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Effect of Caffeine on the Testosterone and Cortisol Levels of University Football Players

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Authors' Contribution
MZIB supervised the investigation and YT designed the study. BMD recruited football players and conducted experimental analysis. MAI did statistical interpretation and helped in biochemical analysis.

Key words
Caffeine, Testosterone, Cortisol, Football players, Steroid hormones

ABSTRACT

This current study investigates the effect of caffeine on the testosterone and cortisol levels of university football players. For this purpose 120 university-level male football players (age: 22.39 ± 1.69 years; height: 172.9 ± 5.85 cm; body mass index: 23.45 ± 1.43 kg/m^2 Mean ± SD) from South Punjab, Pakistan were selected. The players were divided into four groups, each of 30 to determine the effect of caffeine on hormonal levels of the players. Caffeine was administered to three groups (A, B, C) orally in the form of capsules containing caffeine at 3, 6 and 9 mg.kg^{-1}, respectively. Group D was considered a placebo-control group and was given no caffeine. Testosterone and cortisol levels were determined 60 min before and after administration of different doses of caffeine. The results concluded that medium (6 mg.kg^{-1}) and high doses (6, 9 mg.kg^{-1}) caused significant increase in the levels of testosterone and cortisol of university football players. This increase in hormonal level was later reflected in sports efficiency.

INTRODUCTION

Football is the most widely played and popular sport in the world (Schulenkorf *et al.*, 2016). The general performance of football players is influenced by different physical fitness characteristics, playing methods, cognitive ability, team strategies and psychological components (Benítez-Sillero *et al.*, 2021). Over 90% of adults globally use caffeine every day, making it the most widely used drug in the world (dePaula and Farah, 2019). Seventy five percent of the players who compete in elite sports have admitted to using drugs before or during the competition (McDuff *et al.*, 2019). Recently however caffeine has been taken off the World Anti-Doping Agency's (WADA) list of banned substances during the tournament (Aguilar-Navarro *et al.*, 2020). Additionally, the International Olympic Committee mentions in its latest agreed statement on dietary supplements that taking caffeine before exercise in doses between 3 and 6 mg.kg^{-1} enhances performance (Mielgo-Ayuso *et al.*, 2019). Athletes tend to take these doses in a competitive environment since studies indicate that doses of 9 mg.kg^{-1} taken directly resulted in post-exercise urine caffeine levels below the former IOC threshold concentration of 12 mg/L (Magkos and Kavouras, 2005).

Caffeine is rapidly absorbed by the body and its peak levels were detected within 15 to 120 min in the blood. After 3 to 4h, caffeine levels start to decline (Grgic *et al.*, 2019). Like most pharmaceutical and dietary additives, caffeine has an impact on every cell in the body, including those in the central nervous system, muscles and fat (Sellami *et al.*, 2018). Stimulation of nervous system and brain makes the body alert and energetic while lessening tiredness (Lima-Silva *et al.*, 2021). Caffeine may enhance performance by raising the amount of circulating epinephrine (adrenaline), the hormone that triggers the fight or flight response (Barreto *et al.*, 2021). Caffeine is administered usually around an hour before physical exercise in the form of a pill or beverage, with peak plasma level caffeine concentrations occurring 15 to 120 min after intake (Ranchordas *et al.*, 2018).

Testosterone is a male's primary reproductive anabolic steroid hormone, which affects both sexes health and well-being in a variety of ways, including behavior, temperaments and osteoporosis prevention. Low testosterone levels in males may cause anomalies including

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bone loss and frailty (Rochira *et al*., 2018). Testosterone levels may differ considerably within and across people due to the dynamic control of endogenous testosterone synthesis, depending on acute effects of competition and exercise (D'Andrea *et al*., 2020).

Cortisol is a stress hormone linked to pressure or stress response. This steroid hormone is produced in the adrenal cortex and plays a vital role in maintaining normal physiological processes (Berger *et al*., 2019). A few of these include raising blood glucose level, conversion of amino acids to carbohydrates, inhibiting protein synthesis, stimulating gluconeogenesis and increase in muscle protein oxidation (Deng *et al*., 2018). Under extended physical and mental stress, cortisol helps in maintaining cellular and metabolic processes and managing homeostatic equilibrium (Deussing and Chen, 2018). The aim of this study was to investigate the effect of multiple doses of caffeine on the testosterone and cortisol levels of university football players.

