

Dose Effect of Caffeine on Testosterone and Cortisol Responses to Resistance Exercise

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Introduction: Interest in the use of caffeine as an ergogenic aid has increased since the International Olympic Committee lifted the partial ban on its use. Caffeine has beneficial effects on various aspects of athletic performance, but its effects on training have been neglected. **Purpose:** To investigate the acute effect of caffeine on the exercise-associated increases in testosterone and cortisol in a double-blind crossover study. **Methods:** Twenty-four professional rugby-league players ingested caffeine doses of 0, 200, 400, and 800 mg in random order 1 hr before a resistance-exercise session. Saliva was sampled at the time of caffeine ingestion, at 15-min intervals throughout each session, and 15 and 30 min after the session. Data were log-transformed to estimate percent effects with mixed modeling, and effects were standardized to assess magnitudes. **Results:** Testosterone concentration showed a small increase of 15% (90% confidence limits, $\pm 19\%$) during exercise. Caffeine raised this concentration in a dose-dependent manner by a further small 21% ($\pm 24\%$) at the highest dose. The 800-mg dose also produced a moderate 52% ($\pm 44\%$) increase in cortisol. The effect of caffeine on the testosterone:cortisol ratio was a small decline (14%; $\pm 21\%$). **Conclusion:** Caffeine has some potential to benefit training outcomes via the anabolic effects of the increase in testosterone concentration, but this benefit might be counteracted by the opposing catabolic effects of the increase in cortisol and resultant decline in the testosterone:cortisol ratio.

Keywords: anabolic, athlete, catabolic, performance, testosterone:cortisol ratio, strength training

Interest in the use of caffeine as an ergogenic aid has increased since the International Olympic Committee lifted the partial ban on its use in 2004. The positive effects of caffeine in time-to-exhaustion and endurance tests have been demonstrated in laboratory conditions (Norager, Jensen, Madsen, & Laurberg, 2005; Pasman, van Baak, Jeukendrup, & de Haan, 1995) and in experiments that have simulated

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performance conditions. Caffeine-modulated performance enhancements have been reported in cycling (Wiles, Coleman, Tegerdine, & Swaine, 2006), swimming (MacIntosh & Wright, 1995), and running (Bridge & Jones, 2006). Caffeine has also been shown to have beneficial effects on the physical and skill activities required in an intermittent high-intensity team sport (Stuart, Hopkins, Cook, & Cairns, 2005). Furthermore, data from this 2005 study (unpublished observations) suggested that ingested caffeine might elevate endogenous testosterone levels. Previous rodent experiments also demonstrated that high doses of caffeine increased plasma levels of testosterone (Pollard, 1988).

Testosterone is also known to increase in response to resistance exercise, with a relationship between total work and intensity and the degree of acute testosterone response (Ahtiainen, Pakarinen, Kraemer, & Häkkinen, 2004; Kraemer et al., 1990; Kraemer & Ratamess, 2005). Indeed, testosterone, as the primary anabolic hormone, has been linked to strength and muscle gain. The relationship in humans is based on a number of observations that include males showing muscle growth at puberty when testosterone production increases (Ramos, Frontera, Llopart, & Feliciano, 1998), exogenous application of supraphysiologic doses of testosterone in intact males resulting in greater strength and muscle gains from resistance exercise (Bhasin et al., 1996), and pharmacologic blockade of testosterone-specific receptors suppressing exercise-induced hypertrophy of skeletal muscle (Inoue, Yamasaki, Fushiki, Okada, & Sugimoto, 1994). In healthy adults Staron et al. (1994) linked increases in strength and muscle-fiber transformation in males to elevated serum testosterone levels. Furthermore, Hansen, Kvorning, Kjær, and Sjøgaard (2001) concluded that increases in isometric strength were related to the magnitude of testosterone response to resistance exercise in young men. More recent data have demonstrated that suppression of endogenous testosterone attenuates strength and muscle-mass gains over an 8-week strength-training period (Kvorning, Anderson, Brixen, & Madsen, 2006).

