Original Article

# Effects of acute caffeine intake on sex hormones response and repetitions to failure in resistance-trained females during early follicular phase

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## ABSTRACT

This study investigated the acute effects of caffeine intake on sex hormones (follicle-stimulating hormone, luteinizing hormone, prolactin, oestradiol, and progesterone) following resistance exercise performance during the early follicular phase. In addition, the total number of failed repetitions was determined. Ten resistance-trained females performed two consecutive trials (48 h apart). Participants were randomly assigned to receive either caffeine (4 mg/kg) 1 h before exercise or a placebo, using a double-blind crossover design. Each trial included the following resistance exercises: chest press, lat pulldown, triceps pushdown, and rowing torso. Each exercise was performed in three sets of 10 repetitions at 60% of 1-RM, with a 90 s recovery interval, followed by repetitions to failure at the same intensity during the fourth set. Two-minute breaks were allocated between each exercise's third and fourth sets of and between exercises. Blood samples were collected from each participant 1 h after the completion of each trial. Data revealed no statistical difference ($p > .05$) in ergogenic response to caffeine on sex hormones during the early follicular phase between trials. However, serum prolactin level significantly decreased ($p = .039$) after caffeine intake compared to the placebo. The overall repetitions to failure were significantly higher ($p = .023$) in the caffeine trial than in the placebo. In conclusion, caffeine intake (4 mg/kg) 1 h before multiple resistance exercises had no effect on sex hormone responses during the early follicular phase, except for prolactin. However, the overall repetitions until failure were higher following caffeine intake.

**Keywords**: Sport medicine, Health, Muscular endurance, Menstruation, Pituitary, Hypothalamus, Oral contraceptive pills.

**Cite this article as:**
Abumoh'd, M. F. (2024). Effects of acute caffeine intake on sex hormones response and repetitions to failure in resistance-trained females during early follicular phase. *Journal of Human Sport and Exercise, 19*(1), 183-192. https://doi.org/10.14198/jhse.2024.191.16

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Submitted for publication September 07, 2023.
Accepted for publication October 12, 2023.
Published January 01, 2024 *(in press* November 15, 2023).
JOURNAL OF HUMAN SPORT & EXERCISE ISSN 1988-5202.

**doi:10.14198/jhse.2024.191.16**

## INTRODUCTION

The female reproductive cycle represents events that synchronously occur in the ovaries and uterus, and typically ranges from 24 to 35 days (Tortora & Derrickson, 2017). The early follicular phase, a uterine menstruation event, is the first phase of the ovarian cycle, lasting approximately 5 days (Lara et al., 2020; Norum et al., 2020), and it is also defined as the new cycle. On its first day, this phase triggers a negative feedback system, prompting the arcuate nucleus in the hypothalamus to release gonadotropin-releasing hormone towards its target, the anterior pituitary gland (Tortora & Derrickson, 2017). Follicle-stimulating hormone (FSH) and luteinizing hormone (LH) are secreted and reach the ovaries through the bloodstream (Lara et al., 2020) to develop primary, secondary, and mature (Graafian) follicles (Tortora & Derrickson, 2017), which undergo ovulation. These events make the hypothalamic-pituitary-ovarian axis pivotal in eumenorrhea. Alterations in this axis can induce changes in essential female sex hormones, including oestradiol and progesterone (Seyed Saadat et al., 2016). Fluctuations in female sex hormone levels throughout the menstrual cycle can affect neuromuscular function (Ansdell et al., 2019).

During the early follicular phase, females lose approximately 50–150 ml of blood containing tissue fluids, mucus, and epithelial cells (Tortora & Derrickson, 2017) shed from the endometrium. Subsequently, oestradiol and progesterone concentrations decrease, whereas prostaglandin levels rise (Seyed Saadat et al., 2016). Female athletes experience fatigue, wide mood swings, and energy deficits during training (Klentrou & Plyley, 2003; Romero-Moraleda et al., 2019). Resistance exercises require high energy levels to cope with mechanical stress (Heavens et al., 2014). In this context, nutritional supplements, including caffeine, have become inseparable from resistance exercise.