MATERIALS AND METHODS

Sample size

One hundred and twenty football players at the University level (18-25 years of age); height 172.9 ± 5.85 cm; body mass 70.12 ± 5.03 kg; body mass index 23.45 ± 1.43 kg/m^2 Mean ± SD) were selected from Multan zone for this investigation according to the guidelines documented by Fraenkel and Wallen (1993) and rules formulated by Higher Education Commission, Pakistan. Participants were briefed about the pre-participation screening questionnaire adopted by AHA/ACSM Health/Fitness Facility (Balady *et al*., 1998). Players having diabetes, heart disease, low or high blood pressure, irregular heartbeat, kidney and liver disease, mental disorders like anxiety and panic attacks, seizure, ulcer, thyroid disease, trouble in sleep, caffeine allergic reactions were not included in this study.

Research design

The current study is randomized, placebo-controlled single-masked parallel groups trial. Prior to tessting the players were restrained from performing strenuous activities and maintained a normal diet for 48 h. As a routine participants consume 150.1 ± 39.6 mg caffeine daily but they were instructed not to drink caffeine 24 h prior to the start of the experimental session. The participants were divided into four groups each of 30 individuals. Three groups were administered with three different concentrations of caffeine (3, 6, 9 mg.kg^{-1}) orally, while the fourth group, which acted as control group, was not administered with caffeine. Blood samples of all participants were drawn one hour before and one hour after caffeine administration. The blood samples were centrifuged at 8000 rpm for 10 min to separate the serum which was used for analysis of testosterone by the Chemiluminescence Immunoassay Technique and cortisol by the Chemiluminescence Microparticle Immunoassay Technique (Abbott - Alinity Ci).

Statistical analysis

The analysis of the data was conducted on GraphPad Prism version 6.0 software. Data from each research parameter was given as Mean ± SEM and statistically assessed using a two-tailed Paired sample "t"-test and Pearson correlation were used to check the effect of each dose of caffeine on hormonal responses, respectively.

RESULTS

Testosterone levels of football players

Figure 1 shows testosterone (pg/mL) levels of football players administered 3, 6 and 9mg caffeine kg^{-1}. Caffeine at a dose of 3 mg.kg^{-1} does not cause any appreciable change in the levels of testosterone. Prominent elevation (P < 0.0001) of 103% was observed after oral administration of 6mg.kg^{-1}. Likewise, 102% increase (P < 0.0001) was marked in levels of testosterone after oral administration of 9 mg.kg^{-1}.

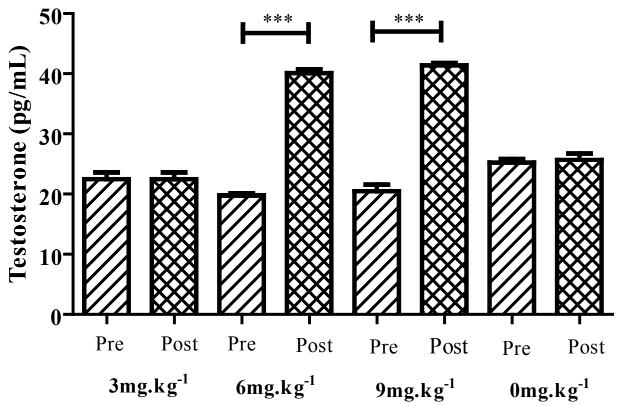

Fig. 1. Effect of different concentrations of caffeine on testosterone levels in blood of football players. Pre, testosterone level before administration of caffeine; Post, testosterone level one hour after administration of caffeine.

Table 1 shows correlation coefficients of testosterone (pg/mL) in different dose groups. There is a positive significant correlation (P < 0.05) among Group A 3mg Pre and Group A 3mg Post, Group B 6mg Pre and Placebo 0mg Post. It is also noted that there is a negative significant correlation (P < 0.05) among Group B 6mg Pre and Group C 9mg Post, Group B 6mg Post and placebo 0mg post.

