±Standard deviation. The level of significance was set at p≤0.05.

Results

In regard to study parameters, results indicated no significant differences in PRL (t=-1.164, p=.271), LH (t=-0.630, p=.543), CK-MB (t=1.849, p=.094), myoglobin (t=0.681, p=.511), and troponin (t=1.796, p=.103) between trials. However, caffeine had a positive effect in FSH (t=2.283, p=.046) and TT (t=2.236, p=.049), and a negative effect in CK (t=3.071, p=.012) compared to the placebo (Table 2).

Table 2. Pituitary-testicular hormonal responses and muscle damage biomarkers in both trials. Data were analyzed using paired sample t test.

	Caffeine	Placebo
PRL (ng/ml)	4.77±2.55	4.91±2.46
FSH (IU/L)	6.63±3.06 *	6.49±2.18
LH (IU/L)	15.09±2.76	14.66±3.74
TT (ng/ml)	7.88±2.06 *	7.21±2.90
CK (U/L)	280.09±5.97	271.82±6.27 *
CK-MB (U/L)	20.23±1.53	19.91±1.43
Myoglobin (µg/L)	78.77±3.72	76.64±4.48
Troponin (ng/ml)	0.11±0.01	0.12±0.02

Note. Significance level was set at p<0.05. Values expressed as Mean±SD. *: Significant difference between caffeine and placebo. PRL: Prolactin; FSH: Follicle-stimulating hormone; LH: Luteinizing hormone; TT: Total testosterone; CK: Creatine kinase; CK-MB: Creatine kinase-MB

For muscular endurance, Table 3 illustrates the repetitions in each of the sets performed in each exercise in each trial. There were no statistical differences in the repetitions to failure of each set between caffeine and placebo (p>0.05). Within-group, however, some different repetitions throughout the sets in all exercises was occurred. In this regard, there was a significant difference in all exercises between the 1st and 2nd sets (p<0.05), 1st and 3rd sets (p<0.05), and between 2nd and 3rd sets (p<0.05). Notably, in caffeine trial, no significant difference was found between 2nd and 3rd sets in only back squat. (p=.302).

Table 3. The number of repetitions in each set of resistance exercises in both trials. Data were analyzed using two-way ANOVA

	Bench press			Biceps curl			Shoulder press		
	Set 1	**Set 2**	**Set 3**	**Set 1**	**Set 2**	**Set 3**	**Set 1**	**Set 2**	**Set 3**
Caffeine	27.36 ±1.91 2,3	22.55 ±1.51 3	17.45 ±2.97	22.73 ±3.85 2,3	19.27 ±2.00 3	15.55 ±1.97	22.09 ±2.21 2,3	18.09 ±3.76 3	13.64 ±2.69
Placebo	26.00 ±2.00 2,3	21.27 ±1.56 3	16.73 ±2.33	21.45 ±2.02 2,3	18.55 ±2.42 3	14.82 ±1.99	20.36 ±4.25 2,3	16.82 ±2.04 3	13.36 ±3.12
	Leg press			**Back squat**			**Leg extension**		
	Set 1	**Set 2**	**Set 3**	**Set 1**	**Set 2**	**Set 3**	**Set 1**	**Set 2**	**Set 3**
Caffeine	23.91 ±2.07 2,3	20.45 ±2.50 3	16.55 ±3.01	17.64 ±5.61 2,3	15.73 ±3.35	15.73 ±3.35	20.00 ±2.10 2,3	15.00 ±4.73 3	11.45 ±1.04
Placebo	21.36 ±2.20 2,3	19.09 ±2.34 3	15.27 ±2.45	17.91 ±4.58 2,3	14.73 ±1.95 3	10.18 ±3.47	17.91 ±1.97 2,3	13.91 ±5.70 3	9.36 ±1.03

Note. Significance level was set at p<0.05. Values expressed as Mean SD. 2: significant different to 2nd set; 3: significant different to 3rd set.