Caffeine, a natural xanthine alkaloid, is the most frequently consumed psychostimulant substance in general and athletic populations (Del Coso et al., 2011; Lara et al., 2020; Norum et al., 2020). It can be obtained from coffee, cacao, tea, energy drinks, chocolate, supplements, and other medicines. Caffeine readily traverses the blood-brain barrier (BBB) (Lara et al., 2020) and inhibits adenosine $A_1$ and $A_{2A}$ receptors in several brain regions (Graham, 2001; Lara et al., 2020; Norum et al., 2020). It can also augment free fatty acid ($FFA_s$) mobilization and cell utilization (Tarnopolsky, 2010), leading to high-energy expenditure during exercise. Furthermore, caffeine enhances performance during resistance exercise (Grgic & Mikulic, 2021; Ruiz-Fernández et al., 2023) by reducing pain sensation (Tinsley et al., 2017). However, the effects of caffeine on performance during any phase of the menstrual cycle remain unclear (Romera-Meraleda et al., 2020; Wilk et al., 2019).

However, few studies have investigated the ergogenic benefits of caffeine during resistance exercise. Most have exclusively involved men (Bowtell et al., 2018; Davis et al., 2012; Grgic & Mikulic, 2017; Polito et al., 2019; Raastad et al., 2000; Smilios et al., 2014; Wilk et al., 2019), and only a limited number have included both men and women (Duncan et al., 2013; Green et al., 2007; Ruiz-Fernández et al., 2023). Interestingly, the statistical data demonstrated an ergogenic effect of caffeine in men. In fact, only 16.3% of the total sample participating in prior studies on the effect of caffeine on resistance exercise were female (Salinero et al., 2019). To the best of my knowledge, only four studies (Filip-Stachnik et al., 2021; Goldstein et al., 2010; Norum et al., 2020; Romero-Moraleda et al., 2019) have investigated caffeine's ergogenic effects on resistance exercise in females. However, only two of these studies examined caffeine's effect on resistance exercise during the early follicular phase. The first study demonstrated a significant increase in movement velocity after caffeine ingestion during all three menstrual cycle phases (Romero-Moraleda et al., 2019). The results of the second study revealed that caffeine ingestion at 4 mg/kg improved the maximal strength and muscular endurance in resistance-trained and caffeine-habituated females during the early follicular phase

 

(Norum et al., 2020). Since there is no available data regarding the effect of caffeine on resistance, especially repetitions to failure, in females, and no study has investigated the ergogenic effect of caffeine on hormonal responses during the early follicular phase, its practical applicability requires further research.

Consequently, this study aimed to investigate the effects of caffeine intake on sex hormone responses after resistance exercise in resistance-trained females during the early follicular phase. In addition, the total repetitions to failure were determined after caffeine and placebo intake. This study hypothesized that caffeine improves performance during the early follicular phase, possibly because of its antagonistic effect on adenosine receptors.

## METHODS

### *Participants*

Ten healthy resistance-trained females (age: 24.53 ± 4.11 years, height:164.22 ± 4.34 cm, body mass: 58.42 ± 3.63 kg, BMI: 21.98 ± 2.44 kg/m$^2$, training experience: 4.33 ± 3.34 years) volunteered for the study. All participants were familiar with the resistance exercises. Multiple inclusionary criteria were considered as follows: a) regular strength exercise for at least 2 years before participation; b) low to moderate caffeine intake (approximately ≤ 200 mg per day); c) normal menstrual duration cycle (24–35 days); and d) absence of medical problem, such as headache, and other factors affecting performance. Volunteers were excluded if they reported a) musculoskeletal injury (3 months before the study); b) menstrual disorder or discomfort (i.e., amenorrhea, mittelschmerz); c) oral contraceptive pill use, or d) ergogenic ingestion (3 days before the study). 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. This study was approved by the Ethics Committee of Al-Ahliyya Amman University (FES-18G-358-2023) and was conducted according to the latest version of the Declaration of Helsinki.

