Chronic exposure to delta-9-tetrahydrocannabinol impacts testicular volume and male reproductive health in rhesus macaques

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Objective: To determine the dose-dependent effect of delta-9-tetrahydrocannabinol (THC) exposure on male testes and reproductive health in a nonhuman primate model.
Design: Research animal study.
Setting: Research institute.
Animal(s): Adult male rhesus macaques 8–10 years of age (n = 6).
Intervention(s): Daily edible THC at medically and recreationally relevant doses.
Main Outcome Measure(s): Testicular volume and epididymal head width, serum levels of inhibin B, albumin, total testosterone, prolactin, follicle-stimulating hormone, estradiol, and luteinizing hormone; semen volume; and sperm motility, morphology, and concentration.
Result(s): For each 1 mg/7 kg/day increase in THC dosing, there was a marked loss in total bilateral testicular volume of 11.8 cm^3 (95% confidence interval [CI]: 8.3–15.4). In total, average bilateral testicular volume decreased by 58%. Significant dose-response decreases in mean total testosterone level by 1.49 ng/mL (95% CI: 0.83–2.15) and in estradiol level by 3.8 pg/mL (95% CI: 2.2–5.4) were observed, but significant increases in the levels of follicle-stimulating hormone by 0.06 ng/mL (95% CI: 0.02–0.10), luteinizing hormone by 0.16 ng/mL (95% CI: 0.08–0.25), and prolactin by 7.4 ng/mL (95% CI: 3.4–11.3) were observed. There were no statistically significant changes in semen parameters.
Conclusion(s): In rhesus macaques, chronic exposure to THC resulted in significant dose-response testicular atrophy, increased serum gonadotropin levels, and decreased serum sex steroids, suggestive of primary testicular failure. Further studies are needed to determine

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if reversal of these observed adverse effects would occur if THC was discontinued and for validation of thefindings in a human cohort. (Fertil Steril® 2022;117:698-707. ©2021 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Cannabis, delta-9-tetrahydrocannabinol, male reproductive health, marijuana, testicular volume

Marijuana is the most commonly used federally illegal drug in the United States and worldwide, with increasing popularity as both a recreational and a medicinal drug, especially among men of reproductive age (1, 2). In 2015, the estimated prevalence of past year use among 18- to 25-year–old men in the United States was 36% (or approximately 7.4 million) (3). This high prevalence is due in part to the recent trend in legalization, which has increased the availability of marijuana products and their perceived safety. The action of the main active ingredient of marijuana, delta-9-tetrahydrocannabinol (THC), is mediated through cannabinoid receptors 1 and 2. The results of published studies on the effect of exposure to marijuana on male infertility are inconsistent. Previous studies demonstrated that cannabinoid receptors are present in the male reproductive tract (4) and on sperm and that the endocannabinoid system has a role in regulating male reproduction, suggesting the potential for marijuana to disrupt sperm function (5, 6). Given the increasing prevalence of marijuana use, it is critical to determine whether chronic marijuana use, a modifiable risk factor, adversely impacts male reproductive health.

The hypothalamic-pituitary-gonadal (HPG) axis plays a critical role in both spermatogenesis and testosterone production. This axis regulates the release of 2 gonadotropin hormones vital for reproduction, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), which act on cells in the testes, including the Leydig cells, which produce testosterone. Animal studies suggest that the impact of the endocannabinoid system on spermatogenesis (7) includes inhibition of Leydig cell function and steroidogenesis, reduction in gonadotropins (8–10), testicular atrophy (11–15), and abnormal sperm morphology (16, 17) after acute exposure to THC. The effect of chronic marijuana use in men is uncertain, with some studies reporting an association with lower testosterone and LH levels (18, 19) and poorer semen quality (20–23), whereas other studies could not replicate these findings (20, 22, 24–26). A large, population-based study of healthy young Danish men reported an increase in testosterone levels, but lower sperm count, among regular marijuana smokers (22). However, the direct effects of regular exposure to marijuana on sperm concentration, motility, and function have not been well studied.

Human studies assessing the effects of exposure to marijuana on the male reproductive system have focused on men typically recruited from assisted reproduction centers or with histories of polysubstance abuse. This limits the generalizability of the findings and precludes determination of a causal effect specific to marijuana (16, 27–30). Most of these studies were observational or retrospective. In addition, unlike alcohol, where a shot, a glass of wine, and a beer have similar alcohol content based on the volume of alcohol they contain, no such equivalency for marijuana exists, because the different strains of marijuana plants and delivery mechanisms vary in potency, and there is no consistent labeling and formulation (31). This heterogeneity in marijuana potency has resulted in limited knowledge available to counsel patients who are unable to abstain regarding a dose-response effect from marijuana use.

