Original Research

Effect of prepubertal caffeine consumption and recovery on adult erectile tissue functions in Wistar rats

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Abstract

The study examines the impact of caffeine consumption during prepubertal development and recovery on the contractile function of the adult corpus cavernosum in Wistar rats. Prepubertal male rats consumed distilled water (vehicle) and caffeine (5 mg/kg). A third group consumed caffeine and was allowed a period of recovery. Cavernosa tissues were excised at adulthood, and their contractile functions in the presence of Ca^{2+}, K^{+}, indomethacin, glibenclamide, methylene blue, L-N-nitro-arginine-methyl-ester (L-NAME), and sodium nitroprusside (SNP) were assessed. K^{+}-induced increase in contraction in the caffeine-treated group was similar to that in the recovery group. Ca^{2+} ions, however, increased contraction significantly in the caffeine group compared to the recovery group. Acetylcholine-mediated relaxation (%) was significantly higher in the recovery as compared to the caffeine treated after incubation with indomethacin and methyl blue. Acetylcholine-mediated relaxation, however, was higher in caffeine as compared to the recovery group after incubation with glibenclamide and L-NAME. Relaxation in the presence of SNP was significantly reduced in recovery than in the caffeine-treated group. Prepubertal caffeine intake had an erectogenic effect on the cavernous tissues in the presence of glibenclamide, nifedipine, and L-NAME. Inhibitors of prostacyclin (indomethacin) and guanylyl cyclase (methylene blue) militate caffeine-induced relaxation. These effects were reversed after recovery.

Keywords

Prepubertal caffeine consumption, erectile tissue function, recovery, methyl blue, indomethacin, L-NAME, glibenclamide

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Učinek predpubertetnega uživanja kofeina in okrevanja na funkcije odraslih erektilnih tkiv pri podganah Wistar

Izvleček

Študija preučuje vpliv uživanja kofeina med predpubertetnim razvojem in okrevanjem na kontraktilno funkcijo odraslega kavernoznega telesa pri podganah Wistar. Podganji samci v predpuberteti so uživali destilirano vodo (nosilec) in kofein (5 mg/kg). Tretja skupina je uživala kofein in ji je bilo dovoljeno obdobje za okrevanje. Tkiva kavernoze so bila izrezana v odrasli dobi in ocenjena je bila njihova kontraktilna funkcija v prisotnosti Ca^{2+}, K^{+}, indometacina, glibenklamida, metil modrega, L-NAME in natrijevega nitroprusida. S K^{+} povzročeno povečanje krčenja v skupini, zdravljeni s kofeinom, je bilo podobno kot v skupini, ki je okrevala. Ioni Ca^{2+} pa so znatno povečali kontrakcijo v skupini s kofeinom v primerjavi s skupino, ki je ozdravela. Relaksacija (%), posredovana z acetilholinom, je bila bistveno višja pri okrevanju v primerjavi s kofeinom, zdravljenim po inkubaciji z indometacinom in metil modrim. Relaksacija, posredovana z acetilholinom, pa je bila večja pri kofeinu v primerjavi s skupino za okrevanje po inkubaciji z glibenklamidom in L-NAME. Sprostitev v prisotnosti natrijevega nitroprusida je bila znatno zmanjšana pri okrevanju kot v skupini, zdravljeni s kofeinom. Predpubertetni vnos kofeina je imel erektogeni učinek na kavernozna tkiva v prisotnosti glibenklamida, nifedipina in L-NAME. Zaviralci prostaciklina (indometacin) in cGMP (metilno modro) spodbujajo sprostitev, ki jo povzroča kofein. Po okrevanju so bili učinki obrnjeni.

Ključne besede

Uživanje kofeina v predpuberteti, funkcija erektilnega tkiva, okrevanje, metilno modro, indometacin, L-NAME, glibenklamid

Introduction

Scientists have been fascinated by research in caffeine for decades. Caffeine ranks as one of the most frequently ingested active substances (Nawrot et al., 2003; Scorza et al., 2022). Apart from coffee, which serves as the commonest source, caffeine is usually present in prescription drugs, soft or energy drinks, gums, tea leaves, and stimulants (Mahoney et al., 2019). Absorption of caffeine in humans is almost optimal, with 100% bioavailability after oral intake (Sepkowitz, 2013).

