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Original Contribution

Caffeine Intake and Semen Quality in a Population of 2,554 Young Danish Men

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The authors examined the association between semen quality and caffeine intake among 2,554 young Danish men recruited when they were examined to determine their fitness for military service in 2001–2005. The men delivered a semen sample and answered a questionnaire including information about caffeine intake from various sources, from which total caffeine intake was calculated. Moderate caffeine and cola intakes (101–800 mg/day and ≤14 0.5-L bottles of cola/week) compared with low intake (≤100 mg/day, no cola intake) were not associated with semen quality. High cola (>14 0.5-L bottles/week) and/or caffeine (>800 mg/day) intake was associated with reduced sperm concentration and total sperm count, although only significant for cola. High-intake cola drinkers had an adjusted sperm concentration and total sperm count of 40 mill/mL (95% confidence interval (CI): 32, 51) and 121 mill (95% CI: 92, 160), respectively, compared with 56 mill/mL (95% CI: 50, 64) and 181 mill (95% CI: 156, 210) in non-cola-drinkers, which could not be attributed to the caffeine they consumed because it was <140 mg/day. Therefore, the authors cannot exclude the possibility of a threshold above which cola, and possibly caffeine, negatively affects semen quality. Alternatively, the less healthy lifestyle of these men may explain these findings.

caffeine; cola; fertility; reproductive medicine; semen analysis

Abbreviation: CI, confidence interval.

Intake of caffeine (1,3,7-trimethylxanthine) (found in coffee, tea, chocolate, and some soft drinks, particularly cola-containing beverages (1, 2)) is high in the industrialized world, and consumption of cola, in particular, has been increasing among children and young adults. Caffeine intake has been associated with increased risk of spontaneous abortions (1, 2), and some studies have reported a decrease in female fertility with increasing caffeine consumption (3, 4), although both these associations remain controversial. No effect on male fertility has been found (5–7).

Previous studies on caffeine intake and semen quality have been contradictory (8–13). However, they have been performed among highly selected groups of either infertile men (9, 10, 12, 13) or fertile men undergoing vasectomy (11). A recent Danish study found some reduction in semen quality among men exposed to maternal caffeine in utero, whereas, in the same study, current caffeine intake had no impact on semen quality, although it was associated with an increase in serum testosterone (7). To our knowledge, no previous studies have investigated associations between semen quality and caffeine intake from multiple sources. Therefore, we investigated these associations in a cross-sectional study among Danish young men from the general population.

MATERIALS AND METHODS

Population

Because of the military draft in Denmark, all men 18 years of age, except those with severe chronic disease, are required to undergo a compulsory physical examination to determine their fitness for military service. Some men postpone their examination to continue their education and are therefore called up to serve when they have completed their education.

Trained staff from the University Department of Growth and Reproduction approached these young men when they

appeared for this compulsory physical examination in Copenhagen and during 2 time periods in Aalborg, Denmark, and invited them to participate in a study of reproductive function. Men recruited from September 2001 to December 2006 were included in the present study (because the questionnaire they completed included detailed information about lifestyle factors). Participants, who were compensated for their time (500 kr = ~US $100), completed a questionnaire, delivered a semen sample, had a blood sample drawn, and underwent a physical examination. Participants did not differ from nonparticipants with regard to age, but they were better educated than nonparticipants (data not shown). Ethical approval was obtained from the local ethical committee. For a detailed description of the study, refer to Andersen et al. (14) and Jørgensen et al. (15).

Semen analysis

All men provided a semen sample by masturbation in a room close to the semen laboratory. The period of ejaculation abstinence was recorded, and the semen sample was analyzed according to the World Health Organization's 1999 guidelines (16), modified in accordance with Jørgensen et al. (17). Since 1996, our laboratory has led a quality control program for assessment of sperm concentration, and the laboratory has kept the interlaboratory difference unchanged in comparison with 2 other laboratories that have also participated since the program started (15, 17, 18).