To understand the impact of chronic marijuana use on male fertility and reproductive health, a relevant translational animal model, such as the nonhuman primate (NHP), can overcome the obstacles and limitations of human studies. Compared with other animal models, the NHP offers many advantages, including plasma THC disposition (32, 33) and physiologic, genetic, anatomic, and endocrine properties similar to those in humans, resulting in observations that are directly translatable to humans (34, 35). In addition, the NHP model allows for minimization of intersubject variability and potential confounders to determine the direct effects of THC only. The objective of our study was to determine the dose-dependent effect of THC exposure on male testes and reproductive health in a NHP model.

MATERIALS AND METHODS

Experimental Design

A cohort of sexually mature, adult male rhesus macaques (*Macaca mulatta)* (n = 6) aged 8 to 10 years and weighing 9.3 to 12.7 kg, with prior proven paternity, were used in this study. The animals were socially housed, and all procedures were approved by the Oregon National Primate Research Center (ONPRC) Institutional Animal Care and Use Committee and conformed to all applicable regulations (IP0001389). The animals were maintained on a standard chow diet (Test-Diet, St. Louis, MO) with a daily cookie containing research-grade edible THC obtained directly from the National Institute of Drug Administration (NIDA) Drug Supply Program, as previously described (36). The animals were fed a diet of fresh chow and produce enrichment, and water was available ad libitum. The cookies were administered before the animals' morning chow to ensure that they were consumed on an empty stomach and to confirm complete ingestion. The dose of THC was slowly titrated up to 2.5 mg/7 kg/day, with a dose increase every 70 days (the life cycle of NHP sperm is approximately 64 days) over a period of approximately 7 months to conform to published medical marijuana acclimation recommendations (37). Specifically, the animals were maintained on a dose of THC of 0.5 mg/7 kg/day on days 1 to 70, 1 mg/7 kg/day (moderate THC dose) on days 71 to 140, and 2.5 mg/7 kg/day (heavy THC dose) on days 141 to

210. This dose was calculated from the recommended THC starting dose of 5 mg (NIDA's designated standard unit of THC for research) for a 68-kg man, followed by titration to 10 mg for moderate users and to 20–30 mg for heavy users (37–39). Most states with legalized marijuana consider 10 mg of THC a single serving of marijuana (37). To minimize potential confounders and interanimal variability, each animal served as its own control during the study.

Two-milliliter samples of blood were obtained at each dose adjustment time point during THC induction, 3 hours (37, 39) after consumption of THC, to determine peak THC concentrations with each increase in THC dosage. Immediately before each increase in dosage, the animals were weighed and scrotal ultrasound and semen analysis were performed.

Scrotal Ultrasound

The animals underwent scrotal ultrasound examination before THC was initiated and at the end of each THC dosing period. Testicular volume was calculated by measurement of the maximal length (longitudinal diameter), width (transverse diameter), and height (anterior-posterior diameter) of the testes. The maximal length and width of the epididymal head were measured in the longitudinal plane. These scans were performed with the animals nonsedated in a sitting position by a single, ultrasound-trained practitioner (J.O.L.), using image-directed pulsed and color Doppler equipment with a 5- to 9-MHz sector probe (Voluson; GE Healthcare, Duluth, Georgia) according to standard human clinical practice protocols. Testicular volume was calculated by the standard clinical formula length × width × height. Color Doppler ultrasonography was used to evaluate the symmetry of flow to both testes, the testicular vasculature, and the presence of varicoceles, which can affect sperm quality and male fertility.

Semen Collection and Processing

Before the initiation of THC, the animals were trained by the ONPRC Behavioral Services Unit in collaborative semen collection by nonsedated electro-ejaculations (40). The collections were performed on 3 separate occasions before THC dosing for baseline semen measurements and at the end of each THC dosing time point. The semen samples were collected and allowed to liquefy at 37°C for 30 minutes before evaluation. The ejaculate of the rhesus macaque is composed of liquid and solid (coagulum) fractions, both of which were measured. The volume of the liquid fraction was measured with a pipette, and the liquid fraction was then transferred into a sterile 15-mL conical tube. The coagulum was rinsed with 2 to 3 mL of warm *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid (Hepes)-buffered tyrode albumin lactate pyruvate (TALP-Hepes) with bovine serum albumin supplemented at 3 mg/mL to recover additional sperm, and the rinse was combined with the liquid fraction before adding TALP-Hepes quantity sufficient to a volume of 12 mL. The sample was washed at 300 × g for 7 minutes. After the first wash, the supernatant (11 mL) was aspirated and the sperm pellet was resuspended in the remaining 1 mL. An aliquot was further diluted 1:20 into TALP-Hepes and analyzed by a computer-assisted sperm analysis system (IVOS II-Animal Motility software, version 1.11; Hamilton Thorne, Beverly, MA), programmed specifically for macaque sperm physiology, to determine sperm motility parameters and concentration. The remaining sample was centrifuged again at 300 × *g* for 7 minutes to obtain a pellet. The supernatant was discarded, and another aliquot was taken and diluted (1:60 to 1:100) for assessment of plasma membrane integrity (Live/Dead Sperm Viability Kit, Catalog #L7011; Molecular Probes, Eugene, OR), mitochondrial membrane potential/mitochondrial activity (MitoTracker Orange, Catalog #M7510; Molecular Probes), and gross sperm morphology (10% Formalin solution). To assess plasma membrane integrity, the washed sperm were mixed with the Live/Dead stain and incubated at 37°C for 10 minutes, and a smear was prepared. A total of 100 spermatozoa were classified as live (intact plasma membrane) or dead (injured plasma membrane) with the use of a fluorescence microscope. Assessment of mitochondrial membrane potential was performed by mixing washed sperm with MitoTracker Orange and incubating them at 37°C for 20 minutes before a smear was prepared. A total of 100 spermatozoa were classified as having active (stained) or inactive (nonstained) mitochondria with the use of a fluorescence microscope. For assessment of gross sperm morphology, sperm were diluted in a prewarmed (37°C) 10% Formalin solution and resuspended, and 10 μL of fixed spermatozoa was placed on a microscope slide under a coverslip and analyzed by standard clinical protocol per World Health Organization criteria (41). A total of 100 spermatozoa were assessed with the use of a phase contrast microscope at ×1,000 magnification under oil immersion. The spermatozoa were classified into 5 main categories (abnormal head, abnormal midpiece, sharp bend/coiled tail, double head/tail, or normal), and the percentages of normal sperm were compared across treatment groups.