A study reported a reduced likelihood of having erectile dysfunction when young adults consume around three saucers of coffee (about 170-375 mg/kg) daily (Lopez et al., 2015). A prospective cohort investigation of 40–75-year-old men found that prolonged coffee intake is not related to the development of erectile dysfunction (Lopez et al., 2018). Yang et al. (2008) also reported that caffeine intake (10 and 20 mg/kg) enhanced the erectile functions of diabetic rats through upscaling cavernous cyclic guanosine monophosphate (cGMP) activity.

On the contrary, the maternal exposure of rats to caffeine was reported to adversely affect the birth weight, cytoarchitecture of the testes, and serum testosterone level of the male offspring in adulthood (Ogunwole et al., 2015). Caffeine treatment for four weeks in adult male rats also impaired the body, reproductive organ weight, sperm characteristics, and testicular cytoarchitecture (Oluwole et al., 2016). The effects were, however, reported to be reversed in recovery groups.

The foregoing reports, along with others in the literature on caffeine consumption and its direct effect on reproductive functions, have not only been contradictory but also inconclusive in both human and animal studies. Furthermore, fewer studies have identified or targeted the significance of prepubertal caffeine exposure on erectile functions in adulthood. The significance of recovery or weaning from caffeine on erectile functions after exposure is also not fully determined.

This study investigates caffeine consumption during prepubertal development and recovery and the contractile activity and function of the adult corpus cavernosum in Wistar rats.

Materials and Method

Caffeine preparation and Wistar rats

The caffeine was purchased from Aesar Johnson Matthew Company, 26 Park Ridge Road, MA, U.S.A. Before daily treatment, the caffeine was freshly prepared using distilled water. The prepubertal rats (2 weeks old) were sourced from the Central Animal House, College of Medicine, Ibadan. They were acclimatized for one week and fed a pelletized rat diet and water. The ethics approval (Ref. No: AREC/2022/050) for this study was granted by the Lagos State University College of Medicine Animal Ethics Committee.

Experimental design and treatment

Eighteen prepubertal male rats were randomly divided into three equal groups. Group 1 (the control) was given distilled water (a vehicle for caffeine). Group 2 was given caffeine orally at a dose of 5 mg/kg (Metro et al., 2017) for the entirety of the study. Group 3 rats were treated with caffeine orally (5 mg/kg) for six weeks but were allowed six weeks of recovery without caffeine administration.

Serum collection for testosterone assay

The serum was carefully aspirated using a Pasteur pipette into an Eppendorf tube and stored at -4°C. Serum testosterone was assayed using a testosterone ELISA kit (Monobind Inc, Lake Forest, CA, U.S.A.)

The excision of the cavernosa tissues and cavernosa contractile functions studies

The cavernosa tissues were excised after the 12th week of the treatment. The procedures for the excision of the cavernosa tissue, composition of the P.S.S., mounting in the organ chamber, and recording of the isometric contraction using a data capsule system were as previously described (Salami et al., 2017). Using a force isometric transducer that is connected to the Ugo Basile (model 17400, Italy) data acquisition system, the contractile responses of the cavernosa tissues to increasing doses of phenylephrine (10^{-9}-10^{-5} M) and acetylcholine (10^{-9}-10^{-5} M) were recorded. In addition, cavernosa contractile responses to increasing doses of Ca^{2+} (10–60 mM) and K^{+} (10–60 mM) in calcium and potassium-free tissue chambers were recorded. Finally, the acetylcholine-mediated relaxation of the cavernosa tissues following incubation with indomethacin, glibenclamide, methylene blue, nifedipine, L-NAME, and sodium nitroprusside (10^{-4} M) was determined and recorded to investigate how prepubertal caffeine ingestion and recovery have influenced contractile mechanisms guided by the individual agents. Care was taken to wash the tissue three times before another drug was introduced.

Statistical Analysis

All the data were expressed as mean ± standard error of the mean (S.E.M.). One-way and two-way analysis of variance (ANOVA) were carried out with Tukey's multiple comparisons using GraphPad Prism 8.0 software. Statistical significance was taken at $p < 0.05$

Results

Table 1 showed that the testosterone level was significantly reduced in the recovery group

Table 1. Effect of caffeine treatment and recovery on testosterone concentration in male Wistar rats.