The same experienced technician assessed sperm morphology according to strict criteria (16) within 8 consecutive working weeks (19). The current analysis includes morphology results for only a subset of men because not all samples had been counted yet. Spermatozoa morphology was assessed in 284 for the 299 men consuming more than 1 bottle (0.5 L) of cola per day, as well as for 97 randomly selected men consuming no cola and 98 randomly selected men consuming less than 1 bottle (0.5 L) of cola per day because we initially found a negative association between cola intake and semen quality.

Physical examination

Four physicians performed all physical examinations. The Tanner stage of pubic hair and genital development, testicular volumes, the possible presence of a varicocele (stage 1 to 3) or hydrocele, the location of the testes in the scrotum, and the consistency of the testis and epididymis were recorded. Weight and height were measured and body mass index calculated as weight in kilograms divided by squared height in meters. Diseases and conditions found at the physical examination that may affect semen quality (varicocele (stage 2 to 3) or abnormal position of the testes) were summarized in a single variable: ''conditions found at the physical examination''; 38 men had more than one condition.

Questionnaire

Prior to the examination, all participants completed a questionnaire containing information on previous and/or current diseases and genital diseases such as inguinal hernia, varicocele, epididymitis, gonorrhea, chlamydia, and surgery for testicular torsion. The men were asked whether they were born with both testicles in the scrotum. In addition, they reported whether they had had a fever of >38°C (100.4°F) during the previous 3 months. Self-reported diseases in the reproductive organs affecting semen quality (operation of varicocele, torsion of testes, epididymitis, or sexually transmitted diseases) were transformed into one variable: ''self-reported genital conditions''; only one man had more than one of these conditions.

The young men responded to a standard questionnaire about parents' social class conceptualized as parents' education and occupation coded according to the standards of the Danish National Institute of Social Research (20), which is almost identical to the United Kingdom Registrar General's categorization into 5 social classes from I (high) to V (low), with an additional category: housewife. The social class of the highest ranking parent was used.

Participants were asked, How much did you consume of the following beverages during the last week? Possible responses were as follows: glasses of wine (units), bottles of beer (0.33 L), number of strong alcoholic drinks (12 cL), bottles of cola (0.5 L), bottles of diet soft drinks (0.5 L), and number of chocolate bars (50 g). In addition, they were asked how many cups of coffee, tea, and chocolate-containing beverages they consumed daily during the last week. Alcohol intake was considered the sum of strong alcoholic drinks (approximately 12 g of alcohol in each), glasses of wine, and bottles of beer per week. Each man's daily caffeine intake was estimated by assuming a cup to contain 150 mL and the caffeine content to be 117 mg in one cup of coffee, 70 mg in one cup of tea, 5 mg in one cup of chocolate beverages, 70 mg in 0.5 L of cola and diet soft drinks, and 7 mg in a 50-g chocolate bar (1). In the analyses, ''weekly intake of cola'' was calculated as the sum of reported drinks of cola and diet soft drinks (assuming diet soft drinks to be cola).

The men were asked about their dietary habits with the following question: How often do you consume cheese, butter, vegetables, fruits, chicken, lamb or beef, burgers, fish, etc.? Answer categories were never, 1–3 times per month, once per month, 2–3 times per week, once per day, and more than once per day.

Statistics

Outcome variables were semen volume, sperm concentration, total sperm count, and percentages of motile and morphologically normal spermatozoa. Exposure variables were average daily caffeine intake included as a continuous variable (ln transformed) and categorized as daily intake of 0–100 mg, 101–200 mg, 201–800 mg (1–800 mg), and >800 mg, which corresponds to approximately 1, 2–7, and >7 cups of coffee per day. All analyses were initially performed with total daily caffeine consumption and then for men who reported caffeine intake from coffee, tea, chocolate beverages or bars, diet soft drinks, or cola separately to determine the independent associations with each. Cola consumption was reported as weekly intake of

number of 0.5-L bottles; therefore, many men reported intake of 7 and 14 bottles per week, corresponding to daily intake of 0.5 L (70 mg of caffeine) and 1 L (140 of mg caffeine). Cola intake was entered as a continuous variable (transformed by natural logarithm) or categorized as no cola, 1–7, 8–14, and >14 bottles per week.