The stress hormone cortisol has also been shown to rise during resistance exercise (Ahtiainen et al., 2004; Smilios, Pilianidis, Karamouzis, & Tokmakidis, 2003) because of the increase in metabolic demand. The catabolic nature of cortisol, however, suggests that an increase in this steroid hormone is not ideal from the perspective of a weight-training athlete whose aim is to increase or maintain muscle mass. Recent work has illustrated the detrimental effects of cortisol on resistance-training outcomes by showing that supplementation that blunted cortisol release resulted in increased muscle-mass gains (Bird, Tarpenning, & Marino, 2006). The ratio of testosterone to cortisol has been used as an index of the relative anabolic or catabolic state (Daly, Rich, & Klein, 1998), and positive changes in this index have been associated with an enhanced environment for muscle growth (Zakas, Mandroukas, Karamouzis, & Panagiotopoulou, 1994).

The effects of caffeine as a training aid have largely been neglected as studies have focused on performance benefits. The effects of caffeine on endogenous testosterone and cortisol responses to resistance exercise are unknown. As a result this placebo-controlled, double-blind, crossover investigation was designed to examine the effect of caffeine ingestion on salivary testosterone and cortisol levels before, during, and after resistance exercise.

Methods

Participants

Twenty-four professional rugby league athletes (age 22.3 ± 3.0 years, mass 94.9 ± 9.8 kg; $M \pm SD$) from a single team were recruited to participate in the study. All participants were fully informed of the nature and possible risks of the study before giving written consent. The protocol was approved by the Auckland University of Technology Ethics Committee. All participants were informed that they could cease their participation in the trial at any time without giving a reason, with no repercussions.

Experimental Protocol

The study was a randomized, double-blind, placebo-controlled, balanced trial. Participants were assessed on four occasions when gelatin capsules containing either lactose (placebo) or caffeine were ingested 1 hr before exercise. Four caffeine doses of 0, 200, 400, and 800 mg were used in the study and represent a range of doses commonly reported in the literature (Bridge & Jones, 2006; Graham & Spriet, 1995). Participants were instructed to refrain from ingesting dietary caffeine such as coffee, tea, chocolate, and caffeine-containing beverages before exercise on the experimental days. A dietary log for the preceding 24 hr was collected to assess caffeine intake, and reminders were given to ensure dietary compliance. Participants also completed a short questionnaire at the conclusion of training to ascertain their perception of the caffeine dose that they had ingested.

Saliva samples were obtained from each participant at the time of caffeine ingestion, before resistance exercise, every 15 min during exercise, and 15 and 30 min postexercise. For each sample, participants were required to expectorate 2 ml of saliva into a sterile container (Labserve, Auckland, NZ). Saliva samples were stored at -20°C until assay. Salivary steroid samples were taken in this study because they are minimally invasive and have the advantage of reflecting free (or bioavailable) steroid concentrations, which are reported to be more physiologically relevant than total blood levels (Obminski & Stupnicki, 1997; Vining, McGinley, & Symons, 1983). To prevent blood contamination of saliva, resulting in an overestimation of hormone concentrations, participants were advised to avoid brushing their teeth and drinking hot fluids in the 2 hr before assessment. Individual participants performed each of the four sessions at the same time of day to control for the effects of circadian rhythm on hormone concentrations.

Saliva Analysis

Saliva samples were analyzed in triplicate for testosterone and cortisol using radioimmunoassay. The methods were modified from those described by Granger, Schwartz, Booth, and Arentz (1999). Briefly, standards from serum diagnostic kits (Diagnostic Systems Laboratories, USA) were diluted in phosphate-buffered saline (Sigma P4417) to cover the ranges of 0–18.56 and 0–1.73 nmol/L for cortisol and testosterone, respectively. Saliva sample sizes of 50 and 100 μl were used for cortisol

and testosterone, respectively. The antibodies were diluted in a phosphate-buffered saline solution containing 0.05% bovine serum albumin. Kit standards were diluted so that approximately 50% binding was achieved compared with the total counts (10,000 and 4,500 counts per minute for cortisol and testosterone, respectively). Detection limits for the assays were 0.013 and 0.002 nmol/L for cortisol and testosterone, respectively. The intra- and interassay coefficients of variation were <9% for cortisol and <10% for testosterone.

