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Original Contribution

Association Between Use of Marijuana and Male Reproductive Hormones and Semen Quality: A Study Among 1,215 Healthy Young Men

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A total of 1,215 young Danish men aged 18–28 years were recruited between 2008 and 2012 when they attended a compulsory medical examination to determine their fitness for military service. The participants delivered a semen sample, had a blood sample drawn, and underwent a physical examination. They responded to questionnaires including information on marijuana and recreational drug use during the past 3 months (no use, use once per week or less, or use more than once per week). A total of 45% had smoked marijuana within the last 3 months. Regular marijuana smoking more than once per week was associated with a 28% (95% confidence interval (CI): −48, −1) lower sperm concentration and a 29% (95% CI: −46, −1) lower total sperm count after adjustment for confounders. The combined use of marijuana more than once per week and other recreational drugs reduced the sperm concentration by 52% (95% CI: −68, −27) and total sperm count by 55% (95% CI: −71, −31). Marijuana smokers had higher levels of testosterone within the same range as cigarette smokers. Our findings are of public interest as marijuana use is common and may be contributing to recent reports of poor semen quality.

drug use; male reproductive health; marijuana; semen quality; sex hormones

Abbreviations: BMI, body mass index; CI, confidence interval; THC, tetrahydrocannabinol.

Editor's note: *An invited commentary on this article appears on page 482.*

Marijuana is the most widely used illicit recreational drug in the Western world with reported use among 13.7% in the United States and 17.6% in Denmark in 2012 and 2013 (1, 2) and users being predominantly males (3). In most countries, marijuana is still an illegal drug, yet more and more countries are legalizing marijuana for recreational use. The active component of marijuana is Δ^9-tetrahydrocannabiol, which has been shown to have receptors in both the brain and the testis (4). Over the past years, marijuana has been found to affect the brain, increasing the risk of psychotic disorders, and to change hormone levels: Chronic abuse of marijuana can lead to cognitive deficits (3, 5). Yet few studies have investigated the association between marijuana and male reproduction. Previous studies have been conducted among men attending infertility clinics, or in small populations of chronic users, and among men suffering from malnutrition and using other recreational drugs (6–8).

We therefore studied the association between marijuana use and semen quality and hormone levels among 1,215 young, healthy, unselected men of whom 45.4% had used marijuana during the past 3 months.

METHODS

Population

Because of the military draft in Denmark, all men aged 18 years, except those suffering from severe chronic disease, are required to undergo a compulsory physical examination to determine their fitness for military service. Some men postpone their examination until completion of their education. Since 1996, trained staffs from the Department of Growth and Reproduction at Copenhagen University Hospital (Rigshospitalet,

Copenhagen, Denmark) have approached draftees when they appeared for their compulsory physical examination and invited them to participate in a study of semen quality. Men recruited from January 2008 to June 2012 were included in the present study because they were asked detailed questions about marijuana and/or recreational drug use. A total of 1,221 men, representing 30% of those who were invited, agreed to take part in the study and completed a questionnaire, delivered a semen sample, had a blood sample drawn, and underwent a physical examination. They received kr500 (approximately US $85) in compensation for their time.

Participants did not differ from nonparticipants with regard to age, but they were generally better educated than nonparticipants (data not shown). Study approval was obtained from the local ethics committee. A detailed description of the study has been published previously (9, 10).

Table 1. Information From Questionnaire and Physical Examination According to Self-Reported Marijuana Use During the Past 3 Months Among 1,221 Young Danish Men, 2008–2012