Serum Hormones

Two-milliliter peripheral blood samples were collected before administration of THC and at each THC dose for measurement of the concentrations of total testosterone, FSH, LH, estradiol (E2), prolactin (PRL), inhibin, and albumin by detailed assays performed by the Endocrine Technologies Core at the ONPRC. These hormones were measured to determine the effect of THC on the HPG axis and their critical role in regulating spermatogenesis.

Total testosterone. Total testosterone concentrations were measured by automatic immunoassay on a Roche Cobas e411 instrument (Roche Diagnostics, Indianapolis, IN). The range of the assay was 0.025–15 ng/mL. The intraassay coefficient of variation (CV) using an in-house NHP serum quality control (QC) pool was 4.2% (n = 1 assay).

FSH and LH. Follicle-stimulating hormone and LH concentrations were measured by a double-antibody radioimmunoassay procedure similar to that described by Niswender and Spies (42). The LH and FSH radioimmunoassay kits were purchased from Dr. Albert Parlow (National Hormone and Pituitary Program, Harbor-University of California Los

Angeles Medical Center, Los Angeles, CA). These are homologous cynomolgus macaque assays with recombinant cynomolgus FSH (AFP-6940A) or LH (AFP-6936A) for both iodination and standards. Rabbit anti-cynomolgus FSH (AFP-782594) or LH (AFP-342994) was used at final dilutions of 1:1,038,462 and 1:750,000 for FSH and LH, respectively. The standard curves ranged between 0.005 and 10 ng/tube for both assays. The detection limit of each assay was 0.005–0.02 ng/tube. The intraassay variation was 6.5% and 4.7% for FSH and LH, respectively (n = 1 assay).

Estradiol. Estradiol concentrations were measured by automatic immunoassay on a Roche Cobas e411 instrument (Roche Diagnostics, Indianapolis, IN). The range of the assay was 5 to 4,300 pg/mL. The intraassay CV using an in-house NHP serum QC pool was 0.7% (n = 1 assay).

Prolactin. Prolactin concentrations were measured by automatic immunoassay on a Roche Cobas e411 system. The range of the assay was 0.047 to 470 ng/mL. The intraassay CV using an in-house NHP serum QC pool was 4.6%.

Inhibin B. Inhibin B concentrations were measured by enzyme-linked immunoassay, following the manufacturer's instructions (Beckman-Coulter, Pasadena, CA). The range of the assay was 11.5 to 1,100 pg/mL. The intraassay CV was 4.3%, and the interassay CV was 6.9% (n = 2 assays).

Albumin. Albumin concentrations were measured by enzyme-linked immunoassay, following the manufacturer's instructions (MilliporeSigma, St. Louis, MO). The samples were diluted by 1:50,000 to 1:1,000,000 for analysis. The range of the assay was 4,915 to 1,200 ng/mL. The intraassay CV was 2.3%, and the interassay CV was 9.0% (n = 2 assays).

Delta-9-tetrahydrocannabinol Testing

Chemicals and reagents. Strata Impact Protein precipitation plates and 2-mL collection plates were obtained from Phenomenex (Torrance, CA). Oasis Prime elution plates and 1-mL round collection plates were obtained from Waters (Milford, MA). Delta-9-tetrahydrocannabinol and metabolites as well as their deuterated internal standards were purchased from Cerilliant (Round Rock, TX). Acetonitrile, methanol, and water were purchased from Honeywell (Mexico City, Mexico), and formic acid, together with sample vials and other high-performance liquid chromatography supplies, was purchased from Fisher Scientific (Rockwood, TN). Human ethylenediaminetetraacetic acid Plasma for standards was purchased from Innovative Research (Novi, Michigan) with a voluntary drug-free affidavit, although multiple samples were tested before a drug-free matrix was obtained. Control rhesus macaque plasma was used for initial testing and quality controls. Preparation of plasma and calibration were performed according to our previously published protocol (36).