Exercise Protocols

All exercises were performed at the stadium gymnasium where the participants were accustomed to training. Participants completed a 5-min warm-up consisting of footwork ladder and cone-based agility drills. A sport-specific skill component was included (catch and pass) in these drills. All players completed four primary lifts comprising the following movements: squat, dead lift, lunge (or variations of it), and some isolated hamstring strengthening (knee flexion). The core lifts were coupled in supersets or complex sets with stability and speed patterns. Speed consisted of 10-m sprints, and players were given quantitative feedback on their sprint performance via timing gates. The primary lifting component of the workout averaged approximately 45 min in duration. All players then completed a circuit of approximately 10 min of core-strengthening exercises (abdominal plus trunk and hip extensors) at the completion of the strength-training session.

Statistical Analysis

Testosterone and cortisol concentrations and their ratio were analyzed with the mixed-model procedure (Proc Mixed) in the Statistical Analysis System (Version 9.1, SAS Institute, Cary, NC). The fixed effects in the model were clock time in hours (to adjust for any linear diurnal change) and the interaction of caffeine dose (four levels: 0, 200, 400, and 800 mg) and exercise time (eight levels: -60, 0, 15, 30, 45, 60, 75, and 90 min). The mean effects of exercise and caffeine were estimated by appropriate combinations of the levels of this interaction. The random effects in the model were the residual (to estimate error of measurement within a session), athlete identity (representing mean differences between participants), the interaction of athlete identity with exercise session (representing within-participant variation between sessions), and the interaction of athlete identity with dummy numeric variables to estimate within-participant variation (individual responses) associated with exercise and with caffeine. The dummy variable for caffeine had values of 0, 1, 2, and 3 to represent the four doses of caffeine, and the resulting individual responses were therefore estimated as if they increased linearly in magnitude with each step increase in caffeine dose.

The dependent variables were log-transformed before analysis; plots of residuals versus predicted values showed that this transformation produced acceptable uniformity of error, and no observations were excluded as outliers. Back-transformation provided estimates of mean effects as percentages and estimates of individual responses and errors as coefficients of variation. For qualitative assessment of magnitude, the log-transformed effects were standardized by dividing by the between-participants *SD* at rest in the no-caffeine condition. The *SD* was the square

root of the sum of the variances for athlete identity; athlete identity interacted with session identity and the residual. Small-sample bias in the standardized effects was adjusted by multiplying by a factor of $(1 - 3)/(4v - 1)$, where v was the degrees of freedom of the SD ($=15$). Magnitudes of the standardized effects were interpreted using thresholds of 0.2, 0.6, and 1.2 for small, moderate, and large, respectively, a modification of Cohen's thresholds of 0.2, 0.5, and 0.8 (Cohen, 1988); the modifications are based primarily on congruence with Cohen's thresholds for correlation coefficients (see <http://newstats.org/effectmag.html>).

The effect of caffeine on sprint performance was analyzed with a mixed model. The dependent variable was log-transformed mean sprint time. The fixed effect was either the actual dose or the guessed dose, with a value 0, 1, 2, or 3. The random effects were the residual, athlete identity, and the interaction of identity with the actual or guessed dose (to allow for individual responses to dose). For qualitative assessment of magnitude, the threshold for a substantial change in sprint time was assumed to be 0.8% (Paton, Hopkins, & Vollebregt, 2001).

In keeping with recent trends in inferential statistics (e.g., Sterne & Smith, 2001), we made magnitude-based inferences about true (population) values of effects by expressing the uncertainty in the effects as 90% confidence limits. For brevity, confidence limits are shown as $\pm x$, where x represents half the confidence interval. An effect was deemed unclear if its confidence interval overlapped the thresholds for substantiveness (that is, if the chances of the effect's being substantially positive and negative were both $>5\%$); otherwise the magnitude of the effect was reported as the magnitude of its observed value (Batterham & Hopkins, 2006).