First, we compared semen quality in men in relation to caffeine and cola intake by the Kruskal-Wallis test. Then, we compared the distributions of the variables from the questionnaires and physical examinations among these groups of men by the chi-square test to identify potential confounders.

Finally, data were examined by using univariate analyses of variance. Normally distributed outcome variables were entered directly as continuous variables in the model, whereas sperm concentration and total sperm count were transformed by use of the natural logarithm to obtain normality and were back-transformed to obtain the percentage change in these semen parameters. Covariates initially included factors possibly associated with semen parameters or caffeine consumption and were then excluded stepwise if they did not change the estimate by more than 10%. Period of abstinence was entered to adjust to 96 hours. The same set of confounders was used for all semen parameters: fever >38°C within the last 3 months, period of abstinence, body mass index, in utero exposure to smoking, conditions found at the physical examinations, self-reported genital conditions and cryptorchidism, and sperm motility time from ejaculation until analysis of the sample. In addition, we estimated the adjusted median of the semen parameters by performing the analyses without including an intercept. Analyses for different types of caffeine intake were performed by including the sources in separate models as well as by simultaneously including all caffeine-containing sources in the same model. In this paper, results are presented as regression coefficients with 95% confidence intervals. We evaluated fit of the regression models by testing the residuals for normality and by inspecting the residual plots.

RESULTS

A total of 2,554 men participated (approximately 31% of those approached). Of these men, 141 (5.5%) had fathered a pregnancy and 15 (0.6%) had been examined for infertility. One hundred forty-nine men who did not provide information about caffeine intake did not differ from the others with respect to other lifestyle factors and semen quality (data not shown). More than 50% of the total caffeine intake was derived from coffee and 20% from cola (Table 1). Caffeine content in cola is quite low compared with coffee; one cup of coffee contains about 117 mg compared with 70 mg in 0.5 L of cola.

Although men who reported no caffeine intake ($n = 72$) had better semen quality (median sperm concentration, total sperm count, and morphological normal sperm: 62 mill/mL, 210 mill, and 7%, respectively), moderate consumption of caffeine was not associated with a reduction in semen quality. However, men with a high caffeine intake (>800 mg of caffeine per day) had a slight reduction in semen quality (not statistically significant) (Table 2). Men whose caffeine intake was >800 mg (about 7 cups of coffee) per day generally had a less healthy diet, eating more burgers and cheese; drank more alcohol; smoked more often; and had a high or low body mass index (Table 3). In addition, they were from a lower social class, more often had self-reported genital conditions in the reproductive organs or conditions found at the physical examination, and more often had been exposed to smoking in utero compared with men whose caffeine consumption was lower (Table 3).

Table 1. Average Daily Caffeine Intake From All Sources Among 2,554 Danish Young, Healthy Men Recruited for the Study in 2001–2005

Source	Average Intake, mg/day	Average % of Total Caffeine
Coffee	105.4 (168.5)[a]	53
Cola	40.6 (46.9)	20
Tea	37.2 (101.2)	19
Chocolate bars	13.8 (22.9)	7
Cocoa	2.1 (5.8)	1
Total	199.1	100

[a] Values in parentheses, standard deviation.

After control for confounders, we found that low (101–200 mg) to moderate (201–800 mg) daily caffeine consumption was not associated with a reduction in semen quality (Table 4). Consumption of >800 mg of caffeine per day resulted in a nonsignificant reduction in semen quality. Caffeine consumption was also entered as a (ln-transformed) continuous variable. Over the entire range, only semen volume decreased significantly with increasing caffeine intake (Table 4).

Analyses were then performed among men consuming caffeine from different sources to determine the associations with each. After we controlled for confounders (including cola consumption), no association of coffee, tea, chocolate beverages or bars, or diet soft drinks with semen quality was observed. When all caffeine sources were included simultaneously in the model, only cola consumption was associated with a significant reduction in semen quality (data available on request).