Liquid chromatography with tandem mass spectrometry analysis of cannabinoid metabolites. Delta-9-tetrahydrocannabinol and metabolites were analyzed with a 5500 Q-TRAP hybrid/triple quadrupole linear ion trap mass spectrometer (SCIEX, Framingham, MA) with electrospray ionization in positive mode according to our previously published protocol (36).

Statistical Analysis

We assessed the average association between THC dose and plasma THC levels, testicular measures, hormone levels, and semen parameters using linear mixed effects modeling with random intercepts by animal. We generated scatter plots of individual animal measurements with the predicted marginal changes from the mixed effects models for all outcomes. All statistical tests were two-sided with an alpha of 0.05. All analyses were performed with Stata, version 15.1 (StataCorp, College Station, TX).

RESULTS

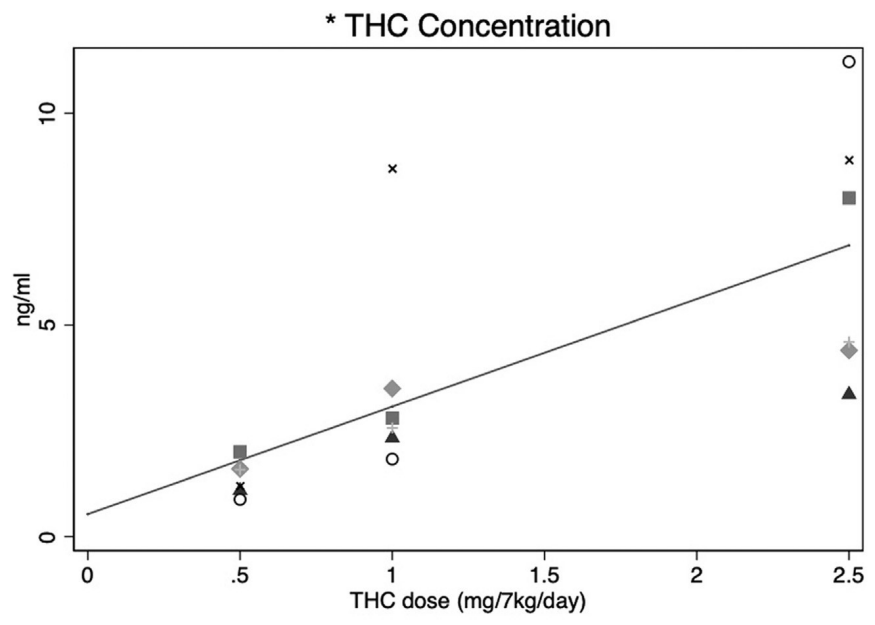
All 6 male rhesus macaques in this study were of reproductive age (mean 9.1 years, SD = 0.6) with prior proven paternity and no previous exposure to THC or any other significant known environmental exposures. The mean baseline weight was 11.6 kg (SD = 1.4) and was 11.9 kg (SD = 1.3) at the highest THC dose; all animals gained weight after starting the THC treatment. For each increase of 1 mg/7 kg/day after starting THC, there was an increase in the average weight of all animals of 0.1 kg, which was not significant (P=.095). The animals' behavior after THC treatment was not noted to be grossly different by the veterinary and animal support staff.

During THC induction, the average plasma THC concentration increased by 2.54 ng/mol for each 1 mg/7 kg/day increase in THC (95% confidence interval [CI]: 1.35–3.73 ng/mol, P<.001) (Fig. 1). With increasing THC dosing, a marked decrease in testicular volume was observed; the average total bilateral testicular volume decreased by 58%. The average total bilateral testicular volume decreased by 12.6 cm^3 for each 1 mg/7 kg/day increase in THC (95% CI: 10.4–14.9, P<.001) (Fig. 2). The left epididymal head width decreased by 0.15 cm (95% CI: 0.10–0.20, P<.001) and the right epididymal head width decreased by 0.13 cm (95% CI: 0.08–0.19, P<.001) for each 1 mg/7 kg/day increase in THC (Fig. 2). No scrotal masses or varicoceles were noted on ultrasound or physical examination.

With regard to the reproductive endocrine axis, serum FSH, LH, and PRL concentrations increased significantly with increasing THC dose. Follicle-stimulating hormone increased by 0.06 ng/mL (95% CI: 0.02–0.10, P=.001), LH by 0.16 ng/mL (95% CI: 0.08–0.25, P<.001), and PRL by 7.4 ng/mL (95% CI: 3.4–11.3, P<.001) for each 1 mg/7 kg/day increase in THC (Fig. 3). In contrast, a significantly decreased dose-response of 1.49 ng/mL (95% CI: 0.83–2.15, P<.001) was observed for testosterone and of 3.8 pg/mL (95% CI: 2.2–5.4, P<.001) for E2 for each 1 mg/7 kg/day increase in THC (Fig. 3). As anticipated, there was no statistically significant change in albumin concentration (P=.735).