### *Experimental design*

A randomized, double-blind, placebo-controlled crossover design was used to determine hormonal responses and repetitions to failure during the early follicular phase in 10 resistance-trained females after caffeine and placebo intake. All participants refrained from strenuous exercise for 48 h before each trial and consumed three main meals during the days between trials. Additionally, they were also instructed not to consume breakfast or morning coffee on the days of the trials. Participants were asked to drink 500 ml of water during each trial to avoid the sensation of thirst. The study trials were conducted at 22–24 °C and a relative humidity of 37–42%. The entire procedure in each trial took roughly 107 minutes.

### *Experimental protocol*

Participants attended four laboratory visits. The first visit involved defining the procedure, collecting demographic data, and determining the one-repetition maximum. The second visit focused on baseline measurements (Table 1) and two main testing trials using the same procedure. To ensure both caffeine and placebo trials were conducted within the early follicular phase, these trials were separated 48 h apart, with participants' observations guiding the phase determination. Each trial consisted of resistance exercises following a warm-up, including chest press, lat pulldown, triceps pushdown, and rowing torso. The warm-up consisted of 5 minutes on a treadmill (TechnoGym, Smart Code Program, A01, Italy) at 7 km/h and 10 repetitions of each exercise at 40% 1-RM, with 60 s recovery interval. After a 5-minute, participants performed three sets of 10 repetitions in each exercise at 60% of 1-RM, with a 90-s rest interval between sets. The number of repetitions was based on previous research indicating that a repetition range (8–12) helps prevent whole-body fatigue (Davis et al., 2012). After completing the third set of each exercise, participants performed

as many repetitions as possible for chest press, lat pulldown, triceps pushdown, and rowing torso at the same intensity to examine repetitions to failure. Two minutes of recovery were allocated between each exercise's third and fourth sets and between exercises. During the test, each repetition was performed at maximal velocity, and the examiner observed and counted the number of repetitions in each exercise during both trials. Failure was defined as an inability to tolerate full repetition. All exercises were performed according to the American College of Sports Medicine guidelines (ACSM, 2010). Blood samples were collected from each participant to analyse sex hormone responses.

Table 1. Baseline measurements (Mean ± SD).

| | **Values** |
|---|---|
| FSH (I/L) | 7.92 ± 2.15 |
| LH (IU/L) | 6.16 ± 1.76 |
| PRL (ng/ml) | 18.23 ± 4.21 |
| Oestradiol (pg/ml) | 78.66 ± 3.57 |
| Progesterone (nmol/L) | 2.22 ± 3.44 |

*Note. FSH, Follicle-stimulating hormone; LH: Luteinizing hormone; PRL: Prolactin.*

### *One repetition maximum*

Five days before the commencement of the trials, each participant underwent a 1-RM test for each exercise. First, participants performed their routine 5-minute warm-up on the treadmill followed by 10 repetitions of the selected exercises using 30% loads of their estimated 1-RM, with a 60-s recovery interval. After a 5-minute passive recovery, the 1-RM test commenced, following the method described by Filip-Stachnik et al. (2021). The 1-RM constituted the maximum weight lifted once, encompassing concentric and eccentric phases, with a 5-minute passive recovery allowed between successful attempts. A pilot test indicated no significant difference in the 1-RM values for chest press ($t = 2.01$, $p = .51$), lat pulldown ($t = 1.32$, $p = .72$), triceps pushdown ($t = -0.11$, $p = .66$), or rowing torso ($t = -0.55$, $p = .71$).