A total of 2,114 men reported that they drank cola during the past week; of these, 93 (4.4%) drank more than 14 bottles per week (>1 L per day, 140 mg of caffeine). Men who drank cola had poorer semen quality than men who did not (Table 2). Men who drank >14 bottles (140 mg of caffeine) of cola per week generally also drank less milk and consumed less fruit, vegetables, and fish, but they more frequently consumed beef and burgers compared with men who drank ≤1 L of cola per day (Table 3). In addition, they reported more diseases in reproductive organs, drank more alcohol, had a high or low body mass index, and more often were smokers or had been exposed to smoking in utero than men who drank fewer than 14 bottles of cola per week.

After control for confounders, semen volume, sperm concentration, total sperm count, and percentage of spermatozoa with normal morphology decreased among cola-drinking men compared with nondrinkers (Table 4)

Table 2. Median and 25th–75th Percentile Values for Unadjusted Semen Parameters Among 2,554 Danish Young, Healthy Men Recruited for the Study in 2001–2005, by Daily Caffeine and Cola Consumption

Caffeine and Cola Consumption	No. of Subjects	Semen Volume, mL		Sperm Concentration, mill/mL		Total Sperm Count, mill		Motile Sperm, %		Morphologically Normal Forms, %[a]	
		Median	25th–75th Percentiles	Median	25th–75th Percentiles	Median	25th–75th Percentiles	Median	25th–75th Percentiles	Median	25th–75th Percentiles
Daily caffeine consumption, mg											
0–100	1,164	3.2	2.3–4.3	46	22–80	146	65–257	66	57–74	6.5	3.3–8.5
101–200	521	3.2	2.4–4.1	42	20–78	133	62–242	67	58–74	7.0	4.3–9.5
201–800	657	3.2	2.4–4.1	47	23–84	149	70–260	68	57–74	6.5	3.5–9.5
>800	63	3.0	2.1–4.1	41	26–64	133	68–192	66	57–72	5.5	3.3–9.3
Weekly cola consumption, no. of 0.5-L bottles											
0	379	3.3	2.4–4.5	50[b]	25–89	171[b]	75–295	66	57–73	8.0[b]	5.0–10.5
1–7	1,759	3.2	2.3–4.2	45[b]	22–80	143[b]	65–254	67	55–74	6.0[b]	3.5–9.5
8–14	262	3.1	2.4–4.1	47[b]	23–76	138[b]	71–241	69	58–76	6.0[b]	3.5–9.0
>14	93	3.0	2.2–4.0	35[b]	17–66	102[b]	42–197	66	58–73	7.0[b]	5.0–10.0

[a] Sperm morphology was assessed for 284 men consuming >1 bottle (0.5 L) of cola per day, 97 men with no weekly cola intake, and 98 men consuming less than 1 bottle (0.5 L) of cola per week using strict criteria.
[b] Significant according to the Kruskal-Wallis test.

and significantly decreased among men who drank more than 14 bottles (1 L) of cola per week. Men whose weekly cola consumption was 0, 1–7, 8–14, and >14 bottles had respective adjusted sperm concentrations (mill/mL) of 56 (95% confidence interval (CI): 50, 64), 47 (95% CI: 44, 51), 49 (95% CI: 43, 57), and 40 (95% CI: 32, 51) and respective total sperm counts (mill) of 181 (95% CI: 156, 210), 144 (95% CI: 132, 157), 153 (95% CI: 129, 182), and 121 (95% CI: 92, 160). When cola was entered as a continuous variable (ln transformed), a significant decline in semen volume, total sperm count, sperm concentration, and sperm morphology was found. No association with sperm motility was observed. The analyses were not adjusted for dietary factors because they did not have a significant impact on the associations. All analyses were repeated by excluding diet soft drinks from cola intake, which did not affect our findings.

