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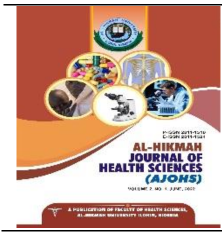

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Original Article

Effect of Chronic Administration of Caffeine on Plasma Testosterone Level and Body Mass Index in Male Wistar Rats

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ABSTRACT

Objectives: Caffeine is one of the most frequently ingested pharmacological agents contained in several beverages and food items. With some positive biological effects on the body system, little knowledge is known of its effect on reproductive parameters. This present study therefore aimed to determine the effect of aqueous solution of caffeine on serum testosterone level and body mass index (BMI) in male Wistar rats.

Methods: Twenty-one (21) male Wistar rats with an average weight of 250 g ± 20g were obtained for this study and randomly grouped into three (3) of seven (7) rats each. Group A, served as the control and received 0.5ml of normal saline while groups B and C served as test groups and received orally 0.38 mg/kg (low dose) and 1.14mg/kg (high dose) of caffeine respectively, once daily for 14 days.

Results: There was a significant increase ($p<0.05$) in body weight, BMI, and plasma testosterone levels of high dose (HD) and low dose (LD) compared to the control, while there was no significant ($p>0.05$) change in length of the animals compared to the control.

Conclusion: Long-time consumption of caffeine has the potential to stimulate the production of testosterone to possibly increase sex drive perhaps via the hypothalamic-pituitary-testicular axis, and also increase BMI.

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Introduction

Caffeine (1,3,7trimethylxanthine) is one of the most widely used psychoactive substances due to its ubiquitous occurrence in commonly consumed beverages such as coffee, tea and cola. Caffeine is a natural stimulant commonly found in coffee, tea, and soda. Per serving, coffee typically contains the highest amount of caffeine (Heckman *et al*., 2010). Of the world's total population, about 85% consume copious amounts of caffeine (Harris, 2004). Approximately 15% of couples are challenged with fertility problems (Sharma *et al*., 2013; Agarwal *et al*., 2015), and male factor seem to contribute for up to 30% of them (O'Flynn O'Brien *et al*., 2010). Caffeine has a number of biological effects including central nervous system stimulation, increased secretion of catecholamines, relaxation of smooth muscle and stimulation of heart

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rate whereas a moderate intake may confer a modest protective effect against some cardiovascular system disease and on the metabolism of carbohydrate and lipids including arrhythmia, diabetes, liver disease (Cano-Marquina*et al*., 2013) and even deleterious health effects (Sepkowitz, 2013).

In recent years, there have been growing concerns about the decrease in male reproductive health as found in declining semen quantity and quality in developed countries (Olesen *et al*., 2018). It has been shown that exposure to environmental, occupational, lifestyle, and dietary factors can adversely affect reproduction(Kumar *et al*., 2019). During recent decades, caffeine, one of the most comprehensively studied ingredients in food supply, has been implicated in a number of epidemiologic studies as a risk factor for infertility (Wilcox *et al*., 1990). Body mass index (BMI) is the fundamental determining tool for the assessment of obesity and its usefulness cuts across many fields of discipline (Am and Sa, 2001).

Testosterone is the primary male sex hormone and an anabolic steroid (Ayinde *et al*., 2014). In addition, it is associated with health and general well-being of an individual. In adults, testosterone effects are more clearly demonstrable in males than in females, but are important to both sexes, however, some of these effects may decline as testosterone levels decrease in the later adult life (Kesley *et al*., 2014). Animal study showed that rat sexual arousal is sensitive to reductions in testosterone. When testosterone-deprived rats were given adequate testosterone, their sexual behaviors (copulation, partner preference, etc.) resumed, but not when administered inadequate strength of the same hormone. In males, testosterone is synthesized primarily by Leydig cells (20% of adult testis) in the interstitium of seminiferous tubules. Even when germinal epithelium of seminiferous tubules is severed, the Leydig cells continue secretion of testosterone. Other sites of production in human are the adrenal glands, and ovaries in females. The amount of testosterone synthesized is regulated by the hypothalamic–pituitary–testicular axis. When testosterone levels are low, gonadotropin-releasing hormone (GnRH) is released by the hypothalamus, which in turn stimulates the pituitary gland to release follicle stimulating hormone (FSH) and luteinizing hormone (LH). These latter two hormones stimulate the testis to synthesize testosterone. Finally, increasing levels of testosterone through a negative feedback loop act on the hypothalamus and pituitary to inhibit the release of GnRH and FSH/LH, respectively. Testosterone levels usually peaks early each day, regardless of sexual activity (van Anders and Dunn, 2009).

Given the contradictory results on the reproductive effects of caffeine and the fact that caffeine is so widely consumed, its health benefits and consequences have been and are still being studied extensively. This study thus, evaluated the effect of chronic administration of caffeine (in coffee drink, Nescafé), on serum levels of testosterone and BMI of male Wistar rats.

