High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population[1–3]

Tina K Jensen, Berit L Heitmann, Martin Blomberg Jensen, Thorhallur I Halldorsson, Anna-Maria Andersson, Niels E Skakkebæk, Ulla N Joensen, Mette P Lauritsen, Peter Christiansen, Christine Dalgård, Tina H Lassen, and Niels Jørgensen

ABSTRACT

Background: Saturated fat intake has been associated with both cardiovascular disease and cancer risk, and a newly published study found an association between saturated fat intake and a lower sperm concentration in infertile men.

Objective: The objective was to examine the association between dietary fat intake and semen quality among 701 young Danish men from the general population.

Design: In this cross-sectional study, men were recruited when they were examined to determine their fitness for military service from 2008 to 2010. They delivered a semen sample, underwent a physical examination, and answered a questionnaire comprising a quantitative food-frequency questionnaire to assess food and nutrient intakes. Multiple linear regression analyses were performed with semen variables as outcomes and dietary fat intakes as exposure variables, adjusted for confounders.

Results: A lower sperm concentration and total sperm count in men with a high intake of saturated fat was found. A significant dose-response association was found, and men in the highest quartile of saturated fat intake had a 38% (95% CI: 0.1%, 61%) lower sperm concentration and a 41% (95% CI: 4%, 64%) lower total sperm count than did men in the lowest quartile. No association between semen quality and intake of other types of fat was found.

Conclusions: Our findings are of potentially great public interest, because changes in diet over the past decades may be part of the explanation for the recently reported high frequency of subnormal human sperm counts. A reduction in saturated fat intake may be beneficial for both general and reproductive health. *Am J Clin Nutr* 2013;97:411–8.

INTRODUCTION

Saturated fat intake is associated with both cardiovascular disease and cancer risk (1, 2). Little is known about the possible influence of diet on semen quality. A recent Cochrane review suggested that treatment of the male partner with antioxidant supplementation may improve live birth and pregnancy rates for infertile couples undergoing infertility treatment, although no convincing effect on semen quality was found (3). Additionally, normospermic controls had a higher intake of carbohydrates and fiber and lower intakes of protein and total fat than did cases with poor semen quality in a Spanish study among infertility patients (4). Furthermore, a traditional Dutch diet characterized by a high intake of meat, potato, and whole grain was found to be positively associated with sperm concentrations (5). A newly published study among 99 US men attending an infertility clinic found that a high intake of saturated fat was negatively related to sperm concentration, whereas a higher intake of omega-3 fatty acids (n−3 fatty acids) was positively related to sperm morphology (6). These studies were, however, conducted among men attending infertility clinics; to our knowledge, no studies have hitherto examined the association between dietary factors and semen quality among men from the general population.

Identifying possible risk factors for infertility is of public health importance because infertility is a common disorder affecting 10–15% of couples attempting to conceive (7). In 2009, ~8% of all Danish children were born after some form of assisted reproduction (7). Poor semen quality is a widespread problem in the population and is part of the etiology in ~50% of couples seeking assistance. Nevertheless, only a few modifiable lifestyle factors associated with reduced semen quality have been identified, although not all have been confirmed by all studies (8–16). We therefore examined the associations between dietary fat intakes and semen quality among 701 young Danish men from the general population, hypothesizing that a high intake of saturated fat is associated with reduced semen quality.

[1] From the University Department of Growth and Reproduction, Rigshospitalet, Denmark (TKJ, MBJ, A-MA, NES, UNJ, MPL, PC, THL, and NJ); the Department of Environmental Medicine, Institute of Public Health, University of Southern Denmark, Odense, Denmark (TKJ and CD); the Research Unit for Dietary Studies, Institute of Preventive Medicine, Copenhagen Capital Region, Copenhagen University Hospitals, Copenhagen, Denmark (BLH); the Unit for Nutrition Research, Faculty of Food Science and Nutrition, University of Iceland, Reykjavik, Iceland (TIH); and the Center for Fetal Programming, Department of Epidemiology Research, Statens Serum, Institute, Copenhagen, Denmark (TIH).

[2] Supported by The Danish Council for Strategic Research, Program Commission on Health, Food and Welfare (project no. 2101-08-0058), Rigshospitalet (grant 961506336), European Union, DEER (grant agreement no. 212844), The Danish Ministry of Health, The Danish Environmental Protection Agency, and Kirsten and Freddy Johansens Foundation (grant 95-103-72087).