Tabela 1. Učinek zdravljenja s kofeinom in okrevanja na koncentracijo testosterona pri samcih podgan pasme Wistar.

	Control	Caffeine	Recovery
Testosterone (ng/ml)	4.16 ± 0.35	4.60 ± 0.28	1.63 ± 0.42*

N= 6, * p<0.05, Value expressed as mean S.E.M.

Effect of prepubertal caffeine ingestion and recovery on cavernosa tissue contraction to phenylephrine (10^{-9}- 10^{-5} M), potassium chloride and calcium chloride.

Phenylephrine-mediated contraction of the cavernosa tissue was significantly greater in the caffeine group than in the recovery group. The contraction was, however, significantly reduced in the control group when compared to the caffeine and recovery groups (Figure 1). Cumulative doses of extracellular potassium chloride influx significantly increased the contraction of the cavernosa tissue

in the caffeine and recovery groups when compared to the control (Figure 2). The extracellular influx of cumulative doses of calcium chloride significantly increased the cavernous tissue contraction in the caffeine group as compared to the recovery group (Figure 3).

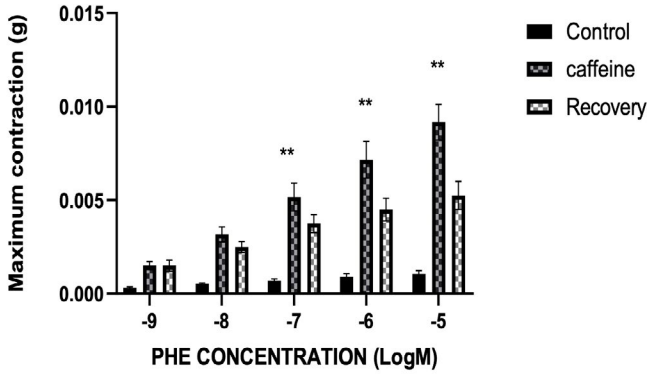

Figure 1. Maximum contraction of cavernosa tissue to cumulative doses of phenylephrine (10^{-9} – 10^{-5} M). N=6, ** = p < 0.01.

Slika 1. Največja kontrakcija kavernoznega tkiva na kumulativne odmerke fenilefrina (10^{-9} – 10^{-5} M).
N=6, ** = p < 0,01.

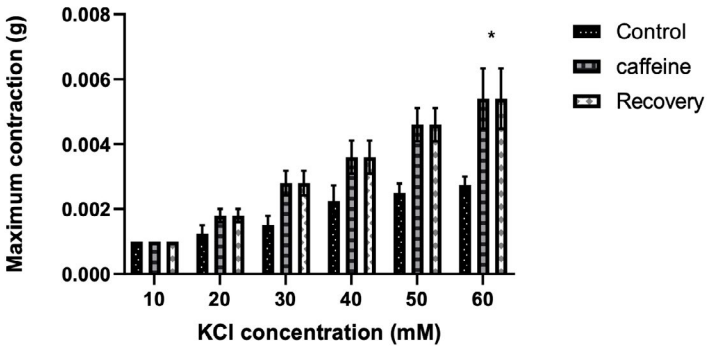

Figure 2. Maximum contraction of cavernosa tissue to influx of potassium chloride (10 – 60 mM), N = 6, *...p < 0.05

Slika 2. Največja kontrakcija kavernoznega tkiva na dotok kalijevega klorida (10 – 60 mM), N = 6, *...p < 0,05

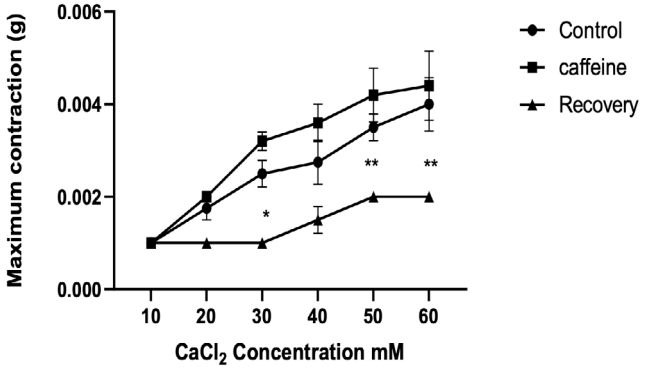

Figure 3. Maximum contraction of cavernosa tissue to influx of calcium chloride (10 – 60 mM), N = 6, *...p < 0.05, **...p < 0.01.