Table 4 shows the sum of repetitions of the sets in each exercise and the total repetitions to failure in each trial. Within-group, Bonferroni post hoc test revealed significantly (p<0.05) differences between bench press and the other exercises after both caffeine and placebo administration. The test also showed that leg press had significantly (p<0.05) greater repetitions than in shoulder press, back squat, and leg extension in both trials. In addition, the sum of repetitions in upper body exercises were significantly (p<0.05) more than in lower body exercises in caffeine and placebo trials. Between-group, results revealed no significant (p>0.05) differences in bench press, biceps curl, and shoulder press, but showed significantly (p<0.05) caffeine's effect in leg press, back squat, and leg extension compared to the placebo. In addition, caffeine had significantly (p<0.05) higher sum of repetitions in upper and lower body exercises than in the placebo. Last, total repetitions to failure were significantly (t=9.41, p=.013) higher after caffeine administration compared to the placebo.

Table 4. Sum of repetitions in each exercise and total repetitions to failure in both trials. Data were analyzed using one-way ANOVA for sum of repetitions between- and within-group and paired sample t test for total repetitions

	Bench press	Biceps curl	Shoulder press	Leg press	Back squat	Leg extension	Total
Caffeine	67.36±4.47 a	57.55±3.52 c	53.82±3.96	60.91±3.93 b	48.29±4.19	46.45±3.91	334.38±9.70 *
	Sum of repetitions (178.73±6.73) @, *			Sum of repetitions (155.65±5.98) *			
Placebo	64.00±4.30 a	54.82±3.46 c	50.54±4.42	55.72±3.41 b	42.82 ± 3.61	41.17±5.88	309.07±9.43
	Sum of repetitions (169.36±7.11) @			Sum of repetitions (139.71±6.44)			

Note. Significance level was set at p<0.05. Values expressed as Mean±SD. a: significant different to other 5 exercises; b: significant different to shoulder press, back squat, and leg extension; c: significant different to back squat and leg extension. @: significant difference between upper and lower body exercises. *: significant difference between caffeine and placebo.

Discussion

The present study investigated the acute effects of caffeine on pituitary-testicular hormonal responses and muscle damage biomarkers following muscular endurance test performed by multiple resistance exercises. The total repetitions to failure was also determined compared to the placebo. The highlight findings of the current study were as follow: 1) acute caffeine (5 mg/kg) administration 1 h prior to muscular endurance test increased positively concentration of FSH and TT in well resistance-trained males; 2) muscle damage biomarkers were similar in caffeine and placebo; 3) the total repetitions to failure were more after caffeine administration compared to the placebo.

In regard to hormonal response, there is a consensus that resistance exercise could increase testosterone concentration (Spiering et al., 2009; Smilios et al., 2013; Stokes et al., 2013). This increment depends on mechanical stress associated with heavy loading workout and adaptation to resistance training (Kraemer & Ratamess, 2005; Heavens et al., 2014). The participants in the present study have experience of a minimum 7 years, and the experimental protocol consisted of high intensity workout, which explain the elevation of TT. In addition, increased testosterone level is linked to the movement velocity (Heavens et al., 2014). Of relevance, HPG axis is activated during slow movement velocity (Lee et al., 2002), and submaximal intensity with high volume and short recovery (60-90 s) have been shown for triggering the HPG axis (Kraemer & Ratamess, 2005; McCaulley et al., 2009). In this context, Smilios et al. (2013) revealed no statistical difference in testosterone level either after submaximal or maximal movement velocity (3:3 vs 1:1). However, the previous study was conducted without caffeine intervention. In the present study, the velocity applied during all of the sets was 1 s for each concentric and eccentric contraction. Probably, several factors may change testosterone concentration, such as the number of sets, the number of exercises, the nature of selected exercises (upper or lower body muscles), and the time of recovery interval. In the present study, the number of exercises is greater than the number in the prior studies. Biologically, testosterone increases as a result of secretion FSH, LH, and sex hormone-binding globulin (SHBG) (Spiering et al., 2009; Wu & Lin, 2010; Stokes et al., 2013). In the current study, FSH was significantly higher in caffeine trial compared to the placebo. However, compared to baseline measurements, FSH and LH decrease in both trials but still within normal range. Although testosterone secretion is not related to the values of FSH and LH since these hormones' values are within normal range, future research is needed to elucidate these findings. Actually, investigation of the acute caffeine effects in resistance exercise has been cited as a further research need.