Variables	Total ($n = 1,221$)		Marijuana Use, %	
	No.	%	No ($n = 661$)	Yes ($n = 554$)
Information obtained at the physical examination				
Examined between October and March	700	58	58	58
Period of abstinence, median hours	1,209	63	62	63
Varicocele stage 2 or 3	97	8	7	9
Body mass index[a]				
<20	212	17	17	18
20–24.99	776	64	63	66
≥25	225	18	20	17
Information obtained from questionnaire				
Age, mean years	1,221	19	19.7	19.5[b]
Alcohol intake >21 units/week[c]	311	26	16	37[b]
Total caffeine intake >300 mg/day	282	23	20	27[b]
Weekly current tobacco smoking	454	37	20	58[b]
Self-reported physical fitness poor or very poor	48	4	8	8
Television watching more than 5 hours per day	422	35	19	20
Stress score above 20	323	27	30	24
Sleep score above 20	163	13	16	11
Exposure in utero to mother's tobacco smoking	270	25	23	27
Maternal educational level, years				
<9	36	3	4	3
9–10	234	19	23	20
>10	803	75	73	77
Missing	142	12	11	13
Fever >38°C within the last 3 months	82	7	7	8
Born with cryptorchidism	74	6	7	5[b]
Self-reported genital conditions[d]	115	10	6	6
Sexually transmitted diseases[e]	115	10	8	12[b]
Use of recreational drugs other than marijuana	132	11	2	21[b]
Mean daily intake of macronutrients based on food frequency questionnaire on 681 men (standard deviation)				
Total energy intake, MJ	9.5	4.3	9.2 (3.9)	10.0 (4.8)
Total fat, % energy	31.2	5.8	31 (5.8)	31.4 (5.7)
Saturated fat, % energy	13.2	2.8	13.1 (2.9)	13.4 (2.6)
Protein, % energy	16.4	3.2	16.3 (3.2)	16.5 (3.1)
Carbohydrate, % energy	56.5	7.5	56.7 (7.6)	56 (7.3)

[a] Weight (kg)/height (m)^2.
[b] $P < 0.05$ by χ^2 test or t test for age.
[c] One unit = 12 g of alcohol.
[d] Self-reported information about torsion of testes, epididymitis, or inguinal hernia.
[e] Sexually transmitted diseases, gonorrhea and chlamydia.

Physical examination

A physician examined the participants for the Tanner stage of pubic hair and genital development, testicular volumes by use of a Prader wooden orchidometer (Pharmacia & Upjohn Company, LLC, London, United Kingdom), location of the testes in the scrotum, and the consistency of the testes and epididymis. The possible presence of variocele (grades 1–3), hydrocele, or any particular malformation was recorded. Height and weight of the men were measured, and body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters.

Questionnaire

Before the examination all the men filled in a questionnaire on previous and/or current genital diseases, such as inguinal hernia, variocele, epididymitis, chlamydia, and gonorrhoea, and whether they had had surgery for testicular torsion. They reported whether they had been responsible for a pregnancy and if they were born with both testes in the scrotum, as well as whether they had had fever above 38°C (100.4°F) in the previous 3 months. Fever was not reported by 6.2% of the men, but they were categorized as not having had fever because their semen quality resembled that of those without fever. Self-reported genital diseases were divided into 2 categories: "self-reported genital conditions" (including torsion of testis, epididymitis, and inguinal hernia) and "sexually transmitted diseases" (including chlamydia and gonorrhea).

They were asked to estimate their fitness by self-reported physical activity converted to watts per week using the method of Craig et al. (11). Men were asked about current tobacco-smoking habits, and daily caffeine intake was estimated on the basis of their reported intake of caffeine-containing beverages the week prior to the visit. They completed a diary reporting their daily intake of red and white wine, beer, strong alcoholic drinks, alcopops, and others alcoholic drinks during the week prior to participation; these were categorized into >20 or ≤20 units per week, the former being the advised maximum intake for men by the Danish Health and Medicines Authority (12). In addition, they were asked about television watching, sleeping habits, and stress 3 months before (categorized as described by Craig et al. (11) and Jensen et al. (13)), and they answered a food frequency questionnaire from which their intake of 681 macronutrients was calculated (12–14). Their mothers answered questions about their tobacco-smoking habits during pregnancy, and the educational level of mothers was coded as below 9 years, 9–10 years, or above 10 years of schooling.

The men were asked, "Have you smoked hashish (pot, marijuana) within the last 3 months?" and "Have you taken recreational drugs other than marijuana (e.g., amphetamine, ecstasy, cocaine) within the past 3 months?" Answer categories were daily, several times per week, at least once per week, less than once per week, and never.