Slika 3. Največja kontrakcija kavernoznega tkiva na dotok kalcijevega klorida (10 – 60 mM), N = 6, *...p < 0,05, **...p < 0,01.

Effect of caffeine ingestion and recovery on acetylcholine-mediated relaxation response following incubation with indomethacin, glibenclamide, and methylene blue

As shown in Figure 4, acetylcholine-mediated relaxation (%) was significantly increased in the recovery group following incubation of the cavernous tissue with indomethacin (10^{-4} M). However, acetylcholine-mediated relaxation (%) was significantly reduced in the recovery group (Figure 5) following the incubation of the cavernous tissue with glibenclamide (10^{-4} M). The relaxation (%) to the cumulative dose of acetylcholine following the incubation of the cavernosa tissue in methyl blue was significantly increased in the recovery group (Figure 6).

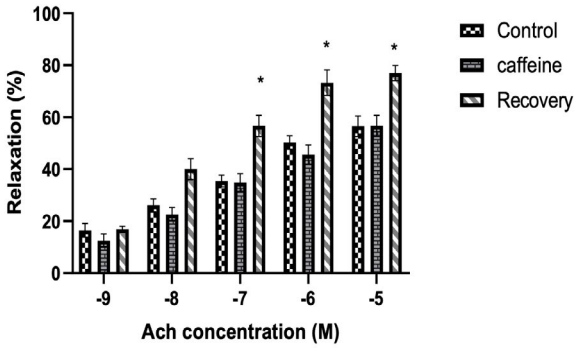

Figure 4. The relaxation (%) of the cavernosa tissue to cumulative doses of acetylcholine (10^{-9} – 10^{-5} M) after the incubation of the cavernosa tissue in 10^{-4} M indomethacin. N = 6, *...p < 0.05.

Slika 4. Relaksacija (%) kavernoznega tkiva na kumulativne odmerke acetilholina (10^{-9} – 10^{-5} M) po inkubaciji kavernoznega tkiva v 10^{-4} M indometacinu. N = 6, *...p < 0,05.

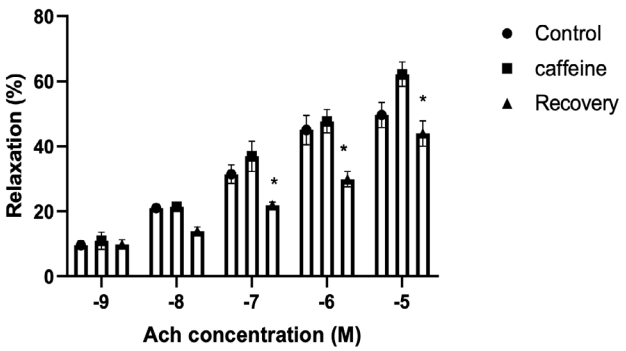

Figure 5. The relaxation (%) of the cavernosa tissue to cumulative doses of acetylcholine (10^{-9} – 10^{-5} M) after the incubation of the cavernosa tissue in glibenclamide (10^{-4} M). N = 6, *...p <0.05.

Slika 5. Relaksacija (%) kavernoznega tkiva na kumulativne odmerke acetilholina (10^{-9} – 10^{-5} M) po inkubaciji kavernoznega tkiva v glibenklamidu (10^{-4} M). N = 6, *...p <0,05.