### *Intervention intake*

Two trials were performed under two consecutive conditions: one involving caffeine and the other a caffeine (placebo). In the experimental trial, participants received 4 mg/kg of caffeine in capsule form (Florida Supplement Caffeine Capsule, Nutrix Research, USA) and swallowed it with 300 ml of water (18 °C) 1 h before the trial began. In the placebo trial, participants ingested empty capsules of similar shape using the same procedure. The capsules were coded and provided by an independent nutritionist, and neither the participants nor the assistants were aware of their content.

### *Blood collections*

Blood samples (5 ml) were drawn from the left antecubital vein of each participant 1 h after completing each trial. Serum samples were used for all parameter analyses and were centrifuged at 3500 rpm for 10 minutes. FSH, LH, and PRL levels were analysed using (Cobas6000-REI, Switzerland). Oestradiol and progesterone levels were analysed via immunochromatography (lateral flow assay, Japan). The reference ranges of parameters during the early follicular phase were as follows: 3.5–12.5 IU/L for FSH, 2.4–12.6 IU/L for LH, 4.79–23.3 ng/ml for PRL, 19.0–140 pg/ml for oestradiol, and 0.181–2.84 nmol/L for progesterone.

### *Statistical analysis*

The normal distribution of the data was verified using the Shapiro-Wilk test, and all analysed variables (female sex hormones) exhibited normal distribution ($p > .05$). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) software (version 18.0). Between-group differences in sex hormone

 

responses and total repetitions were determined using paired-sample t-tests. One-way ANOVA with repeated measures and Bonferroni post-hoc analysis were conducted to investigate significant differences both between and within groups in repetitions to failure. Cohen's effect size (d) was calculated for all statistically significant pairwise comparisons, with the magnitude of effect size interpreted as follows: ≤0.2 (trivial), >0.2–0.6 (small), >0.6–1.2 (moderate), >1.2–2.0 (large), >2.0 (very large) (Lara et al., 2019). Descriptive statistics are presented as Mean ± standard deviation. Statistical significance was set at $p < .05$.

## RESULTS

Regarding sex hormone responses, the results indicated no significant differences in FSH (t = 0.573, $p$ = .412, Effect size [d] = 0.21), LH (t = -1.641, $p$ = .084, d = 0.19), oestradiol (t = 1.382, $p$ = .441, d = 0.20), and progesterone (t = -0.421, $p$ = .204, d = 0.19) between the two trials. However, caffeine had a positive effect on PRL (t = 2.307, $p$ = .039, d = 0.71) compared to the placebo (Table 2).

Table 2. Ergogenic response to caffeine on sex hormones. Data were analysed using a Paired sample *t*-test.

| | Caffeine | Placebo |
|---|---|---|
| FSH (IU/L) | 8.01 ± 1.08 | 7.97 ± 1.26 |
| LH (IU/L) | 6.23 ± 1.77 | 6.39 ± 2.02 |
| PRL (ng/ml) | 16.81 ± 2.94 * | 18.53 ± 3.83 |
| Oestradiol (pg/ml) | 81.87 ± 3.42 | 80.94 ± 4.36 |
| Progesterone (nmol/L) | 2.36 ± 4.22 | 2.44 ± 3.89 |

*Note. The significance level was set at p < .05. Values expressed as Mean ± SD. FSH, follicle-stimulating hormone; LH: Luteinizing hormone; PRL: Prolactin. * Significant difference compared to placebo.*

Table 3 presents the number of repetitions to failure performed following three sets of 10 repetitions for each exercise in both trials. There were no statistically significant differences ($p > .05$) in the repetitions to failure of each exercise between caffeine and placebo groups. In the within-group analysis, there were no differences ($p > .05$) between exercises in the placebo group. However, the Bonferroni post hoc test indicated a significant difference (F = 2.64, $p$ = .026) only between the chest press and lat pulldown after caffeine intake. Furthermore, using a Paired sample t-test, the data revealed that the overall repetitions to failure were significantly (t = 3.22, $p$ = .023) greater with caffeine intake than with the placebo.