Blood sample

Blood samples were drawn from a cubital vein and centrifuged, and the serum was separated and frozen. Serum levels

Table 2. Semen Quality According to Use of Marijuana Within the Last 3 Months Among 1,221 Young Danish Men, 2008–2012

Frequency of Marijuana Use	No.	Semen Volume, mL			Sperm Concentration, million/mL			Total Sperm Count, million			Motile Sperm, %			Morphologically Normal Forms, %		
		Median	5th Percentile	95th Percentile	Median	5th Percentile	95th Percentile	Median	5th Percentile	95th Percentile	Median	5th Percentile	95th Percentile	Median	5th Percentile	95th Percentile
No use	671	3.3	1.2	6.5	48	4	169	156	12	555	58	24	78	7.0	0.5	15.9
≤1 time per week	409	3.2	1.4	6.1	47	5	165	146	19	527	58	24	79	7.5	1.0	16.0
>1 time per week	136	3.2	1.0	6.3	41	0.3	128	107	0.5	441	56	12	80	6.5	0.0	15.3

of follicle-stimulating hormone, luteinizing hormone, and sex hormone-binding globulin were determined by using a time-resolved immunofluorometric assay (Delfia; Wallac Oy, Turku, Finland). Testosterone and estradiol levels were determined by using time-resolved fluoroimmunoassays (Delfia; Wallac Oy). The inhibin B level was determined by means of a specific 2-sided enzyme immunometric assay (Inhibin B Gen II; Beckman Coulter, Ltd., High Wycombe, United Kingdom). The hormones were all measured within same time period and in the same assay batches. Free testosterone was calculated on the basis of the measured serum concentrations of total testosterone and sex hormone-binding globulin by using the method of Vermeulen et al. (15) and a fixed albumin concentration of 43.8 g/L (15).

Semen analysis

Participants of the study provided a semen sample by means of masturbation in a room close to the semen laboratory. The period of ejaculation abstinence (time since last ejaculation) and the month of delivery of the sample (divided into October–March or April–September intervals) were recorded. The semen samples were analyzed for volume, sperm concentration, total sperm count, percentage of motile spermatozoa, and percentage of morphologically normal spermatozoa as described by Jørgensen et al. (16) in accordance with the most recent World Health Organization guidelines (17). Since 1996, our laboratory has led a quality-control program for the assessment of sperm concentration; the laboratory has kept the interlaboratory difference unchanged (14), and the variation between technicians was less than 10%. The sperm morphology has currently been assessed by experienced technicians according to strict criteria for the first 838 men.

Statistical methods

Outcome variables for semen quality were semen volume, sperm concentration, total sperm count, and percentages of motile and morphologically normal spermatozoa. Outcome variables for hormone analyses were testosterone, luteinizing hormone, follicle-stimulating hormone, sex hormone-binding globulin, and calculated free testosterone. Exposure variables included frequency of smoking marijuana during the past 3 months, specified as none (reference value), ≤1 time per week, and >1 time per week. In addition, a variable including the combined use of marijuana and other recreational drugs was created as marijuana use ≤1 time per week and no use of other recreational drugs (reference value), marijuana use >1 time per week and no use of other recreational drugs, and marijuana use >1 time per week and use of other recreational drugs.

First, semen quality and reproductive hormone levels were compared among men with and without marijuana use. Then variables from the physical examination and questionnaire were compared for men with and without marijuana smoking by use of the χ^2 or *t* test to identify potential confounders. The data were then examined by multiple linear regression. Because of the nonnormal (skewed) distributions of semen quality and serum reproductive hormones, all variables except for motile sperm and morphologically normal forms were transformed by a natural logarithmic scale and back-transformed to obtain the expected percentage change for marijuana use. The variables for motile sperm and morphologically normal forms were analyzed untransformed because of the normal distribution to obtain the change in percentage points. To select confounders, we initially included those associated with semen parameters, hormone levels, or use of marijuana.