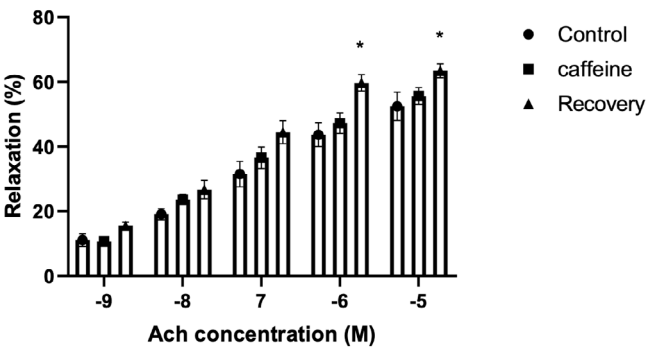

Figure 6. The relaxation (%) of the cavernosa tissue to cumulative doses of acetylcholine (10^{-9} – 10^{-5} M) after the incubation of the cavernosa tissue in methylene blue (30 µM). N = 6, *...p < 0,05.

Slika 6. Sprostitev (%) kavernoznega tkiva na kumulativne odmerke acetilholina (10^{-9} – 10^{-5} M) po inkubaciji kavernoznega tkiva v metilensko modrem (30 µM). N = 6, *...p < 0,05.

Effect of caffeine ingestion and recovery on acetylcholine-mediated relaxation response after incubation with nifedipine, L-NAME, and sodium nitroprusside (SNP)

Acetylcholine-mediated relaxation (%) showed no significant changes in the caffeine and recovery groups when compared with the control group following incubation of the cavernous tissue with nifedipine (Figure 7). Furthermore, acetylcholine-mediated relaxation (%) was higher in the caffeine compared to recovery and control following the incubation of the cavernous tissue in L-NAME (Figure 8). The relaxation (%) response of cavernosa tissue to increasing doses of SNP after the precontraction of the cavernosa tissue with phenylephrine was significantly reduced in the recovery group (Figure 9).

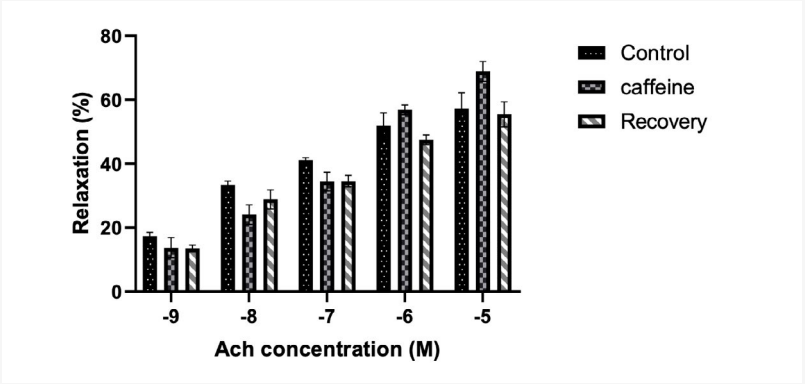

Figure 7. The relaxation (%) of the cavernosa tissue to cumulative doses of acetylcholine after the incubation of the cavernosa tissue in nifedipine (10^{-4} M)

Slika 7. Sprostitev (%) kavernoznega tkiva na kumulativne odmerke acetilholina po inkubaciji kavernoznega tkiva v nifedipinu (10^{-4} M)

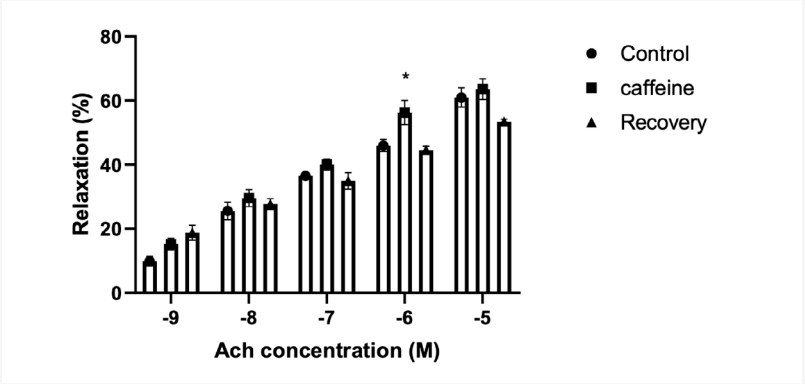

Figure 8. The relaxation (%) of the cavernosa tissue to cumulative doses of acetylcholine after the incubation of the cavernosa tissue in L-NAME (10^{-4} M). N = 6, *...p < 0,05.