Table I. Coefficient of correlation of testosterone levels (pg/mL) in different dose groups.

	Group A 3mg Pre	Group A 3mg Post	Group B 6mg Pre	Group B 6mg Post	Group C 9mg Pre	Group C 9mg Post	Placebo 0mg Pre	Placebo 0 mg Post
Group A 3mg Pre	1	.942**	.153	.102	.054	.040	-.133	.058
Group A 3mg Post		1	.217	.020	-.011	-.004	-.183	.173
Group B 6mg Pre			1	.015	.077	-.361*	.001	.364*
Group B 6mg Post				1	.114	.122	-.220	-.411*
Group C 9mg Pre					1	.326	.140	.272
Group C 9mg Post						1	-.357	-.109
Placebo Pre							1	.320
Placebo Post								1

** Indicate Significant at $P \leq 0.01$ (2-tailed); * indicate significant at $P \leq 0.05$ (2-tailed). For details of abbreviation see Figure 1.

Table II. Coefficient of correlation of Cortisol level (μg/dL) in different dose groups.

	Group A 3mg Pre	Group A 3mg Post	Group B 6mg Pre	Group B 6mg Post	Group C 9mg Pre	Group C 9mg Post	Placebo Pre	Placebo Post
G A 3mg Pre	1	.179	.378*	-.116	.122	-.037	.113	.099
GA 3mg Post		1	-.050	-.256	.003	.042	-.217	.256
G B 6mg Pre			1	.242	.334	-.024	.010	-.041
G B 6mg Post				1	.086	.036	-.022	.025
G C 9mg Pre					1	-.158	-.097	-.093
G C 9mg Post						1	.196	.362*
Placebo Pre							1	.224
Placebo Post								1

* Indicate Significant at $P \leq 0.05$ (2-tailed); G, Group; mg, milligram; Pre, Pretest; Post, Posttest; μg/dL, micrograms per deciliter. For details of abbreviations see Figure 1.

Cortisol level in football players

Figure 2 shows pre-test vs post-test levels of cortisol after different doses of caffeine. There is 2% increase in the levels of cortisol (μg/dL) after oral administration of 3 $mg.kg^{-1}$. Prominent elevation ($P < 0.0001$) of 56% and 52% has been observed in the levels of cortisol after oral administration of 6 and 9 $mg.kg^{-1}$.

Table II shows correlation coefficient of cortisol in different treatment group. There is a positive significant correlation ($P < 0.05$) between Group A 3mg pre and Group B 6mg Pre, Group C 9mg Post and Placebo Post. It is also noted that the correlation among Group A 3mg Pre and Group B 6mg Post, Group A 3 mg Pre and Group C 9mg Post, Group A 3 mg Post and Group B 6mg Pre, Group A 3 mg Post and Group B 6mg Post, Group A 3 mg Post and Placebo 0mg Pre, Group B 6mg Pre and Group C 9mg Post, Group B 6mg Pre and Placebo 0 mg Post, Group B 6mg Post and Placebo 0mg Pre, Group C 9mg Pre and Placebo 0mg Pre, Group C 9mg Pre and Placebo 0mg Post had become an inverse sign.

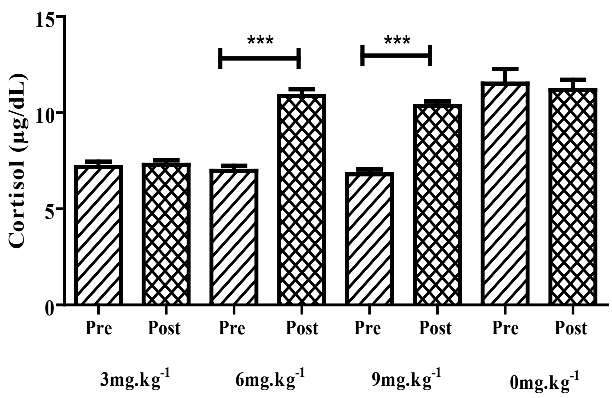

Fig. 2. Effect of different concentrations of caffeine on cortisol level in blood of football players. For details of abbreviations see Figure 1.