Table 3. Semen Quality According to Marijuana Use and Simultaneous Use of Other Recreational Drugs Among 1,221 Young Danish Men, 2008–2012[a]

	No. of Men	Semen Volume, mL		Sperm Concentration, million/mL		Total Sperm Count, million		Motile Sperm, %		Morphologically Normal Forms, %	
		%	95% CI	%	95% CI	%	95% CI	β	95% CI	β	95% CI
Adjusted results for marijuana use[b]											
No use	643	Referent		Referent		Referent		Referent		Referent	
≤1 time per week	399	2	−4, 9	14	−7, 39	19	−3, 46	1.9	−0.4, 4.2	0.5	−0,3, 1.2
>1 time per week	130	1	−9, 12	−28	−48, −1	−29	−46, −1	−0.3	−4.0, 3.5	−0.6	−1.8, 0.6
Adjusted results for marijuana and recreational drug use[c]											
Marijuana ≤1 time per week, no recreational drugs	971	Referent		Referent		Referent		Referent		Referent	
Marijuana >1 time per week, no recreational drugs	53	2	−9, 15	−32	−53, −3	−33	−54, −3	−0.7	−4.9, 3.4	−0.9	−2.3, 0.5
Marijuana ≤1 or >1 time per week and recreational drugs	43	−3	−15, 10	−52	−68, −27	−55	−71, −31	−5.8	−10.6, −1.0	−1.1	−2.7, 0.5

Abbreviation: CI, confidence interval.

[a] Results from linear regression analyses (adjusted β coefficients or percent change and 95% confidence interval).

[b] Adjusted for hours of abstinence (<48 hours, 48–95 hours, >95 hours), alcohol intake (>21 units per week; 1 unit = 12 g of alcohol), tobacco smoking, sexually transmitted diseases, and use of recreational drugs (yes or no).

[c] Adjusted for hours of abstinence (<48 hours, 48–95 hours, >95 hours), alcohol intake (>21 units per week), tobacco smoking, and sexually transmitted diseases.

Potential confounders were then stepwise excluded, on the basis of their changing the estimates by more than 10%. One set of confounders was used for all semen quality analyses: hours of abstinence (subdivided into <48 hours, 48–95 hours, and >95 hours), tobacco smoking, alcohol consumption, self-reported sexually transmitted diseases, and use of other recreational drugs. There was 1 set of confounders for all hormone analyses: BMI levels (<20, 20–24.99, and ≥25), tobacco smoking, hour of day of blood sampling, and use of other recreational drugs. In the analyses of the combined association of marijuana and other recreational drug use, use of recreational drugs was not included as a confounder.

We evaluated a trend in the association of marijuana use by inserting the categorical marijuana variable (never, less than once per week, at least once per week, several times per week, and daily) into the model assuming the association to be linear. Finally, we compared marijuana use for men with semen concentration below World Health Organization reference values (17) (15 and 20 million/mL) by binary logistic regression analyses adjusting for the same set of confounders.

The maternal educational level was left out in the final analysis as answers were missing in 12% of the cases. However, we repeated all of the analyses with inclusion of maternal education. For the semen quality analyses, we tested for interaction with BMI, alcohol intake, and smoking, which was not significant. However, we stratified the analyses on BMI, alcohol intake, and tobacco smoking in order to evaluate if these had any modifying effect.

The fit of regression models was evaluated by testing the residuals for normality and by inspecting the residual plots. All of the analyses were conducted as general linear models in SPSS, version 19, statistical software (SPSS, Inc., Chicago, Illinois), and the results are presented with 95% confidence intervals.

RESULTS

A total of 1,215 of the 1,221 men responded to the questions on marijuana use. Their mean age was 19.1 years, and 8% had been responsible for a pregnancy. Among the 1,215 men, 45.4% had smoked marijuana within the last 3 months, and 61% of these smoked less than once per week. A total of 10.9% had used recreational drugs other than marijuana (93.9% of these for less than once per week), and. 9.6% had used both marijuana and other recreational drugs within the last 3 months (data not shown).