Slika 8. Sprostitev (%) kavernoznega tkiva na kumulativne odmerke acetilholina po inkubaciji kavernoznega tkiva v L-NAME (10^{-4} M). N = 6, *...p < 0,05.

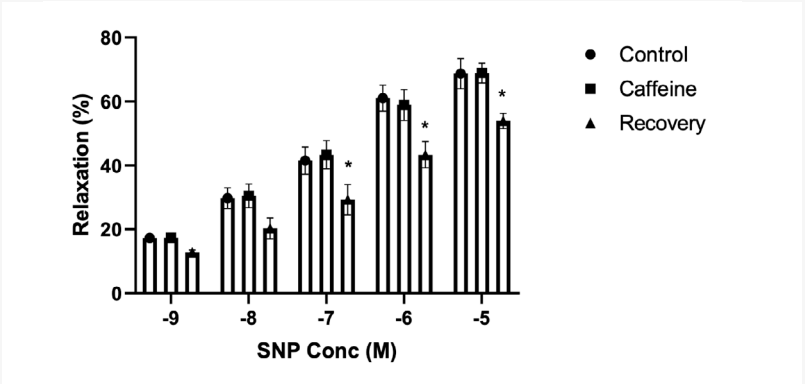

Figure 9. The relaxation (%) of the cavernosa tissue to cumulative doses of sodium nitroprusside (SNP) after the precontraction of the cavernosa tissue in phenylephrine. N = 6, *...p < 0,05.

Slika 9. Sprostitev (%) kavernoznega tkiva na kumulativne odmerke natrijevega nitroprusida (SNP) po predkontrakciji kavernoznega tkiva v fenilfrinu. N = 6, *...p < 0,05.

Discussion

The testosterone level of the recovery group in this study was significantly lower than the caffeine and control groups (table 1). Oluwole et al. (2016) earlier reported an increase in testosterone levels in caffeine and an insignificant decrease in the recovery group, albeit during adult exposure to caffeine. Furthermore, the dosage of caffeine administered in the study was far higher (20 mg/kg and 40 mg/kg) than that reported in the current study (5 mg/kg). The juxtaposition of our current study and that by Oluwole et al. (2016) emphasizes the importance of caffeine dosage and prepubertal caffeine exposure on testosterone levels. It is possible that caffeine's inhibition of aromatase activity caused the increase in serum testosterone levels in this study. Aromatase is the major enzyme involved in the conversion of androgens into estrogens in adult Leydig cells (Carreau, 2007). Additional studies have linked coffee to high levels of testosterone, sex hormone-binding globulin, and low levels of estrogen (Svartberg et al., 2003; Ramlau-Hansen et al., 2008). The absence of the inhibitory impact of caffeine during the recovery period may also account for the significantly lower testosterone levels in the recovery group compared to the control and caffeine groups.

This study found that phenylephrine-mediated contractions increased significantly in the caffeine group as compared to the control and recovery groups (Figure 1). The increase in contraction of the cavernosa tissue of the caffeine group may be due to the caffeine-induced presence of catecholamines, adenyl cyclase, and cyclic adenosine monophosphate (cAMP). Although these factors were not estimated in the current study, caffeine consumption has been reported to increase circulating levels of catecholamine, adenyl cyclase, and cAMP (Nabofa and Alada, 2020). The elevation of these factors during the period of caffeine exposure and their gradual decline in recovery helped shape the contractile responses of the cavernosa tissues to phenylephrine in this study.

The contraction of the cavernosa tissue after adding doses of potassium chloride caused significantly higher contractions in the caffeine and recovery groups (Figure 2). This suggests that a caffeine-induced increase in the activity of the voltage-operated Ca^{2+} channels (stimulated by the K^{+} influx) persisted in the cavernosa tissue during the recovery period. However, cumulative doses of calcium chloride resulted in significantly reduced contraction in the recovery group when compared to the caffeine and control groups (Figure 3). Caffeine-containing coffee was reported to inhibit K^{+}-induced contractions in aortic and ileal smooth muscle. Caffeine also inhibited Ca^{2+}-induced contraction in vascular and intestinal smooth muscle (Watanabe et al., 1992). Our results show that caffeine-induced contraction varies in the cavernosa tissue as compared to the vascular and intestinal smooth muscle.