Table 3. Ergogenic response to caffeine on repetitions to failure performed by four exercises. Data were analysed using one-way ANOVA for the sum of repetitions between- and within-group and paired sample *t*-test for total repetitions.

| | Chest press | Lat pulldown | Triceps pushdown | Rowing torso | Total repetitions |
|---|---|---|---|---|---|
| Caffeine | 23.12 ± 2.29 [a] | 18.33 ± 3.28 | 22.77 ± 1.77 | 20.63 ± 2.07 | 84.85 ± 5.66 * |
| Placebo | 21.45 ± 3.11 | 18.83 ± 4.31 | 20.21 ± 1.89 | 18.84 ± 3.22 | 79.33 ± 6.43 |

*Note. The significance level was set at p < .05. Values expressed as Mean ± SD. [a]: Significant difference compared to late pulldown; *: Significant difference compared to placebo.*

## DISCUSSION AND CONCLUSIONS

This study investigated the effect of an acute ergogenic response to caffeine on sex hormone responses during chest press, lat pulldown, triceps pushdown, and rowing torso performance in resistance-trained females during the early follicular phase. Repetitions of failure were counted only during the fourth set in each exercise compared to the placebo. The main findings of this investigation were as follows: a) serum PRL

level was significantly lower in the caffeine trial than in the placebo trial, and b) total repetitions to failure were greater after caffeine consumption compared to the placebo.

Regarding sex hormone responses, the results indicated no ergogenic effect of caffeine on any of the study parameters, except PRL, after completing the study procedure during the early follicular phase. This may be attributed to the delayed negative feedback system activity during this phase. Generally, during the follicular phase, the concentrations of the main sex hormones, including oestradiol and progesterone, are lower than those in the other phases of the menstrual cycle (Dasa et al., 2021; Romero-Moraleda et al., 2019). Although a few studies have investigated the ergogenic effects of caffeine during resistance exercise in women, none have examined the response of sex hormones to caffeine during resistance exercise. This research gap results in unclear explanations and speculation. Further research is required in this area.

The scarcity of prior studies on caffeine in resistance exercise for women is attributed to the influence of oral contraceptive pills and disordered menstrual cycles on caffeine metabolites, such as paraxanthine and theophylline (Martins et al., 2020; Romero-Moraleda et al., 2019). However, many female athletes do not experience amenorrhea, oligomenorrhea, or symptoms during different menstrual cycle phases or use oral contraceptive pills. Therefore, these athletes may engage in resistance exercises during menstruation. In this context, it has been speculated that progesterone is linked to catabolic hormones (Dasa et al., 2021; Lara et al., 2020; Romero-Moraleda et al., 2019) and has a lower concentration in the early follicular phase. Engaging female athletes during this phase may enhance muscle performance compared to other phases of the menstrual cycle when progesterone levels are already high (Romero-Moraleda et al., 2019). Another proposed explanation for the weak effect of caffeine on women's performance is that one substance included in oral contraceptive pills (ethinylestradiol) can decrease caffeine metabolism (Filip-Stachnik et al., 2021). Furthermore, all hormone values measured in this study were within the normal range at 3-point measures, including baseline measurements, after the caffeine and placebo trials were completed. These values indicate that the participants who volunteered in this study were not affected by a disordered menstrual cycle throughout the study.

Additionally, the results showed no statistical difference in hormones between trials, indicating that caffeine had no ergogenic effect on female sex hormones; this may be attributed to critical ovum development during the early follicular phase, the ingested dose (4 mg/kg), the timing of venipuncture (1 h after the completion of the test), the number of blood collections (only one sample in each trial), and the nature of the resistance exercise (only upper body parts). Surprisingly, the results indicated that serum PRL levels were significantly lower after caffeine intake than after a placebo. This result can be explained by caffeine's role in activating dopaminergic neurons (Wu & Lin, 2010), which attenuates PRL concentration.