In addition, activation of large muscle mass can induce high testosterone response (Kraemer & Ratamess, 2005). The participants in the present study have a great muscle mass depended on their BMI. Incorporation of lower muscles during resistance training can augment blood testosterone level 1 hour after the end of session (McCaulley et al., 2009). In the current study, the participants predisposed to perform leg press, back squat, and leg extension and these exercises along with large muscle mass may explain the increment of TT in both trials compared to baseline measurements. The higher the blood testosterone level after caffeine administration may also, in part, be attributed to greater heavy load (more repetitions to failure) (McCaulley et al., 2009), which is caused by reduction in RPE (Ferreira et al., 2021).

In resistance training, myofibrillar anabolic rate should be higher than catabolism when exercising repetition to failure to synthesis satellite calls and thus repair damaged fibers (Heavens et al., 2014). This process, however, requires high blood testosterone concentration. On the other hand, physical stress can induce elevation in blood PRL levels (Spiering et al., 2009), predisposing muscle fiber to challenge between repair of damaged sarcomeres and physiological demands for restoring testosterone. In the present study, the value of PRL in both trials has reached just to upper limits of normal range. The explanation of this response may be attributed to increased dopamine levels because of the study's protocol. Of relevance, caffeine could elicit catecholamines during resistance exercise which raise free fatty acids (Wu & Lin, 2010), facilitating high energy expenditure during performance.

For muscle damage, CK levels were higher after caffeine administration compared to the placebo. What is more important, the values of CK in both trials and baseline measurements are within the acceptable criterion in athletes. The other muscle damage biomarkers measured in the current study were also within normal range. Hence, there is difficult to explain that caffeine had a positive effect on damage biomarkers since all the biomarkers values in both trials were in the near upper limits. The explanation of this result may, in part, be attributed to the participant's high adaptation phase. Some studies have demonstrated that caffeine had no effect on muscle damage biomarkers. For instance, AbuMoh'd et al. (2021) demonstrated that 6 mg/kg of caffeine had no beneficial effects in attenuation of muscle damage biomarkers. However, the protocol in that study consisted of resistance exercises (3 sets × 10 repetitions, with 2 min recovery interval) followed by an incremental treadmill test in long-distance runners. Machado et al. (2008) reported that 4.5 mg/kg of caffeine had no effect in CK, lactic dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) following resistance exercises compared to placebo, but in male soccer players. Soleimani et al. (2018) also found no significant effect in CK and serum high sensitivity C-reactive protein (hs-crp) after caffeine supplementation (2 g per day for 14 days) in overweight students. These prior studies did not measure muscular endurance and the population were not resistance trained athletes. Basically, the elevations of biomarkers following exercise are usually higher in untrained and/or recreationally trained players than competitive athletes.

In agreement with prior studies (Green et al., 2007; Davis et al., 2012; Ferreira et al., 2021; Ruiz-Fernández et al., 2023), caffeine had beneficial effect on muscular endurance. The explanation of this result might be attributed to the biologic role of caffeine in antagonizing adenosine receptors (Duncan et al., 2013; Ferreira et al., 2021), reducing PRE and pain sensation (Owens et al., 2019; Ferreira et al., 2021). In this sense, Duncan et al. (2013) reported lower RPE after ingestion of 5 mg/kg of caffeine compared to placebo. However, they suggested that pain sensation was significantly higher in lower body (deadlift, and back squat) compared to bench press and prone row. In the present study, although the total repetitions in the lower body exercises (leg press, back squat, and leg extension) were more in the caffeine trial compared to the placebo, the total repetitions in the lower muscles were significantly less than the upper muscles in both caffeine and placebo trials, agreeing

with the prior study. In addition, the result of the current study may also be caused by the nature of muscle fibers. In fact, the lower body muscles have a greatest motor unit size than the upper muscles, but the type of lower muscle fibers are, in anatomy nature, slow oxidative, potentiating participants intolerant to repeat more repetitions. Hence, repeated eccentric and concentric contractions until momentary muscle failure per set making the number of repetitions per set and the number of sets per exercise decrease.