The relaxation of the cavernosa tissue was diminished (Figure 4) in the presence of a prostacyclin inhibitor (indomethacin) in the caffeine-treated group. It could be suggested that caffeine promotes the output of prostacyclin in cavernosa tissue, similar to what was reported by Eccheverri et al. (2010). The prostacyclin-releasing effect of caffeine was not observed in the recovery group (Figure 4).

There is no difference in the relaxation response of cavernosa tissue following incubation with methylene blue (a guanylyl cyclase inhibitor) in the caffeine-treated group or the control. Relaxation (%) of the cavernosa tissue was, however, higher in the recovery group (Figure 6). This shows that caffeine exposure may impair the activity of the soluble guanylyl cyclase-nitric oxide (NO)-cGMP pathway during recovery from caffeine. The incubation of the cavernosa tissue with an adenosine triphosphate-sensitive K^{+}-channel inhibitor (glibenclamide) significantly reduces the acetylcholine-mediated cavernosa relaxation in the recovery group as compared to the caffeine group (Figure 5). This suggests that caffeine consumption mitigates the detrimental effect of glibenclamide on cavernosa relaxation.

The acetylcholine-mediated relaxations (%) in the cavernosa tissues were higher in the caffeine group than in the recovery group after incubation with nifedipine (Figure 7). Nifedipine stimulates relaxation by inhibiting voltage-gated large-conductance calcium channel activity (Elliott and Ram, 2011). According to Williams et al. (2018), nifedipine and diltiazem appear to increase sexual function. Nifedipine had no negative impact on sexual function, according to Doumas and Douma (2006). The current study demonstrates that caffeine treatment potentiates nifedipine-induced cavernosa tissue relaxation.

Neurons, sinusoidal endothelium, and corporeal smooth muscle cells are potential nitric oxide producers in the penis (Cartledge et al., 2001). Nitric oxide is thought to interact with certain molecular targets to elicit a variety of functional effects. Numerous regulatory variables affect both its production and activity in the penis (Priviero et al., 2007). Through increased NO bioavailability, caffeine improves endothelial function (Higashi, 2019). In the current study,

after the incubation of cavernosa tissues with a nitric oxide synthase inhibitor (L-NAME), the caffeine-treated group showed a considerably higher relaxation response to acetylcholine than the recovery and control groups (Figure 8). The observed increase in the caffeine group could be the result of alternative nitric oxide synthase-independent ways of caffeine-induced relaxation. Caffeine has been found to boost prostacyclin output from the perfused rat mesenteric vascular bed (Echevarri et al., 2010). According to Joshi et al. (2012), the ryanodine receptor found in cavernosa tissue is crucial for relaxation. Ryanodine also enhanced prostaglandin production, indicating that caffeine may be stimulating a ryanodine receptor (Kong et al., 2008) to cause the relaxation seen in the caffeine-treated group and aid in the release of nitric oxide, as reported by Umemura et al. (2007). This was observably absent in the recovery group.

Lastly, the maximum (%) relaxation response of the cavernosa tissue to SNP, which was comparable to the control in the caffeine-treated group, may indicate that caffeine has no adverse effects on erectile function and that its withdrawal can decrease the release of nitric oxide, as was seen in the recovery group (Figure 9).

Conclusion

Prepubertal caffeine intake improved testosterone levels. Caffeine-induced cavernosa tissue relaxation was not impaired with glibenclamide, nifedipine, or L-NAME. Caffeine-induced cavernosa tissue relaxation was impaired with indomethacin and methylene blue. These effects were reversed during the recovery.

Author Contributions

Conceptualization: S.A.S., Methodology: S.A.S. & H.M.S., Software: S.A.S., B.A.M., Validation: S.A.S., Formal Analysis: S.A.S., H.M.S. & B..AM., Investigation: B..OK., B.A.M., Resources: S.A.S., H.M.S., B.O.K., Data Curation: S.A.S., H.M.S., B.O.K., M.O.A., Writing – Original Draft: S.A.S., Writing – Review & Editing: S.A.S., H.M.S., B.A.M., M.O.A., Supervision: S.A.S. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Ethical Disclosures

The study was also certified by the Lagos State University College of Medicine Animal Ethics Committee with reference number AREC/2022/054.

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