Men who had used marijuana during the last 3 months had a higher alcohol and caffeine intake, were more often smokers and had been exposed to their mothers' tobacco smoking in utero, had a higher stress and sleep score, were less often born with cryptorchidism, had a higher prevalence of sexually transmitted diseases, and had a higher use of recreational drugs (Table 1).

Sperm concentration, total sperm count, percentage of motile sperm, and percentage of morphologically normal forms were all lower among men smoking marijuana more than once per week (Table 2). In addition, use of both marijuana and recreational drugs was associated with a further decrease in semen quality. Men using marijuana more than once a week had a 28% (95% confidence interval (CI): −48, −1) and 29% (95% CI: −46, −1) lower sperm concentration and total sperm

Table 4. Hormone Levels According to Use of Marijuana Within the Past 3 Months Among 1,221 Young Danish Men, 2008–2012

Frequency of Marijuana Use	No. of Men	Testosterone, mmol/L			Free Testosterone, nmol/L			LH, IU/L			FSH, IU/L			SHBG, nmol/L		
		Median	5th Percentile	95th Percentile	Median	5th Percentile	95th Percentile	Median	5th Percentile	95th Percentile	Median	5th Percentile	95th Percentile	Median	5th Percentile	95th Percentile
No use	658	19.9	11.2	34.1	459.9	262.2	767.6	3.4	1.5	6.8	2.5	1	6	28	12	51
≤1 time per week	405	21.0	12.5	33.7	473.3	295.3	764.9	3.3	1.6	6.6	2.3	0.9	5.9	28	15	50.8
>1 time per week	131	22.9	12.5	35.1	498.3	302.6	811.1	3.7	1.6	6.6	2.5	0.9	6.6	31	15	51.4

Abbreviations: FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin.

Table 5. Hormone Levels According to Use of Marijuana and Other Recreational Drugs Within the Past 3 Months Among 1,221 Young Danish Men, 2008–2012[a]

Frequency of Marijuana Use	No. of Men	Testosterone, mmol/L		Free Testosterone, nmol/L		LH, IU/L		FSH, IU/L		SHBG, nmol/L	
		%	95% CI	%	95% CI	%	95% CI	%	95% CI	%	95% CI
Adjusted results for marijuana use[b]											
No use	651	Referent		Referent		Referent		Referent		Referent	
≤1 time per week	403	3	−2, 7	2	−2, 7	−3	−9, 3	−3	−10, 4	2	−3, 7
>1 time per week	129	7	0, 14	5	−2, 12	7	−2, 18	−1	−12, 11	7	−1, 16
Adjusted results for marijuana and recreational drug use[c]											
Marijuana ≤1 time per week, no recreational drugs	977	Referent		Referent		Referent		Referent		Referent	
Marijuana >1 time per week, no recreational drugs	74	8	−0, 17	5	−3, 13	7	−4, 18	−8	−20, 5	8	−1, 19
Marijuana >1 time per week and recreational drugs	55	3	−6, 13	1	−7, 11	10	−3, 24	11	−5, 30	5	−6, 17

Abbreviations: CI, confidence interval; FSH, follicle-stimulating hormone; LH, luteinizing hormone; SHBG, sex hormone-binding globulin.

[a] Results from linear regression analyses of hormone levels (adjusted percent change and 95% confidence interval) according to marijuana use and simultaneous use of other recreational drugs.

[b] Adjusted for body mass index (weight (kg)/height (m)^2) levels (<20, 20–24.99, and ≥25), tobacco smoking, hour of day of blood sampling, and use of other recreational drugs.

[c] Adjusted for body mass index levels (<20, 20–24.99, and ≥25), tobacco smoking, and hour of day of blood sampling.

count, respectively, compared with nonmarijuana smokers after adjustment for hours of abstinence, tobacco smoking, alcohol intake, sexually transmitted diseases, and other recreational drugs (Table 3). Combined use of both marijuana more than once per week and other recreational drugs significantly reduced the sperm concentration by 52% (95% CI: −68, −27), total sperm count by 55% (95% CI: −71, −31), and sperm motility by 5.8 percentage points (CI: −10.6, −1.0) (Table 3). Semen volume was not associated with marijuana or recreational drug use.