There were no statistically significant changes in any semen characteristics (Table 1), including weight of coagulum (P=.084), liquid fraction volume (P=.177), sperm concentration (P=.187), total sperm count (P=.128), sperm motility (P=.394), and sperm morphology (P=.438), with increasing THC dose.

FIGURE 1



Plasma delta-9-tetrahydrocannabinol (THC) concentrations with increasing THC dosing. Individual (*symbols*) and average fixed effect (*line*) plasma THC concentrations (ng/mol) in response to increasing oral THC dosage (0–2.5 mg/7 kg/day) in 6 male rhesus macaques. *$P<.001$.

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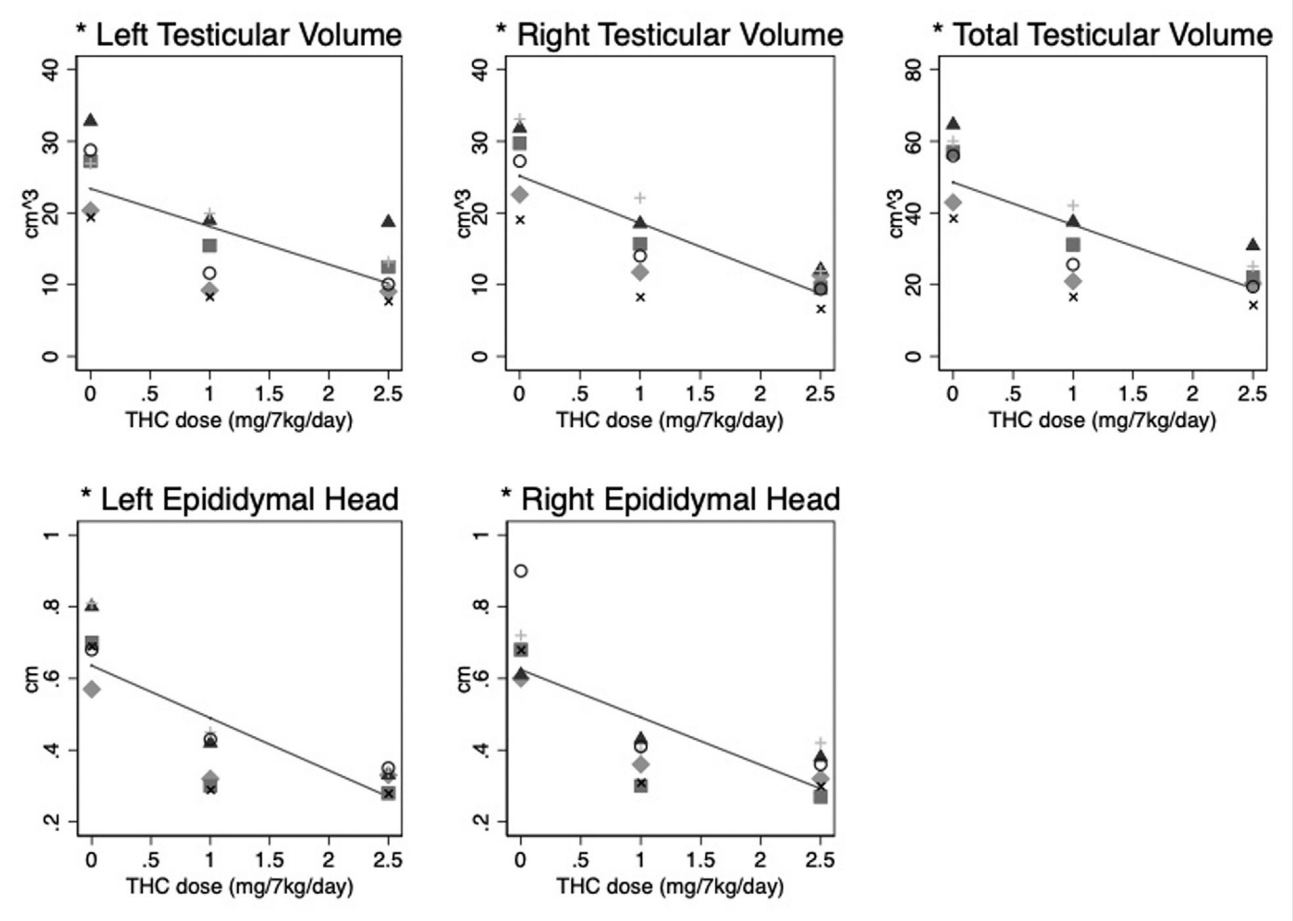
Discussion

To our knowledge, this is the first study using a NHP model to examine the impact of chronic THC use on male reproductive health. Our study found a positive correlation between oral THC dosage and plasma THC concentration, as previously demonstrated. Our study used plasma (36). The average male plasma THC concentrations for the highest oral THC edible dose were within the expected dosing range reported in humans 3 hours after consuming a similar oral THC dose (39, 43). As observed in our previous study (36) and consistent with the existing literature, we did not find a significant effect of THC on weight gain (44).