Further, the biomarkers in the present study were measured only 1h after the end of each trial. So that a one limitation of the present study was that muscle damage biomarkers were not measured after 24 hours. Thus far, the ergogenic effect of caffeine on muscular endurance remains uncertain. Importantly, the discrepant evidence in the previous studies regarding muscular endurance probably due to the type of contraction (isometric, isotonic, or isokinetic), the amount of dose, the nature of participants (elite or untrained-resistance exercise), habitual or inhabitual caffeine intake, gender, and the number of sets per exercise. Hence, further research is needed to measure these variables at several points.

Conclusion

Administration of 5 mg/kg of caffeine 1 h prior to muscular endurance test performed by multiple resistance exercises enhances significantly hormonal response, namely follicle-stimulating hormone and total testosterone and increases total number of repetitions to failure in 11 well resistance-trained males compared to the placebo. However, the findings do not support the acute caffeine prevent muscle damage biomarkers following muscular endurance. Hence, male strength athletes may consider using this dose pre-training as an effective ergogenic aid before muscle endurance exercise.

Acknowledgments

The author would like to thank the strength athletes for partaking in this study. The author is also grateful to Dr. Roya Debae for consultation in statistical analysis and nutritionist Nidal for preparation of caffeine and placebo capsules.

Declaration of conflicting interests

The Author declares that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

Research ethics and athlete consent

The study was approved by the Al-Ahliyya Amman University Ethical Committee (FES-18G-280-2023). All study procedures and potential side effects of caffeine consumption, such as temporary tachycardia, hypertension, and numbness, were explained, and participants provided written informed consent.

Received: 11 August 2023 | **Accepted:** 22 September 2023 | **Published:** 01 October 2023

References

Abumoh'd, M. F., Shamrokh, N., Bataineh, A. S., & Al-Horani, R. (2021). Effects of acute caffeine on muscle damage biomarkers and time to exhaustion after a single session of resistance exercises followed by exhaustive incremental test in long-distance runners. *Journal of Human Sport and Exercise*, *16*(2), 361-372. https://doi.org/10.14198/jhse.2021.162.11

Astorino, T. A., Rohmann, R. L., & Firth, K. (2008). Effect of caffeine ingestion on one-repetition maximum muscular strength. *European Journal of Applied Physiology, 102,* 127-132. https://doi.org/10.1007/s00421-007-0557-x

Cadegiani, F. A., & Kater, C. E. (2019). Enhancement of hypothalamic-pituitary activity in male athletes: evidence of a novel hormonal mechanism of physical conditioning. *BMC Endocrine Disorders*, *19*(1), 117, 1-11. https://doi.org/10.1186/s12902-019-0443-7

Davis, J.-K., Green, J. M., & Laurent, C. M. (2012). Effects of Caffeine on Resistance Training Performance on Repetitions to Failure. *Journal of Caffeine Research*, *2*(1), 31–37. https://doi.org/10.1089/jcr.2012.0005

Duncan, M. J., Stanley, M., Parkhouse, N., Cook, K., & Smith, M. (2013). Acute caffeine ingestion enhances strength performance and reduces perceived exertion and muscle pain perception during resistance exercise. *European Journal of Sport Science*, *13*(4), 392–399. https://doi.org/10.1080/17461391.2011.635811