A negative association with sperm concentration and total sperm count was found; however, trends were not significant ($P = 0.12$ and $P = 0.17$), indicating that the adverse association was found at a threshold with regular use of marijuana more than once per week. When the sperm concentration was categorized according to World Health Organization reference values, the adjusted odds ratios of having a concentration below 15 and 20 million/mL when using marijuana more than once per week were, respectively, 1.07 (95% CI: 0.62, 1.87) and 1.14 (95% CI: 0.69, 1.89). Serum testosterone, free testosterone, and sex hormone-binding globulin were higher among marijuana users than nonusers (Table 4). However, after adjustment for BMI, tobacco smoking, and time of day of blood draw, only testosterone was higher in marijuana users with a level of 7% (95% CI: 0, 14) (Table 5).

We repeated the analyses among smokers and nonsmokers and among men with high and normal BMI separately, which did not significantly change our findings. Moreover, adjustment for maternal education did not affect the findings. Men with a low weekly alcohol intake (1–5 units) using marijuana more than once per week had a reduction of 42% (95% CI: −73, 21) and 39% (95% CI: −72, 34) in sperm concentration and total sperm count, respectively, compared with nonmarijuana smokers after adjustment, whereas the association was attenuated among men with a higher alcohol intake (>5 units per week).

DISCUSSION

In this study on more than 1,200 healthy young men, of whom 45% had smoked marijuana during the past 3 months, we found associations between regular use of marijuana more than once per week during the past 3 months and reduced semen quality, whereas no adverse association was found for irregular use. The combined use of marijuana and other recreational drugs decreased semen quality further. In addition, marijuana use was associated with increased serum testosterone to the same level as cigarette smoking. We cannot exclude the possibility that the men who used marijuana generally have an unhealthier lifestyle and health behavior, which may also affect their semen quality and hormone levels. We, however, adjusted for known lifestyle factors.

A study (6) investigated chronic marijuana users who, after 4 weeks of abstinence from marijuana, smoked between 8 and 20 marijuana-containing joints per week for 4 weeks. An association between marijuana use and decreased sperm count was detected, which persisted in the following 4-week recovery period. This is in accordance with our findings. On the contrary, a study of 159 men attending an infertility clinic found a positive correlation between marijuana use and percentage of motile sperm (18). The tendencies remained unchanged after adjusting for other substance use and history of sexually transmitted diseases. The discrepancies may be due to the fact that we studied healthy young men with a higher marijuana intake than

infertile men, who may have changed their intake because of fertility problems. Only 2 previous studies have assessed the combined effects of marijuana and recreational drug use on semen quality. A case study (19) on a multidrug addict, investigated before and 2 years after cessation of the abuse, showed long-lasting semen abnormalities. Another study of 6 male multidrug addicts showed semen abnormalities (8). All subjects were underweight and malnourished, making it impossible to compare them with our healthy, young men.

The biological mechanisms by which marijuana affects semen quality and hormone levels are not fully known. The active component of cannabis, Δ^9-tetrahydrocannabinol (THC), binds to the human cannabinoid receptors CB_1 and CB_2. CB_1 receptors are found in the anterior pituitary but have also been identified in the testis, vas deferens, and human sperm cells (20), leading to a dose-dependent decreased sperm motility and decreased mitochondrial activity in spermatozoa when activated (21). This makes it possible for marijuana to affect hormone levels and spermatogenesis, as well as the mature sperm cells. In vitro studies have shown that low doses of THC hyperactivated the spermatozoa and that high doses of THC had an inactivating effect (5), mimicking the human cannabinoid anandamide (22). THC could affect the normal balance of anandamide leading to impaired semen quality.