Results

All the participants completed the trial. The mean salivary testosterone concentration measured before each session was 0.29 nmol/L and ranged between 0.07 and 0.97 nmol/L (21.6 and 281 pg/ml). Salivary cortisol ranged between 0.07 and 1.99 nmol/L (0.2 and 5.5 ng/ml) with a mean of 0.57 nmol/L. Only 18% of participants were able to correctly identify the caffeine dose they ingested on the questionnaire they completed after exercise. Furthermore, only 10% of participants correctly guessed the occasion on which they were prescribed the 800-mg caffeine dose. Caffeine was associated with a small improvement in sprint performance, with the 800-mg dose decreasing sprint times (1.7%; 90% confidence limits $\pm 2.5\%$).

A small diurnal decline in testosterone (11%/hr; $\pm 7\%/hr$) occurred over the experimental period. After adjustment for this effect, testosterone concentration showed a small exercise-induced increase (15%; $\pm 19\%$). The effect of caffeine dose on testosterone during exercise is presented in Figure 1. Caffeine doses of ≥ 400 mg tended to cause a small decrease in testosterone after ingestion, followed by a rapid increase after the commencement of resistance exercise. The 800-mg caffeine dose produced a 61% ($\pm 33\%$) increase in testosterone after 60 min of resistance exercise. The 800-mg caffeine dose produced a 51% ($\pm 41\%$) increase in cortisol over the exercise period (Figure 1) that continued to increase to 93% ($\pm 62\%$) above baseline during the recovery period monitored. As a result of the elevated cortisol seen in the postexercise period, the percentage change in the

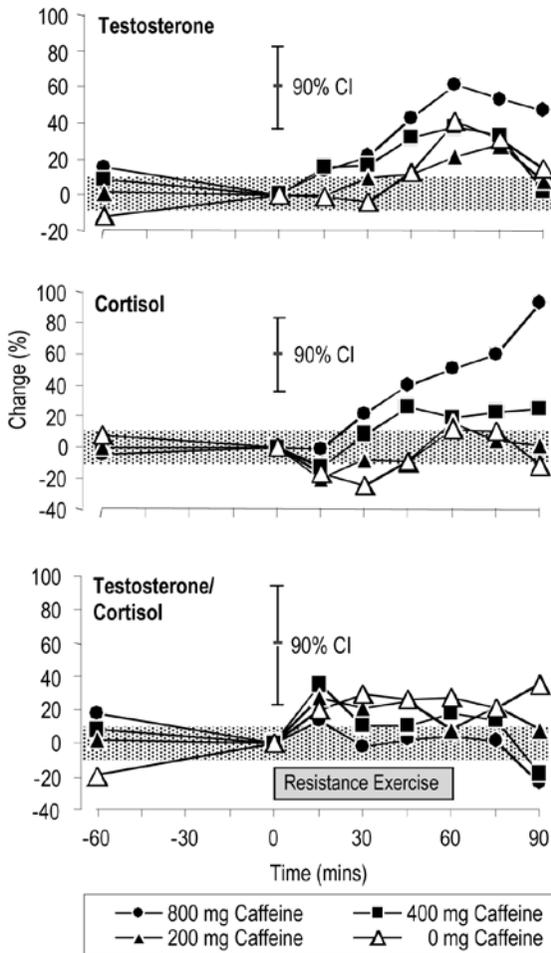


Figure 1 — Temporal profile of hormonal response to four caffeine doses. A Cohen’s effect size of less than 0.2 is represented by the shaded area, indicating a trivial percentage change. The error bar represents an approximately 90% confidence interval (CI) for each point other than the zero time point.

testosterone:cortisol ratio at caffeine doses of ≥ 400 mg was negative compared with the placebo condition (Figure 1).

When hormonal responses were averaged over the exercise and recovery period, caffeine doses of ≥ 400 mg raised testosterone concentration in a dose-dependent manner and by 39% ($\pm 24\%$) at the highest dose (Figure 2). Of this 39% response, 21% ($\pm 24\%$) can be attributed to the caffeine dose. The 800-mg dose of caffeine also caused a moderate 52% ($\pm 44\%$) increase in cortisol during exercise and recovery when compared with exercise alone (Figure 2).