Ferreira, T. T., da Silva, J. V. F., & Bueno, N. B. (2021). Effects of caffeine supplementation on muscle endurance, maximum strength, and perceived exertion in adults submitted to strength training: a systematic review and meta-analyses. *Critical Reviews in Food Science and Nutrition*, *61*(15), 2587–2600. https://doi.org/10.1080/10408398.2020.1781051

Fragala, M. S., Kraemer, W. J., Denegar, C. R., Maresh, C. M., Mastro, A. M., & Volek, J. S. (2011). Neuroendocrine-Immune Interactions and Responses to Exercise. *Sports Medicine*, *41*(8), 621–639. https://doi.org/10.2165/11590430-000000000-00000

Glover, F. E., Caudle, W. M., Del Giudice, F., Belladelli, F., Mulloy, E., Lawal, E., & Eisenberg, M. L. (2022). The association between caffeine intake and testosterone: NHANES 2013–2014. *Nutrition Journal*, *21*(1), 33. https://doi.org/10.1186/s12937-022-00783-z

Graham T. E. (2001). Caffeine and exercise: metabolism, endurance and performance. *Sports Medicine (Auckland, N.Z.)*, *31*(11), 785–807. https://doi.org/10.2165/00007256-200131110-00002

Green, J., Wickwire, P., McLester, J., Gendle, S., Hudson, G., Pritchett, R., & Laurent, M. (2007). Effects of Caffeine on Repetitions to Failure and Ratings of Perceived Exertion During Resistance Training. *International Journal of Sports Physiology and Performance*, *2*, 250–259. https://doi.org/10.1123/ijspp.2.3.250

Grgic, J., & Mikulic, P. (2021). Acute effects of caffeine supplementation on resistance exercise, jumping, and Wingate performance: no influence of habitual caffeine intake. *European Journal of Sport Science*, *21*(8), 1165–1175. https://doi.org/10.1080/17461391.2020.1817155

Heavens, K. R., Szivak, T. K., Hooper, D. R., Dunn-Lewis, C., Comstock, B. A., Flanagan, S. D., Looney, D. P., Kupchak, B. R., Maresh, C. M., Volek, J. S., & Kraemer, W. J. (2014). The Effects of High Intensity Short Rest Resistance Exercise on Muscle Damage Markers in Men and Women. *The Journal of Strength & Conditioning Research*, *28*(4). https://journals.lww.com/nsca-jscr/fulltext/2014/04000/the_effects_of_high_intensity_short_rest.23.aspx

Hurley, C. F., Hatfield, D. L., & Riebe, D. A. (2013). The Effect of Caffeine Ingestion on Delayed Onset Muscle Soreness. *The Journal of Strength & Conditioning Research*, *27*(11). https://journals.lww.com/nsca-jscr/fulltext/2013/11000/the_effect_of_caffeine_ingestion_on_delayed_onset.24.aspx

Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal Responses and Adaptations to Resistance Exercise and Training. *Sports Medicine*, *35*(4), 339–361. https://doi.org/10.2165/00007256-200535040-00004

Lee, S., Miselis, R., & Rivier, C. (2002). Anatomical and Functional Evidence for a Neural Hypothalamic-Testicular Pathway that Is Independent of the Pituitary. *Endocrinology*, *143*(11), 4447–4454. https://doi.org/10.1210/en.2002-220392

Machado, M., Vinícios, P., Zovico, C., Paulucio, D., Pereira, L., Barreto, J., & Pereira, R. (2008). Caffeine Does Not Increase Resistance Exercise-Induced Microdamage. *Journal of Exercise Science and Fitness*, 6(2), 115-120.