The hypophyseal hormones are known to affect spermatogenesis, and marijuana may affect semen quality by influencing both this axis and the testis. Marijuana has been found to reduce testosterone and luteinizing hormone (23, 24). However, previous studies did not take into account cigarette smoking and other possible confounders. In this study, we found a significant increase in testosterone correlated to the use of marijuana, contrary to the other studies. However, this increase could not be separated from the effect of tobacco smoking alone, found in other studies to raise testosterone levels (25, 26), making it impossible to separate the adverse effects of marijuana and cigarette smoking in this study.

Our study has several strengths, as it was large and included unselected young men of whom many used marijuana. A total of 45% of our men reported having tried marijuana during the past 3 months, and 33% used marijuana less than once per week, which is comparable to Danish population studies of men aged 16–34 years in which 46% reported ever having tried marijuana (1) and 17% during the past year. Only 6% of young Danes aged 16–34 years reported having used marijuana during the last month, which is lower than in our study. However, our men are younger, and the response categories are not directly comparable.

Our participation-rate was 30%, which is higher than participation rates in other population-based studies on semen quality (9, 27). Most of the men were unaware of their own fertility potential (8% had been responsible for a pregnancy), making this unlikely to have affected their motivation to participate. Approximately 15% of the men had a sperm concentration at a level that would indicate a high risk of needing future fertility treatment if they want to father a child (10). However, we believe that they represent the general population of young Danish men as we conducted a study where reproductive hormones among participants and nonparticipants were compared (28). We found no significant difference with regard to reproductive hormones in the 2 groups, indicating that our participants represented the general population with respect to reproductive health. In addition, this study compared semen quality and reproductive hormones in groups of men with different marijuana use, and it is therefore of less importance whether the groups of men in fact represented the general population.

Participants were asked to evaluate their use of marijuana in the last 3 months and, though the study was anonymous, there may have been a tendency to underestimate the consumption. However, this seems unlikely, as 45% of the young men reported marijuana use during the past 3 months, which may even indicate overreporting. The potential sources of exposure misclassification are unlikely to be related to the semen parameters or hormone levels, because the men responded to the questionnaire before knowing the results of their semen and serum analysis. Such nondifferential misclassifications would underestimate the results and can therefore not explain our findings. It would have been preferable to measure the carboxy THC metabolite levels in urine samples from the men in order to confirm the results from the questionnaire. However, studies suggest that urine-, blood-, and oral-fluid tests designed to measure marijuana use are unlikely to detect use further back than 1 month, whereas in this study we assessed use during the past 3 months. Furthermore, research has shown large intersubject differences in measurable levels in urine, making urine-sample testing less useful in this study (29).

Doses and time of abstinence from marijuana and other recreational drugs can vary individually and occasionally. We did not obtain information on these factors, but previous studies have suggested a dose and time dependence (6, 22, 30–32). In addition, spermatozoa mature within 3 months, and estimated marijuana intake over the past 3 months is therefore a good study design for assessing the association between semen quality and marijuana and recreational drug use.

Men who used marijuana had an unhealthier lifestyle and health behavior, were often smokers, consumed more alcohol, had a higher caffeine intake, were more likely to have had a sexually transmitted disease, and were more likely to have used recreational drugs other than marijuana. The adverse association between marijuana use and semen quality tended to be larger among men with low alcohol intake, although the results were not statistically significant. The negative association between marijuana use and semen quality and hormone levels may be attributed to differences in lifestyle, health behavior, and diet found among users, and even though we adjusted for many lifestyle factors, residual confounding is still possible.

In conclusion, to the best of our knowledge, this is the first study showing adverse associations between regular marijuana use more than once weekly and semen quality among healthy young men, the association being even more pronounced among men taking other recreational drugs as well. We found an increase in testosterone levels among marijuana smokers within the same range as for tobacco smokers. As the study was cross-sectional, it was not possible to test whether semen quality and hormone levels are restored after cessation of the use, but until further studies have been conducted, men should be informed of the possibility that habitual marijuana use might be detrimental to their semen parameters. Our findings are potentially of great public interest, as almost half of

the young Danish men use marijuana, which may contribute to the etiology for the recently reported high frequency of subnormal human sperm counts.

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