DISCUSSION

Effect of caffeine on testosterone level

In hormonal analysis, caffeine administration's effect on testosterone and cortisol levels was assessed in pre and post-test conditions. No significant difference was evidenced in the testosterone level in the post-test condition in the group administered 3mg.kg^{-1} body weight of caffeine, hence, depicting that a 3mg.kg^{-1} caffeine dose did not manifest any change in testosterone levels in football players.

As testosterone is a hormone that depicts aggression (Batrinos, 2012) in players, aggression is a prerequisite for increased performance; it can be understood that administering 3mg.kg^{-1} body weight of caffeine is not recommendable to achieve the best performance in the players. Moreover, administering 6mg.kg^{-1} and 9mg.kg^{-1} body weight of caffeine to football players markedly elevated serum testosterone levels. This increase in serum testosterone levels undoubtedly develops more aggression in football players, which enhances their overall performance in the playing areas. Hence, caffeine doses ranging from 6mg.kg^{-1} to 9mg.kg^{-1} can suit players in our region. Additionally, the placebo dose group administered 0mg.kg^{-1} body weight of caffeine showed no significant increase in testosterone levels. Hence, the placebo dose group results suggest that the player's performance can be compromised without caffeine supplementation due to a lack of aggression.

Wu (2014), Paton *et al*. (2010), Beaven *et al*. (2008), and Svartberg *et al*. (2003), have reported the same trends of elevated testosterone in the caffeine administered to the group. In a previous investigation by Wu (2014), twelve resistance training male players received low (2 mg/kg^{-1}), medium (4 mg/kg^{-1}), high (6 mg/kg^{-1}) and placebo treatments of caffeine. Results of this investigation demonstrate that high doses of 6mg/kg^{-1} of caffeine increased testosterone responses in resistance training players. In another study Paton *et al*. (2010) examined the effects of caffeine-containing chewing gum on hormone response involving nine elite male cyclists provided caffeine (240 mg) or a placebo; the result noted that caffeine increases the testosterone levels in cyclists. Beaven *et al*. (2008) looked at the immediate impact of caffeine on the increases in testosterone in twenty professional rugby league players who took coffee doses of 0mg, 200mg, 400mg and 800mg. The result discovered that 800mg of caffeine may enhance performance due to the elevation in testosterone levels. A previous epidemiological investigation by Svartberg *et al*. (2003) found that adult males who drink a lot of coffee (more than four cups per day) also had higher total testosterone levels.

Moreover, supplementation of the players in the permitted dose can enhance the players overall performance. In the overall group comparison, the 9mg.kg^{-1} body weight of the caffeine-administered group presented a visible increase in testosterone in the post-test condition. Hence, this suggests that this dose maximizes the players overall performance.

Effect of caffeine on cortisol level

Adrenocorticotrophin, secreted by the pituitary gland, is responsible for regulating cortisol, which is a hormone with catabolic properties. The primary function of cortisol is believed to be the promotion of gluconeogenesis. No significant difference was evidenced in cortisol concentration in post-test conditions after administering 3mg.kg^{-1} body weight of caffeine to the football players. Understandably, this dose of caffeine did not produce any prominent effect on the biosynthesis of cortisol. Meanwhile, in group B, where 6mg.kg^{-1} body weight of caffeine was administered, a marked elevation of cortisol in the post-test condition was observed.

This increase can be explained by the fact that caffeine also has an anxiogenic effect on mental conditions in players supplemented with its doses. Hence, this concentration of caffeine administration can be a cause of elevation in cortisol levels. Generally, cortisol is known to elevate during or after exercise. This increase can be explained by the fact that blood glucose and free fatty acid levels may affect cortisol responses during increased metabolic activity. While some studies demonstrated this effect, other studies showed no significant effects. Therefore, whether nutritional supplementation before and during exercise suppresses the cortisol response is still debatable. Hence, it is still debatable whether dietary supplements can suppress the cortisol response before and during exercise.