Materials and Methods

Source of animals

Twenty-one (21) male Wistar rats with an average weight of 250g ± 20g were used in this study. These rats were purchased from the Animal Holding Unit of Ladoke Akintola University of Technology (LAUTECH), Ogbomoso, Oyo state.

Source of Elisa kit/caffeine

Caffeine was gotten from Nescafe classic coffee tin of 50g, product of Nestle, purchased from Shoprite, Ilorin.

Testosterone kit was a product of Fortress Diagnostics Limited, Antrim Technology Park, Antrim, BT41 1Q3, United Kingdom.

Animal Care and Ethical Approval

The animals were housed in metabolic cages in the animal house of the Faculty of Basic Medical Sciences under standard laboratory conditions The room was well ventilated and kept at a constant room temperature of about 25^0C ± 2^0C. They were given traditional rat feeds and water *ad libitum*. The research was approved to be in compliance with internationally accepted laboratory animal use and care guidelines, and the guidelines for the institution research Ethical Review Committee of College of Health Sciences, University of Ilorin, Ilorin Nigeria.

Animal grouping

The 21 animals were randomly divided into three (3) groups of 7 rats each. Animals in Group A served as control group. Groups B and C were given caffeine orally through oral cannula at doses of 0.38mg/kg body weight (low dose) and 1.14mg/kg body weight (high dose) respectively for an experimental period of two weeks (14 days).

Experimental procedure

Coffee solution was prepared daily by weighing out appropriate weight of the coffee granules and dissolving in 0.5ml of normal saline (0.9% NaCl) and was administered orally to the experimental groups using oral cannula for a period of 14 days.

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Group A (control group) was given 0.5ml NaCl only once daily. Group B (low dose) was given 0.38mg/kg body weight of caffeine dissolved in 0.5mlNaCl orally once daily, while Group C (high dose) was given 1.14mg/kg of caffeine dissolved in 0.5mlNaCl orally once daily. The weight and length of the animals were measured on days 0 and 14 to calculate the BMI for control, LD and HD on the above-mentioned days. However, on day 14, the animals were euthanized under ketamine anaesthesia. Blood samples were collected via cardiac puncture with needle and syringe into lithium heparin sample bottles for plasma testosterone assay.

Body mass index (BMI)

The weight and length of the animals were determined by using digital weighing balance and tape rule respectively. The weight was measured in gramme(g), while the length was measured cranio-caudally from the mouth to the anus in centimeter (cm). The measurements were done at the commencement (Day 0) and the end of the experiment (Day 14) just before sacrifice. The BMI was calculated by dividing the body weight in gramme by the body length in centimeter squared. BMI = Body weight (g) / Length $(cm)^2$.

Determination of plasma levels of testosterone

The working solutions of the testosterone-HRP conjugate and wash buffer were prepared ready for use. Fifty microlitre (50µl) of each calibrator, control and specimen sample was pippetted into correspondingly labeled wells in duplicate. One hundred microlitre (100µl) of the conjugate working solution was then pipetted into each well using a multichannel pipette. Incubation on a plate shaker (approximately 200 rpm) for 1 hour at room temperature was established. The wells were then washed 3 times with 300 µl of diluted wash buffer per well the plate tapped firmly against absorbent paper to ensure that it is dry. One hundred and fifty microlitre (150µl) of TMB substrate was then pipetted into each well at timed intervals. Incubation on a plate shaker for 10-15 minutes at room temperature was again established. Fifty microlitre (50µl) of stop solution was then pipetted into each well at the same timed intervals as previously done above. The plates were then read on a microwell plate reader at 450nm within 20 minutes after addition of the stop solution.

Statistical analysis

The statistical analysis was done with the aid of the statistical package for social science (SPSS) version 20. Difference in means was obtained using ANOVA and Duncan post hoc test. All values reported in the study were expressed as Mean ± SEM (standard error of mean). Differences were taken to be significant at $P < 0.05$.

Results

The results obtained from the analysis of the effect of chronic administration of caffeine on BMI and plasma testosterone levels in male Wistar rats are presented in table 1- 4.

Table1: Effect of Aqueous Solution of Caffeine on Body Length of Male Wistar Rats

Group/Day	A (cm)	B (cm)	C (cm)
0	22.61±1.09	21.58±1.56	22.37±1.82
14	22.64±1.18	21.59±0.98	22.39±0.93

Values are expressed as Mean ± SEM

The result showed that there was no significant change in body lengths of LD or HD from the control, and no significant change was also observed from day 0 to day 14.

Table 2: Effect of Aqueous Solution of Caffeine on Body Weight of Male Wistar Rats

Group	Dose	Day 0 (g)	Day 14 (g)
A	0.9% NaCl	254.30±0.46	260.04±0.21
B	0.38mg/kg	255.17±0.47	267.14±1.03[a]
C	1.14mg/kg	268.38±1.76	283.24±0.71[*ab]

Values are expressed as Mean ± SEM

*Significant at $p<0.05$ when compared with control

[a]Significant at $p<0.05$ comparing day 14 to day 0

[b]Significant at $p<0.05$ comparing HD to LD

There was significant increase in body weight of HD compared to the control and LD.