A significant dose-response impact of chronic THC use on testicular volume and epididymal head width was observed. The average bilateral total testicular volume decreased by 58%. The males were exposed to THC for a total of approximately 7 months (i.e., 3 sperm life cycles), with only the last 2.5 months at an equivalent heavy medical marijuana dose. Previous animal studies have suggested a dose-dependent association between chronic exposure to marijuana and decreased prostate and seminal vesicle weight (12, 14, 15, 36, 45) in mice and rats as well as testicular atrophy in dogs (11). The underlying mechanism of these observed testicular effects is unclear, but it is likely a result of exposure to THC, given that expression of cannabinoid receptors has been demonstrated throughout the male reproductive tract, including the testis, specifically the Sertoli cells, epididymis, seminal vesicles, and prostate (4). Previous studies have suggested that exposure to THC is associated with oxidative stress and decreased antioxidant enzymes in the affected testicles (46, 47). Further histologic studies examining THC-exposed testicular tissue for morphologic regional changes are needed to understand the mechanisms involved.

We observed a significant impact of THC on male reproductive hormones. The rise in LH and FSH with increased exposure to THC, coupled with decreased testosterone and E2, suggests that primary testicular failure is the mechanism of hormonal dysregulation by THC. The few studies (48) that have investigated the effect of acute or chronic exposure to marijuana on FSH levels in males have found minimal effects, whereas there is consistent evidence to support the inhibitory effect of marijuana on LH. Plasma LH is significantly acutely and chronically depressed after a person smokes marijuana (48), and there is a weaker LH response to exogenous gonadotropin-releasing hormone in marijuana smokers compared with nonsmokers (49). In a study comparing 2 groups of chronic THC users, no difference in LH levels was noted between those who smoked 5–9 cigarettes weekly and those who smoked more than 10 cigarettes weekly, suggesting an upper limit of the dose-dependent effect of THC on LH (50). Studies of the effect of THC on testosterone levels in humans and animals have produced mixed findings (19, 51). Gundersen et al. (22) reported 7% higher testosterone levels in male THC users than in nonusers, whereas a subsequent study using the National Health and Nutrition Examination Survey database found no difference in testosterone levels between those who had ever used marijuana and those who had never used marijuana (52) and found that the effect of THC on testosterone may be acute and transient. In comparison, our study results showed that

FIGURE 2



Significantly decreased testicular volume and epididymal head width with increasing delta-9-tetrahydrocannabinol (THC) dosing. Individual (*symbols*) and average fixed effect (*lines*) testicular volume (cm^3) and epididymal head width (cm) in response to increasing oral THC dosage (0–2.5 mg/7 kg/day) in 6 male rhesus macaques. *$P<.001$.