According to the findings of previous studies, Wu (2014), Gavrieli *et al*. (2011), Beaven *et al*. (2008), Woolf *et al*. (2008), Lovallo *et al*. (2006), Knoll *et al*. (1984) showed similar results of increased cortisol in the group receiving caffeine. In a previous study, Wu (2014) treated twelve university males with caffeine at different doses: low (2 mg.kg^{-1}), medium (4 mg.kg^{-1}), high (6 mg.kg^{-1}) and placebo. The findings of this previous study show that resistance training athletes cortisol responses are increased by high doses of caffeine 6 mg.kg^{-1}. In an earlier study, a morning snack was provided with caffeine doses of 3mg.kg^{-1}, stimulating the central nervous system and raising cortisol levels in the resting state (Gavrieli *et al*., 2011). Another study demonstrated a mild increase in cortisol 30 min after resistance exercise (RE) after a heavy caffeine intake of 800mg (Beaven *et al*., 2008). Another research results revealed that both breakfast and caffeine 5mg.kg^{-1} before exercising dramatically increased the amount of cortisol in the blood after exercising (Woolf *et al*., 2008).

Before dynamic exercise, a high dose of coffee (250 mg x 3) was demonstrated to increase cortisol responses (Lovallo *et al*., 2006). Yet prior research showed that eating raises cortisol, especially after a midday meal (Knoll *et al*., 1984). Hence, the results of previous research indicate that consuming low to high levels of caffeine before exercising may boost the cortisol response. In these studies, significant differences in serum cortisol concentration were observed instantly after administering a medium and high dose of caffeine. The results of this study also suggest that only medium and high doses of caffeine ingestion (6 $mg.kg^{-1}$ and 9$mg.kg^{-1}$) may considerably increase cortisol responses. In this research, the caffeine ingestion, pre-test training, the individual's gender and level of hydration may have affected the acute hormonal concentrations. Moreover, the same increasing trend of cortisol concentration was evidenced in Group C, where 9$mg.kg^{-1}$ body weight of caffeine was given. Regarding the placebo dose, no significant difference was evidenced in football players' cortisol levels in the post-test condition compared to the pre-test. This indicates that it became prominent when players were administered 6 or 9 $mg.kg^{-1}$ of caffeine.

Practical application

In this investigation, university football players ingested caffeine capsules. In this investigation, three different doses (3, 6, and 9 mg/kg^{-1}) of caffeine were used for players. Moreover, 6 and 9 $mg.kg^{-1}$ dose are more beneficial for university football players. Caffeine capsules are the safest way to intake caffeine. Caffeine capsule ingestion peaks plasma level after 84-120 min. If football players take caffeine capsules 15 min before the start of the match, caffeine in capsule form begins digestion after 15 min, and 15-120 min after intake, the plasma caffeine concentration reaches its peak. Within 45 min after intake, absorption is almost complete (Kamimori *et al*., 2002). However, according to FIFA rules, an interval break cannot last more than 15 min for players. In the second half of a football match, players feel more tired and need an ergogenic aid for energy. If the player takes caffeine in this way, it will benefit them throughout the second part of the game.

Limitation

To have more authentic and reliable results, the sample size for our investigation should be increased. Moreover, to have a better assessment of caffeine effect on players performance other biochemical parameters like adrenaline should also be assessed.

CONCLUSION

Results concluded that a significant difference was evidenced in the level of testosterone and cortisol in the post-test condition in the group administered 6 and 9 $mg.kg^{-1}$ body weight of caffeine. Overall results of this study showed that medium doses (6 $mg.kg^{-1}$) and high doses (9 $mg.kg^{-1}$) had prominent effects on the hormonal responses of university football players. Moreover, these medium and high doses of caffeine do improve the performance of university football players.

DECLARATIONS

Acknowledgement

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IRB approval

The study was approved by the Institutional Ethical Review Board of the University of Punjab, Lahore (No.D/ 342/ FIMS; Dated: 29-9-2022).

Ethical statement

Each player was required to give written informed consent to participate in this research. On consent form oral intake of caffeine and subsequent provision of blood sampling was explained to each participant.

Statement of conflicts of interest

The authors have declared no conflict of interest.

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