The analyses were repeated for caffeine intake from sources other than cola (Table 4) to determine whether the association between caffeine and semen quality was attributable to an adverse effect of cola. The same magnitude of effect as for total caffeine intake was found for men whose caffeine intake was not derived from cola (Table 4).

We also examined associations of caffeine and cola consumption with serum reproductive hormones (testosterone, inhibin B, follicle-stimulating hormone, and luteinizing hormone). However, we found no statistically significant associations (data not shown).

DISCUSSION

In this study of more than 2,500 Danish young men, caffeine intake of ≤800 mg per day and cola consumption of ≤14 0.5-L bottles per week was not associated with reduced semen quality. However, we observed an apparent ''threshold'' after which especially cola consumption (1 L per day) was associated with a reduction in semen quality. The reduction in semen quality among high-quantity cola drinkers, if causal, must be attributed to constituents in cola other than caffeine because the caffeine content of cola is not high. Alternatively, these associations may be attributed to the less healthy lifestyle and diet of high-quantity consumers.

Coffee has been associated with low levels of estrogen (21) and high levels of testosterone and sex hormone-binding globulin (22). Previous studies of caffeine intake and semen quality have shown contradictory results (7–13) but had limited control for confounders. One study suggested no associations (12), whereas others found increased motility (9–11). Vine et al. (8) found weak evidence for an association between caffeine intake from coffee, tea, and soft drinks and sperm nuclear morphometry, and Parazzini et al. (13) found an increasing risk of poor semen quality with increasing coffee consumption. The only other known study conducted among 343 unselected young Danish men found no adverse effect of caffeine intake on semen quality (7) but increased testosterone levels with increasing caffeine intake (7), which we did not find in our data. However, that study obtained information only about coffee and tea intake, which may underestimate caffeine intake because many young men drink appreciable amounts of cola. Besides coffee, tea, and cola, we obtained information about chocolate-containing drinks, diet soft drinks, and chocolate bar consumption, providing a more precise estimate of caffeine intake and enabling us to examine the associations with different types of caffeinated products.

Table 3. Information (%) Obtained From Questionnaires and Physical Examination of 2,554 Danish Young, Healthy Men Recruited for the Study in 2001–2005 Consuming Different Quantities of Caffeine Daily or Cola Weekly

Variable Distribution	Daily Caffeine Consumption, mg				Weekly Cola Consumption, no. of 0.5-L bottles			
	0–100 (n = 1,164)	101–200 (n = 521)	201–800 (n = 657)	>800 (n = 63)	0 (n = 379)	1–7 (n = 1,759)	8–14 (n = 262)	>14 (n = 93)
Information obtained at physical examination								
Season of examination between October and March	89	87	87	91	86	89	87	85
Examined in Copenhagen	96	95	95	100	96	96	97	96
Conditions found at the physical examination[a]	13	10	12	16	14	12	10	11
Fever >38°C within the last 3 months	5	5	5	3	5	4	5	5
Period of abstinence >48 hours	92	91	90	92	92	91	91	85
Body mass index, kg/m^2								
<20	14*	19*	16*	19*	14	16	19	17
20–24.99	66*	62*	64*	52*	68	64	57	57
≥25	21*	19*	20*	29*	18	20	24	26
Information obtained from the questionnaire								
Older than age 20 years at the time of the examination	17*	15*	22*	21*	21	17	21	20
Alcohol intake >21 units/week[b]	20*	20*	27*	32*	21	22	24	33
Total caffeine intake ≥400 mg/day					7*	8*	10*	20*
More than 2 cups of coffee/day	0*	0*	32*	92*	10	12	9	7
More than 0.5 L of cola/day	5*	26*	21*	18*				
Parental social class[c]								
1	24*	28*	29*	13*	30	26	24	21
2	27*	28*	25*	19*	25	27	26	24
3	11*	10*	10*	15*	11	10	8	11
4	29*	28*	29*	32*	27	29	31	38
5	8*	5*	7*	19*	6	7	9	4
Housewife	1*	2*	1*	2*	2	1	1	1
Current smoking	31*	41*	54*	66*	41	40	39	47
Exposure to mother's smoking in utero	39	38	40	50	38*	39*	41*	55*
Self-reported genital conditions[d]	5*	7*	6*	12*	5*	5*	10*	8*
Born with cryptorchidism[e]	3	4	3	3	3	3		2
Drinks milk	94	95	95	92	96*	95*	92*	84*
Consumes cheese ≥1 times/day	17*	19*	24*	36*	22	20	17	17
Consumes butter on bread ≥1 times/day	53	56	56	51	46*	56*	57*	51*
Consumes fish at least 2–3 times/week	22	23	24	26	24	23	18	20
Consumes chicken or turkey ≥1 times/day	3	2	2	0	2	3	2	2
Consumes veal or beef ≥1 times/day	2*	3*	4*	7*	2	3	4	6
Consumes burgers at least 2–3 times/week	10	11	11	20	3*	10*	20*	34*
Consumes fruit and vegetables >1 time/day	18	20	22	21	26*	20*	13*	8*