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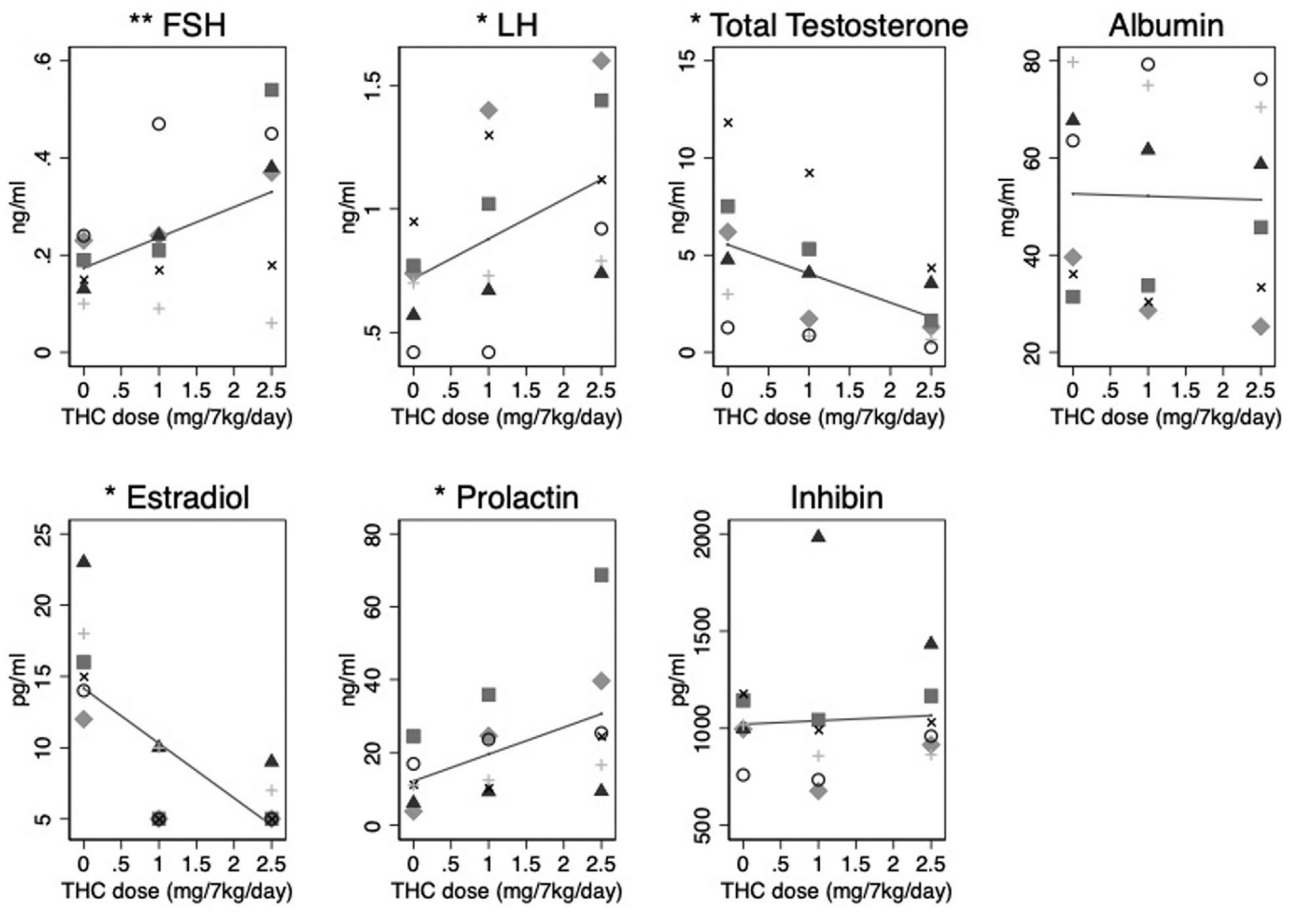
direct, dose-dependent effects of THC on the HPG axis, not confounded by smoking marijuana, were significant and showed a pattern of primary testicular failure. In addition, although serum PRL levels were increased with higher THC doses, the levels were not high enough to inhibit the release of gonadotropins from the anterior pituitary gland and affect spermatogenesis. In contrast, other animal and human studies have found no differences in PRL levels between marijuana users and controls (49, 53).

We did not observe clear changes in semen characteristics with exposure to THC. The absence of statistically significant changes seen in our study may be because of interanimal variability, consistent with the reported high variability of the results of analysis of semen samples taken from the same human subject (54). This variability is present even with strict abstinence intervals, which is why 2 semen analyses are performed in the evaluation of infertile men. Other animal and human studies have reported an impact of marijuana on semen parameters, including changes in motility, morphology, and sperm count and concentration (22, 55–57), but their study designs utilized a single ejaculate collection. Given the degree of variation observed between multiple semen analyses in a single subject, caution should be taken when comparing studies using single vs. multiple ejaculates to assess changes or trends in semen characteristics. In addition, the absence of change in semen characteristics, despite a significant decrease in total testosterone, may be because a critical level of total testosterone was still produced by the Leydig cells to interact with androgen-binding protein secreted by the Sertoli cells to maintain adequate total testosterone concentrations in the seminiferous tubules to support sperm production and development. Although the semen parameters did not change appreciably, we do not know the direct effects of THC on the ability of sperm to undergo capacitation, traverse the female reproductive tract, and fertilize an egg to form a normal embryo. This would be important to pursue in a future study.

Our study had several strengths. To our knowledge, it is the first NHP study examining a dose-response effect of THC on male reproductive characteristics. This animal model

FIGURE 3



Follicle-stimulating hormone (FSH), luteinizing hormone (LH), total testosterone, albumin, estradiol, prolactin, and inhibin concentrations with increasing delta-9-tetrahydrocannabinol (THC) dosing. Individual (*symbols*) and average fixed effect (*lines*) FSH (ng/mL), LH (ng/mL), total testosterone (ng/mL), albumin (μg/mL), estradiol (pg/mL), prolactin (ng/mL), and inhibin (pg/mL) concentrations in response to increasing oral THC dosage (0–2.5 mg/7 kg/day) in 6 male rhesus macaques. *$P<.001$, **$P=.001$. All other hormone associations are not significant at the.05 level.

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provides precise control over experimental variables such as age, weight, prior proven paternity, and quantity of THC administered. To ensure rigor and reproducibility, the THC administered in this study was in an edible form to avoid toxins from smoke; it was from a single source, the NIDA Drug Supply Program; and it utilized the standardized THC unit for research following recent NIDA guidelines. To minimize interanimal variability, each male was his own control with similar environmental exposures, including diet and housing. In addition, seasonal changes in semen parameters have been reported in NHPs that were mostly housed outdoors, unlike our study, in which the animals were housed indoors. These variables are often inconsistent in humans and confound the results of human studies. Compared with other smaller animal studies, this NHP study provides further understanding of the impact of increasing THC dose on male testes and reproductive health that is translatable and applicable to humans.