Machado, M., Zini, E. N., Valadão, S. D., Amorim, M. Z., Barroso, T. Z., & de Oliveira, W. (2012). Relationship of glomerular filtration rate and serum CK activity after resistance exercise in women. *International Urology and Nephrology*, *44*(2), 515–521. https://doi.org/10.1007/s11255-011-9963-4

McCaulley, G. O., McBride, J. M., Cormie, P., Hudson, M. B., Nuzzo, J. L., Quindry, J. C., & Travis Triplett, N. (2009). Acute hormonal and neuromuscular responses to hypertrophy, strength and power type resistance exercise. *European Journal of Applied Physiology*, *105*(5), 695–704. https://doi.org/10.1007/s00421-008-0951-z

Owens, D. J., Twist, C., Cobley, J. N., Howatson, G., & Close, G. L. (2019). Exercise-induced muscle damage: What is it, what causes it and what are the nutritional solutions? *European Journal of Sport Science*, *19*(1), 71–85. https://doi.org/10.1080/17461391.2018.1505957

Polito, M. D., Grandolfi, K., & de Souza, D. B. (2019). Caffeine and resistance exercise: the effects of two caffeine doses and the influence of individual perception of caffeine. *European Journal of Sport Science*, *19*(10), 1342–1348. https://doi.org/10.1080/17461391.2019.1596166

Raastad, T., Bjøro, T., & Hallén, J. (2000). Hormonal responses to high- and moderate-intensity strength exercise. *European Journal of Applied Physiology*, *82*(1), 121–128. https://doi.org/10.1007/s004210050661

Ruiz-Fernández, I., Valadés, D., Dominguez, R., Ferragut, C., & Pérez-López,

A. (2023). Load and muscle group size influence the ergogenic effect of acute caffeine intake in muscular strength, power and endurance. *European Journal of Nutrition*, *62*(4), 1783–1794. https://doi.org/10.1007/s00394-023-03109-9

Smilios, I., Tsoukos, P., Zafeiridis, A., Spassis, A., & Tokmakidis, S. P. (2013). Hormonal responses after resistance exercise performed with maximum and submaximum movement velocities. *Applied Physiology, Nutrition, and Metabolism*, *39*(3), 351–357. https://doi.org/10.1139/apnm-2013-0147

Soleimani, A., Shakerian, S., & Ranjbar, R. (2018). The effect of caffeine supplementation on serum high-sensitivity C-reactive protein and creatine kinase after exhausted aerobic exercise in active overweight university students. *Journal of Birjand University of Medical Sciences*, 24(2), 84-93. https://api.semanticscholar.org/CorpusID:207821214

Spiering, B. A., Kraemer, W. J., Vingren, J. L., Ratamess, N. A., Anderson, J. M., Armstrong, L. E., … & Maresh, C. M. (2009). Elevated endogenous testosterone concentrations potentiate muscle androgen receptor responses to resistance exercise. *The Journal of Steroid Biochemistry and Molecular Biology*, *114*(3), 195–199. https://doi.org/https://doi.org/10.1016/j.jsbmb.2009.02.005

Stokes, K. A., Gilbert, K. L., Hall, G. M., Andrews, R. C., & Thompson, D. (2013). Different responses of selected hormones to three types of exercise in young men. *European Journal of Applied Physiology*, *113*(3), 775–783. https://doi.org/10.1007/s00421-012-2487-5

Vieira, J. M., Carvalho, F. B., Gutierres, J. M., Soares, M. S. P., Oliveira, P. S., Rubin, M. A., … & Spanevello, R. M. (2017). Caffeine prevents high-intensity exercise-induced increase in enzymatic antioxidant and Na+-K+-ATPase activities and reduction of anxiolytic like-behaviour in rats. *Redox Report*, *22*(6), 493–500. https://doi.org/10.1080/13510002.2017.1322739

Willoughby, D. S., Spillane, M., & Schwarz, N. (2014). Heavy Resistance Training and Supplementation With the Alleged Testosterone Booster Nmda has No Effect on Body Composition, Muscle Performance, and Serum Hormones Associated With the Hypothalamo-Pituitary-Gonadal Axis in Resistance-Trained Males. *Journal of Sports Science & Medicine, 13*(1), 192–199. PMCID: PMC3918557

Wu, B. H., & Lin, J. C. (2010). Caffeine attenuates acute growth hormone response to a single bout of resistance exercise. *Journal of Sports Science & Medicine, 9*(2), 262–269. PMCID: PMC3761732