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There was significant increase in body weight of LD and HD on day 14 compared to day 0, while there was no significant increase in body weight of LD compared to the control.

Table 3: Effect of Aqueous Solution of Caffeine on Body Mass Index (BMI) of Male Wistar Rats

Group	Dose	Day 0 (g/cm^2)	Day 14 (g/cm^2)
A	0.9% NaCl	0.402±0.04	0.587±0.02
B	0.38mg/kg	0.411±0.16	0.598±1.03^{*a}
C	1.14mg/kg	0.420±0.07	0.605±0.71^{*ab}

Values are expressed as Mean ± SEM
*Significant at $p<0.05$ when compared with control
[a]Significant at $p<0.05$ comparing day 14 to day 0
[b]Significant at $p<0.05$ comparing HD to LD

There was significant increase in BMI of LD and HD compared to the control, and a significant increase in HD compared to LD was also noticed. There was also a significant increase in LD and HD on day 14 compared to day 0.

Table 4: Effect of Aqueous Solution of Caffeine Plasma on Testosterone Levels in Male Wistar Rats

Group	Dose	Day 14 (nmol/l)
A (Control)	0.9% NaCl	0.60 ± 0.29
B (Low dose)	0.38mg/kg	1.98± 0.63*
C (High dose)	1.14mg/kg	5.16 ± 0.61^{*b}

Values are expressed as Mean ± SEM
*Significant at $p<0.05$ when compared with control
[b]Significant at $p<0.05$ comparing HD to LD

There was significant increase in plasma testosterone levels of LD and HD group compared to the control. In the same vein, there was significant increase in plasma levels of testosterone in the HD group compared to LD groups.

Discussion

The result showed that consuming caffeine at high dose alone was able to precipitate increased body weight. Whereas, ingestion of caffeine at low dose only precipitated increased body weight when taken over prolonged time. This could have been possible due to food craving potentials associated with caffeine. This ability may also be related to the lateral nuclei which is the feeding-regulating centre in the hypothalamus, though, not assessed in the current study. However, there is association of consumption of caffeinated drinks in some studies with increased eating habit, and thus associated added weight. This is not in support of Henn *et al*., 2023 who reported that consumption of coffee and caffeine is linked to less weight gain. It also disagrees with another previous study which concluded that moderate coffee consumption has no effect on weight gain, dietary intake and appetite related feeding (Gavrieli *et al*., 2013).

The increased BMI seen in LD and HD is not surprising. This could have been due to the increased in body weight of the animals due to consumption of caffeine throughout the period of this study. This shows that prolonged caffeine ingestion could increase BMI. Thus, some levels of caution should be taken when consuming caffeine, and not to be carried away with its associated aroma. In this regard, it will be better to even be avoided in an already established obese individual whose BMI is already compromised with its attending manifestations.

The current study also showed that caffeine consumption raised plasma levels of testosterone irrespective of the dose administered as long as it is ingested over a prolonged duration. This is in tandem with a previous study, though in human which found out that those who drink coffee regularly were found to have higher levels of testosterone than men who do not. This testosterone increase could possibly have been due to interaction of caffeine with the hypothalamic-pituitary-testicular axis because of its centrally acting activity, which in turn leads release of GnRH which then causes stimulation of gonadotropins from the pituitary. This in turn leads to stimulation of the Leydig cells to secrete more testosterone. As observed in this study, the significant weight increase could also be accounted for by the anabolic effect of high testosterone orchestrated by the prolonged caffeine consumption. Although, there was associated increased BMI observed in this study, added muscle buck by testosterone could have been more responsible rather than fat deposit or infiltrations. This

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is in agreement with a meta-analysis that revealed that higher coffee intake is not primarily associated with adiposity, particularly in men (Lee *et al*., 2019). The current increase plasma testosterone seen in this work could also be corroborated with another recent study that showed the association between caffeinated and decaffeinated coffee on sex hormone binding globulin and endogenous sex hormone levels at the end of 4-week interval, in which men showed an increase in total testosterone (Wedick *et al*., 2012). Another human study also revealed that men who drank coffee experienced less erectile dysfunction than those who did not. This is in agreement with a previous study which revealed that male coffee/caffeine consumption has been associated with high levels of sex hormone binding globulin (SHBG) and adenosine dependent pathway which have the capacity to mediate sex hormones activity (Goto *et al*., 2011; Glover *et al*., 2022). In another study conducted by Dias *et al*. (2015), caffeine was observed to act indirectly in both adult and fetal systems by influencing the hypothalamo-pituitary-testicular system or through a direct and noxious effect on the germinative epithelium.

Conclusion

Long time consumption of caffeine has potential to stimulate the production of testosterone to possibly increase sex drive and erectile function perhaps via hypothalamo-pituitary-testicular axis. The increased weight gain, and thus, BMI might be due to the anabolic effect of testosterone on muscle buck, and not fat depots accumulation.

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