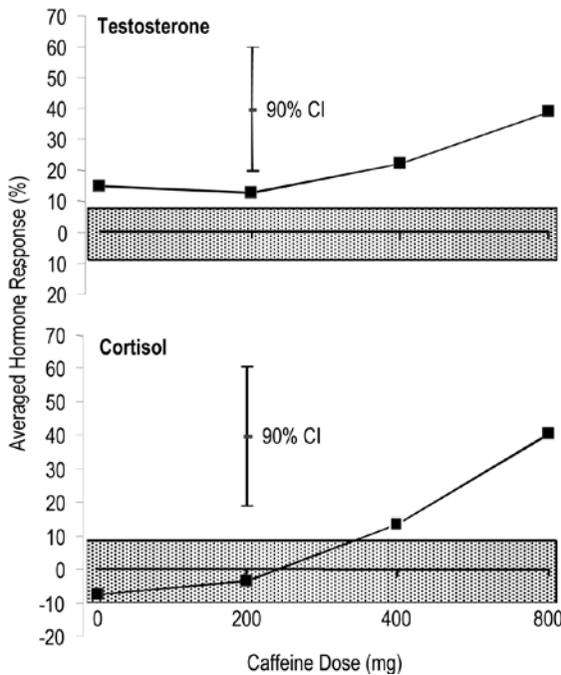


Figure 2 — Hormonal response averaged over the exercise and recovery period. A Cohen's effect size of less than 0.2 is represented by the shaded area, indicating a trivial change. The error bar represents an approximately 90% confidence interval (CI) for each point.

Discussion

The novel finding of this study was that caffeine increased the exercise-induced testosterone response to resistance exercise in a dose-dependent manner. Caffeine ingestion also increased the cortisol response to resistance exercise, and, at the highest caffeine dose, cortisol continued to increase postexercise. Epidemiological studies have reported a positive association with caffeine intake and elevated bioavailable testosterone levels in adult men (e.g., Svartberg et al., 2003). Despite the widespread use of caffeinated products, we are unaware of any studies on the effect of caffeine on testosterone concentrations during and after resistance exercise. The anxiogenic properties of caffeine have been reported previously, and caffeine is reported to augment the increase in cortisol concentration in response to mental stress and exercise (al'Absi et al., 1998; Lovallo, Farag, Vincent, Thomas, & Wilson, 2006).

There is growing evidence of the performance gains that can be achieved by athletes' using caffeine for both endurance and sprint performance (Bridge & Jones, 2006; MacIntosh & Wright, 1995; Stuart et al., 2005). A large number of reported physiological benefits of caffeine *in vivo* are now thought to be a result of the

pharmacological antagonistic actions of caffeine on adenosine receptors, including potentiation of muscle contractions (Magkos & Kavouras, 2005), delayed fatigue via mechanisms in the central nervous system (Davis et al., 2003), direct neuroendocrine activation (al'Absi et al., 1998), and direct actions on skeletal-muscle function (Tarnopolsky & Cupido, 2000). The physiological relevance of adenosine in exercise proposed by Biaggioni (2004) is that the increasing concentrations of adenosine produced during exercise act to inhibit sympathetic efferents and activate afferent nerves. The net effect of this inhibition is to protect the muscle tissue during ischemia or exhaustive exercise. Thus, the antagonistic actions of caffeine on adenosine receptors have the potential to reduce the normal inhibitory effect of adenosine on motor efferents.

Testosterone and cortisol synthesis and release are classically described as having negative feedback control via the hypothalamic-pituitary-gonadal and hypothalamic-pituitary-adrenal axes, respectively. In addition to these classical pathways, however, recent research *in vivo* has identified direct neural links between the paraventricular nucleus of the hypothalamus and testosterone (Selvage, Lee, Parsons, Seo, & Rivier, 2004) and cortisol secretion (Zaki & Barrett-Jolley, 2002). The implications of a direct neural link have particular relevance regarding a mechanism for caffeine-modulated effects if the neurons use adenosine as a neuromodulator.