* $P < 0.05$ by chi-square test.
[a] Varicocele or abnormal testes found at physical examination.
[b] 1 unit = 12 g of alcohol.
[c] Categorized according to national Danish standards (20).
[d] Self-reported information about torsion of testes, epididymitis, varicocele, or sexually transmitted diseases.
[e] If information was missing, the man was categorized as not having cryptorchidism.

Our participation rate was 31%, which is higher than in other population-based semen-quality studies (15, 18, 23, 24). In addition, because the majority of our young men had no knowledge of their own fertility potential, this factor is unlikely to have affected their motivation to participate. In addition, our goal was to compare semen quality among

Table 4. Adjusted Results From the Regression Analyses of Semen Quality of 2,554 Danish Young, Healthy Men Recruited for the Study in 2001–2005, by Caffeine and Cola Intake

Caffeine and Cola Intake	No. of Subjects	Semen Volume, mL[a]		Sperm Concentration[a,b]		Total Sperm Count[a,b]		Motile Spermatozoa, %[a,c]		Morphologically Normal Spermatozoa, %[a,d]	
		β	95% CI	β	95% CI	β	95% CI	β	95% CI	β	95% CI
Total daily caffeine consumption, mg											
Adjusted difference[e]											
0–100	982	Reference		Reference		Reference		Reference		Reference	
101–200	428	−0.1	−0.3, 0.1	−6	−17, 6	−10	−21, 3	−0.1	−1.7, 1.4	0.6	−0.4, 1.6
201–800	548	−0.1	−0.2, 0.1	4	−6, 16	2	−10, 15	−0.1	−1.5, 1.4	−0.2	−1.3, 0.8
≥800	50	−0.2	−0.6, 0.2	−16	−38, 13	−23	−38, 13	−0.5	−4.5, 3.6	−1.1	−3.6, 1.4
Adjusted median[a]											
0–100	982	3.4	3.4, 3.6	48	44, 52	152	138, 167	65.8	64.6, 66.9	6.7	5.9, 7.5
101–200	428	3.5	3.2, 3.5	45	40, 51	137	120, 156	65.6	64.1, 67.1	7.3	6.4, 8.2
201–800	548	3.4	3.3, 3.6	50	45, 56	154	137, 164	65.7	64.4, 67.1	6.5	5.6, 7.3
≥800	50	3.5	2.9, 3.8	40	30, 55	117	83, 164	65.3	61.2, 69.3	5.6	3.1, 8.1
Entered as a continuous variable[a,f]		−0.05	−0.09, −0.01	−1.6	−4.6, 1.5	−3.4	−6.9, 0.00	0.10	−0.31, 0.51	−0.12	−0.42, 0.17
Weekly cola consumption, no. of 0.5-L bottles											
Adjusted difference[e]											
0	312	Reference		Reference		Reference		Reference		Reference	
1–7	1,468	−0.2	−0.4, −0.01	−16	−27, −5	−20	−31, −8	0.3	−1.4, 2.0	−1.8	−3.1, −0.4
8–14	220	−0.2	−0.4, 0.1	−12	−27, 5	−15	−31, 4	-1.1	−1.3, 3.5	−2.3	−3.6, −1.1
>14	78	−0.1	−0.5, 0.3	−29	−45, −8	−33	−50, −10	-0.5	−2.9, 3.9	−1.3	−2.7, 0.2
Adjusted median[a]											
0	312	3.6	3.5, 3.8	56	50, 64	181	156, 210	65.5	63.8, 67.1	8.4	7.3, 9.5
1–7	1,468	3.4	3.3, 3.5	47	44, 51	144	132, 157	65.7	64.7, 66.8	6.6	5.7, 7.5
8–14	220	3.4	3.2, 3.6	49	43, 57	153	129, 182	66.6	64.6, 68.5	6.0	5.2, 6.8
>14	78	3.5	3.2, 3.9	40	32, 51	121	92, 160	66.0	62.8, 69.1	7.1	6.0, 8.3
Entered as a continuous variable[a,f]		−0.08	−0.16, −0.01	−7.0	−12.2, −1.69	−9.5	−15.2, −3.44	0.17	−0.57, 0.91	−0.50	−0.91, −0.09
Daily caffeine consumption excluding caffeine from cola, mg											
Adjusted difference[e]											
0–100	1,192	Reference		Reference		Reference		Reference		Reference	
101–200	338	−0.2	−0.4, 0.0	−8	−19, 5	−14	−26, −1	−0.9	−2.5, 0.8	−0.4	−1.6, 0.7
201–800	432	−0.1	−0.3, 0.0	7	−5, 20	3	−10, 18	−0.2	−1.7, 1.3	0.1	−1.0, 1.2
≥800	46	−0.2	−0.6, 0.2	−12	−35, 20	−20	−44, 14	−0.6	−4.8, 3.6	−1.7	−4.5, 1.2