A limitation of this study was the size of the animal cohort, but this was addressed by using a single-case experimental design in which each male served as his own control. The observed significant impact on male reproductive hormones and testicular volume in our study was suggestive of a dose-response effect. However, because the THC dose was increased after 70 days (i.e., one sperm life cycle), it is possible that the changes seen were in part because of the duration of exposure to THC rather than the size of the THC dose.

In summary, our study indicates a significant adverse dose-response effect on male testes and reproductive health from chronic THC exposure. Further studies are needed to determine the impact of a longer duration of exposure and whether these observed effects are permanent or can be reversed by abstinence from THC. Because marijuana use is becoming more common, with significantly increased potency, our study provides important insight regarding the potential consequences of marijuana use that would help

TABLE 1

Mean semen characteristics (±SD) of 6 rhesus macaques at 3 doses of oral delta-9-tetrahydrocannabinol (0–2.5 mg/7 kg/day) and change with each 1 mg/7 kg/day increase in delta-9-tetrahydrocannabinol dose, with 95% confidence intervals and associated *P* values, from random intercept mixed effects model.

Characteristic	0 mg/7 kg/day THC	1 mg/7 kg/day THC	2.5 mg/7 kg/day THC	Change per 1 mg/7 kg/day THC dose	95% CI	*P*-value
Weight of coagulum (g)	0.80 ± 0.31	0.47 ± 0.13	0.54 ± 0.16	−0.09	−0.20 to 0.01	.084
Liquid fraction volume (mL)	0.45 ± 0.14	0.26 ± 0.16	0.32 ± 0.18	−0.05	−0.12 to 0.02	.177
Sperm concentration (millions/mL)	795 ± 326	1,483 ± 1,005	1,388 ± 1,464	214	−104 to 531	.187
Total sperm count (millions)	338 ± 135	274 ± 166	269 ± 189	−26	−59 to 7	.128
Motility (%)	88.9 ± 6.2	86.3 ± 8.4	87.0 ± 7.9	−0.7	−2.3 to 0.9	.394
Morphology (% normal)	57.8 ± 26.0	55.0 ± 18.8	51.2 ± 22.1	−3.0	−10.6 to 4.6	.438

Note: CI = confidence interval; THC = delta-9-tetrahydrocannabinol.

Hedges. Marijuana and male reproductive health. Fertil Steril 2021.

providers guide couples who are interested in conception or are affected by male infertility.

CONCLUSION

These data suggest that increasing the amount of chronic THC consumption, even at moderate doses, has an adverse impact on male reproductive health and results in a significant dose-response relationship to testicular atrophy, increased gonadotropins, decreased serum sex steroids, and decreased semen volume.

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La exposición crónica al delta-9-tetrahidrocanabinol impacta el volumen testicular y la salud reproductiva del macho en macacos Rhesus.

Objetivo: Determinar el efecto dosis-dependiente de la exposición a delta-9-etrahidrocanabinol (THC) en testículos de macho y en la salud reproductiva en un modelo de primate no humano.

Diseño: Estudio de investigación en animales.

Marco: Instituto de investigación.

Animales: Macacos Rhesus macho adultos de 8 a 10 años de edad (n = 6).

Intervenciones: THC comestible diario en dosis médica y recreacionalmente relevantes.

Principal medida de resultado: Volumen testicular y anchura de la cabeza del epidídimo, niveles séricos de inhibina B, albúmina, testosterona total, prolactina, hormona foliculoestimulante, estradiol y hormona luteinizante; volumen de semen y movilidad, morfología y concentración del esperma.

Resultados: Por cada incremento de 1 mg/7 kg/día en la dosis de THC, hubo una marcada pérdida de volumen testicular de 11,8 cm3 (intervalo de confianza del 95% [IC95]: 8,3 – 15,4). En total, el volumen testicular bilateral medio decreció un 58%. Se observaron bajadas significativas y dosis-dependientes en nivel medio de testosterona total de 1,49 ng/mL (IC95: 0,83 - 2,15) y de estradiol de 3,8 pg/mL (IC95: 2,2 – 5,4), pero se observaron incrementos significativos en los niveles de hormona foliculoestimulante de 0,06 ng/mL (IC95: 0,02 – 0,10), hormona luteinizante de 0,16 ng/mL (IC95: 0,08 – 0,25) y prolactina de 7,4 ng/mL (IC95: 3,4 – 11,3). No hubo cambios estadísticamente significativos en los parámetros de semen.

Conclusiones: En macacos Rhesus, la exposición crónica al TCH resultó en atrofia testicular, aumento de niveles de gonadotropinas y disminución de niveles séricos de esteroides sexuales de manera significativa y dosis-dependiente, sugiriendo un fallo testicular primario. Se necesitan futuros estudios para determinar si estos cambios son reversibles al descontinuar el THC y para validar estos hallazgos en una cohorte humana.