The caffeine doses used were such that each subsequent dose doubled the previous one, and they covered the range of commonly reported dosages (Bridge & Jones, 2006; Graham & Spriet, 1995; Stuart et al., 2005). Researchers have stated that doses of 9 mg/kg ingested orally resulted in postexercise urinary caffeine levels below the former IOC cutoff concentration of 12 mg/L (Magkos & Kavouras, 2004; Pasman et al., 1995), making it likely that athletes would use this dose in a competitive environment. The current study demonstrates that doses of ≥ 400 mg caffeine have some potential to enhance the beneficial effects of training via an increase in bioavailable testosterone. Testosterone is a potent inductor of contractile protein synthesis and is necessary for muscle hypertrophy (Inoue et al., 1994). Despite heterogeneity in hormonal responses to resistance training, it is accepted that testosterone increases in response to resistance exercise and that intensity and volume are important determinants of this response (Kraemer et al., 1990).

The practical importance of acute testosterone responses to resistance training is evidenced by research showing that the magnitude of isometric strength increases are related to the magnitude of anabolic-hormone response (Hansen et al., 2001). More recently Kvorning et al. (2006) demonstrated that suppression of endogenous testosterone attenuated strength gains achieved over an 8-week training period. Such examples stress the importance of acute anabolic responses to training and, combined with the data presented in the current study, suggest some potential for caffeine to enhance training outcomes.

Our data also show a moderate effect of caffeine in elevating cortisol concentrations during resistance exercise. The cortisol elevation observed is consistent with the results of Lane, Pieper, Phillips-Bute, Bryant, and Kuhn (2002), and this elevation might counter the anabolic effects of testosterone. Those researchers also commented on the persistence of the caffeine effect, with the effects of a divided caffeine dose of 500 mg being apparent 10 or more hours after ingestion. Our experimental design only captured data for 150 min from the time of ingestion, but

it was apparent that the 800-mg dose produced a substantial and long-lasting effect on cortisol concentration. Furthermore, our data indicate that caffeine potentiated the stress response to resistance exercise in a dose-dependent manner.

Recent research has reported that suppressing cortisol during resistance exercise by nutritional supplementation is associated with greater gains in muscle cross-sectional area (Bird et al., 2006). These observations suggest that the cortisol increase associated with caffeine ingestion might counteract to some degree any benefit derived from the increase in exercise-induced testosterone concentration. Indeed, the anabolic–catabolic balance, as illustrated by the testosterone:cortisol ratio, showed an overall caffeine-induced decrease, with the decrease being most pronounced at the highest caffeine dose. These data suggest that catabolic processes were predominant in the recovery phase after resistance exercise when higher doses of caffeine were ingested.

Increases in testosterone at the 400- and 800-mg caffeine doses occurred within 15 min of commencing resistance exercise. Similar levels of testosterone were not seen in the placebo group until the 45-min time point. The immediacy of the testosterone rise suggests that caffeine can decrease in the latency of hormonal response to exercise stimuli. A caffeine-mediated potentiation would have important ramifications for training and training outcomes; however, this observation requires further investigation because it is possible that it is a result of the greater magnitude of response rather than a difference in the time course of the response.

The small improvement in sprint performance at the 800-mg dose is of a magnitude similar to that of the improvement in sprint performance demonstrated previously in cyclists (Wiles et al., 2006) and team-sport athletes (Paton et al., 2001). It should be noted that previous research has demonstrated performance benefits in endurance exercise at doses of 3–6 mg/kg (Bridge & Jones, 2006; Graham & Spriet, 1995). Indeed, Graham and Spriet noted that “light” caffeine users struggled with a caffeine dose of 9 mg/kg while “users” experienced large increases in endurance performance relative to a placebo condition. Evidently caffeine habituation and tolerance, in addition to exercise type, are important considerations when identifying optimal doses for performance enhancement.

The primary aim of the current study was to investigate the acute effect of caffeine on the exercise-associated increases in testosterone and cortisol. There are a growing number of studies showing performance benefits from caffeine. The consequences of routine use of high levels of caffeine in training are unknown. Although this study reports a potentially important anabolic advantage from caffeine via the dose-dependent testosterone increase observed, it is tempered by a concurrent increase in cortisol, raising questions about possible functional gains. A longer term training study investigating the impact of caffeine on training outcomes is required.

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