Adjusted median[a]											
0–100	1,192	3.5	3.4, 3.7	48	44, 52	152	139, 167	65.9	64.8, 67.0	6.9	6.2, 7.6
101–200	338	3.4	3.2, 3.5	44	39, 50	131	113, 151	65.0	63.4, 66.7	6.7	5.4, 7.5
201–800	432	3.4	3.3, 3.6	52	46, 58	157	138, 178	65.8	64.3, 67.2	6.4	5.8, 8.1
≥800	46	3.4	2.9, 3.8	42	31, 58	121	85, 173	65.3	61.1, 69.5	5.2	2.3, 8.1
Entered as a continuous variable[a,f]		−0.02	−0.05, 0.01	0.30	−1.8, 2.3	−0.4	−2.7, 1.9	−0.03	−0.29, 0.24	−0.07	−0.25, 0.12

[a] Adjusted for fever >38°C within the last 3 months, period of abstinence, body mass index, in utero exposure to smoking, conditions found at the physical examinations, self-reported genital conditions, and cryptorchidism categorized according to Table 2.
[b] Sperm concentration and total sperm count were transformed by the use of natural logarithm and were back-transformed, giving the percentage change.
[c] Also adjusted for time until assessment categorized according to Table 2.
[d] Sperm morphology was assessed for 284 men consuming >0.5 L (70 mg) of cola per day, 97 men with no weekly cola intake, and 98 men consuming <0.5 L (70 mg) of cola per week using strict criteria.
[e] Adjusted difference from the reference group in semen parameters (sperm concentration and total sperm count in %).
[f] Caffeine and cola intake were transformed by the use of natural logarithm.

men with different caffeine and cola intakes, so whether the men were representative of the general population is of secondary importance.

The men in our study reported caffeine consumption the week before they completed the questionnaire because we assumed that to be more accurate to recall than average intake. If this consumption differed from the typical intake, misclassification of exposure may have occurred. We estimated that a cup of coffee contains 150 mL and 117 mg of caffeine, but it will vary depending on cup size, method of preparation, and product brand. In addition, we did not obtain information on type of tea consumed.