For resistance exercises, caffeine significantly increased the overall repetitions to failure compared to the placebo. This result can be explained by caffeine's benefits in dampening the pain sensation (Filip-Stachnik et al., 2021). It has been evidenced that caffeine act as an antagonist of adenosine $A_1$ and $A_{2a}$ receptors (Dodd et al., 2015; Filip-Stachnik et al., 2021; Graham, 2001; Martins et al., 2020), generating energy expenditure and reinforcing arousal system (Filip-Stachnik et al., 2021; Norum et al., 2020) and activating the release of calcium from sarcoplasmic reticulum (Martins et al., 2020), thereby activating excitation-contraction coupling system.

The results of this study disagree with those of Filip-Stachnik et al. (2021), who reported no statistical difference ($p$ = .18) in repetitions to failure completed after 3 mg/kg of caffeine, 6 mg/kg of caffeine or placebo (33.81, 35.29, 33.05 repetitions, respectively) in 21 resistance-trained females. They observed a significant

 

improvement in strength-endurance and 1-RM-strength, indicating that caffeine has a lower effect on muscular endurance in women than men. However, participants in that study completed only a bench press with a single set at 50% 1-RM, which makes it challenging to conclude that caffeine had no ergogenic effect. Goldstein et al. (2010) also reported significantly greater muscle strength with caffeine only in the bench press (52.1 kg), with no statistical difference in repetitions to failure between caffeine and placebo trials (23.0 vs. 23.1 repetitions, respectively) in 15 resistance-trained females. However, three participants felt "*exhibiting intense emotional responses*." Conversely, this study agrees with the results of Norum et al. (2020), who demonstrated that 4 mg/kg of caffeine significantly increased repetitions to failure during squat and bench press compared to placebo (45 vs. 39, $p = .003$; 23 vs. 21, $p = .010$, respectively). They indicated that female athletes could consume 4 mg/kg of caffeine before competition or training for an effective ergogenic benefit. The slight difference between the results of this study and that of Norum et al. (2020) can be attributed to the side effects of caffeine. In their study, three participants complained of "*shaky*," and the participant's high habitual caffeine intake can explain this response. Romero-Moraleda et al. (2019) assessed the effect of 3 mg/kg caffeine on the mean and peak velocity of half-squats at 20%, 40%, 60%, and 80% of 1-RM during three phases of the menstrual cycle (early follicular, late follicular, and mid-luteal phases) in 13 trained athletes with eumenorrhea. They found that caffeine significantly increased the mean velocity by 60% in the early and late follicular phases compared to placebo and indicated that caffeine induced a small ergogenic effect in all menstrual phases. In anaerobic research, ingestion of 3 mg/kg of caffeine, compared to placebo, increased peak and mean cycling power during a 15-s Wingate anaerobic performance test in the early follicular phase (8.6 vs. 8.9 W/kg, $p = .04$), pre-ovulatory phase (8.6 vs. 8.9 W/kg, $p = .04$), and mid-luteal phase (8.6 vs. 8.9 W/kg, $p < .01$) in 13 well-trained eumenorrheic triathletes (Lara et al., 2020).

In conclusion, the results of this study do not support the intake of caffeine (4 mg/kg) 1 h before resistance exercise to change the response of sex hormones during the early follicular phase compared with placebo. However, repetitions to failure were greater after caffeine intake during the early follicular phase in resistance-trained females. Hence, female strength athletes may use this pre-training dose as an effective ergogenic aid.

## SUPPORTING AGENCIES

No funding agencies were reported by the author.

## DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author.

## RESEARCH ETHICS AND ATHLETE CONSENT

The study was approved by the Al-Ahliyya Amman University Ethical Committee (FES-18G-358-2023). 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.

## ACKNOWLEDGMENTS

The author would like to thank the female 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.

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