The questions about cola and diet soft drink consumption were not very accurate because the men were asked about cola or diet soft drink intake only, not about type of drinks. The caffeine content of diet soft drinks was estimated to be similar to that of cola (70 mg and 0.5 L), although not all soft drinks contain caffeine. The Danish Brewers Association reports that 64% of the sales of soft drinks in Denmark are of cola (25). In addition, very few men had a high intake of ''diet soft drinks,'' which had no independent effect on semen quality. We also repeated the analyses excluding diet soft drinks from total caffeine and cola intake, which did not change the findings. These potential sources of exposure misclassification are all likely to be random and not related to semen quality, since the men responded to the questionnaire before they knew the result of their semen analysis, and therefore underestimate the associations between caffeine and semen parameters. In addition, the dietary questionnaire was not validated, and the men were just asked approximately how often they consumed different food items.

It is well known that interobserver variability in semen analysis exists and is particularly high for motility assessment, which may help explain the lack of an association of caffeine and cola consumption with motility. However, all analyses were performed blinded, and the same technician assessed all morphology slides. Furthermore, our laboratory participated in an external quality control program. We obtained only one semen sample for each man, and intraindividual variability exists, which may have introduced nondifferential misclassification and thereby underestimated the effects.

Men who consumed no caffeine had better semen quality but also a more healthy lifestyle. High-quantity consumers of cola or caffeine had an unhealthier lifestyle, which has previously been associated with poorer semen quality (26–30). To the extent possible, we considered these factors in the analyses, and they did not appear to explain the caffeine and cola associations. High-quantity caffeine and cola consumers also had a less healthy diet, and previous studies have found reduced semen quality among men who consumed few fruits and vegetables (31) and had a low intake of antioxidant and trace minerals (32, 33). We repeated the analyses taking into account these factors (data not shown), but they did not explain the negative association we observed with caffeine and cola intake. High caffeine and cola consumption may also be related to in utero exposure to caffeine (7), working in a sedentary position (34, 35), being less physical active (12, 36, 37), or being more stressed (38), variables that have previously been associated with poorer semen quality. Unfortunately, we did not obtain information about these factors.

Habitual moderate coffee drinking has been associated with a reduced risk of chronic diseases, including cancer, and reduced mortality, whereas high intake has been associated with increased risk (39). The effect of cola intake on reproduction has not been intensively studied, but it has been associated with increased incidence of osteoporosis (40). Colas were originally blends of extracts of the coca leaf and the cola nut, mixed with sugar water. The coca leaf is no longer used, but the cola nut remains in the recipes that are public, and it is reportedly also still in the secret Coca-Cola recipe (The Coca-Cola Company, Atlanta, Georgia). In addition, these drinks contain large quantities of sugar. A report from The Danish Institute for Food and Veterinary Research showed that consumption of sugar-sweetened soft drinks increased from 133 mL to 184 mL per day among Danish teenagers and from 110 mL to 121 mL per day among adults from 1995 to 2001 (25). In our study, mean daily cola intake was 290 mL, which was higher than among Danish teenagers in 2001, indicating that intake increased from 2001 to 2005. Therefore, a possible adverse association with semen quality is of public interest, particularly since poor semen quality in young Danish men is unexplained (14, 15, 41, 42).

In conclusion, we found that moderate caffeine or cola consumption ($\leq$800 mg or 1 L of cola per day) was not associated with a reduction in semen quality. However, among the small fraction of men (3%) who consumed ''high'' quantities of cola, and possibly caffeine, daily (exceeding 800 mg or $>$1.0 L, respectively), several semen parameters were reduced. The associations found for high-quantity cola drinkers could not be attributed to the caffeine content in cola, which was not high. We cannot exclude the possibility of a threshold above which cola (and possibly caffeine) negatively affects semen quality. Alternatively, a less healthy lifestyle among these men may explain the findings. Since cola consumption is high and has been increasing among young Danes, our findings, if confirmed, may be of public health concern.

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