[3] Address correspondence to TK Jensen, University Department of Growth and Reproduction, Rigshospitalet, Blegdamsvej 9, 2100 Copenhagen, Denmark. E-mail: tkjensen@health.sdu.dk.

Received May 2, 2012. Accepted for publication October 26, 2012.
First published online December 26, 2012; doi: 10.3945/ajcn.112.042432.

Supplemental Material can be found at:
http://ajcn.nutrition.org/content/suppl/2013/02/07/ajcn.112.042432.DC1.html

SUBJECTS AND METHODS

Because of the military drafting system in Denmark, all 18-y-old men, 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 because of education and are therefore not called up until they graduate. From 1996 onward, trained staff from the University Department of Growth and Reproduction approached the draftees when they appeared for the compulsory physical examination in Copenhagen, Denmark, and invited them to participate in a study of semen quality. Participants from April 2008 to June 2010 were included in the current study as the questionnaire they completed included a food-frequency questionnaire. Participants were compensated for their time (500 DKK ≈ US$85). They completed a questionnaire, delivered a semen sample, had a blood sample drawn, and underwent a physical examination (17, 18). The exact participation rate could not be calculated but was between 20% and 30%. We had access to basic information on the age and educational status of nonparticipants, and they did not differ from participants with regard to age, but the participants were better educated than the nonparticipants (data not shown). Approval was obtained from the local ethical committee.

Semen analysis

All participants provided a semen sample by masturbation in a room close to the semen laboratory. The period of ejaculation abstinence was recorded. Semen analysis was performed according to the WHO guidelines (19, 20). In brief, semen volume was estimated by weighing the collection tube with the semen sample and subtracting the predetermined weight of the empty tube, assuming that 1 mL semen = 1 g. For the sperm motility assessment, 10 μL well-mixed semen was placed on a clean glass slide, kept at 37°C, and covered with a 22 × 22 mm^2 coverslip. The preparation was placed on the heated stage of a microscope at 37°C and was immediately examined at ×400 magnification. The sperm were classified manually as progressively motile, locally motile, or immotile. For the assessment of the sperm concentration, the samples were diluted in a solution of 0.6 mol $NaHCO_3$/L and 0.4% (vol:vol) formaldehyde in distilled water. The sperm concentration was subsequently assessed by using a Bürker-Türk hemocytometer (Paul Marienfeld GmbH & Co KG). Only sperm with tails were counted. Smears were prepared for morphologic evaluation, Papanicolaou stained, and finally assessed according to strict criteria by 2 experienced technicians (21, 22). Since the start of the program in 1996, our laboratory has directed a quality-control program to assess sperm concentrations and to ensure that interlaboratory differences remained unchanged in comparison with 2 other laboratories (18, 21).

Physical examination

All physical examinations were performed by the same 9 physicians, recording Tanner stage of pubic hair and genital development, testicular volumes (determined by use of a Prader orchidometer), absence or presence of varicocele (stages 1–3) or hydrocele, location of the testes in the scrotum, and consistency of the testis and epididymis. Weight and height were measured, and BMI was calculated as weight (in kg) divided by squared height (in m).

Questionnaire

All participants completed a questionnaire on health, lifestyle, and diet before the examination. This included information on previous and/or current diseases and genital diseases such as inguinal hernia, varicocele, epididymitis, gonorrhea, *Chlamydia*, and surgery for testicular torsion. They 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) within the previous 3 mo. Self-reported diseases in the reproductive organs that may affect semen quality (torsion of testes, epididymitis, or inguinal hernia) were transformed into 2 variables: "self-reported genital conditions" and "sexually transmitted diseases" (gonorrhea or *Chlamydia*).

The participants responded to questions about their mothers' education level, coded as <9 y, 9–10 y, or >10 y of attendance. The estimated caffeine intake from cola, other sodas, diet cola, other diet soft drinks, caffeine-containing energy drinks (eg, Red Bull), chocolate, coffee, tea, and chocolate-containing beverages were calculated (23). The men were asked about daily unit intakes of red and white wine, beer, strong alcoholic drinks, and alcohol pops during the past week, and weekly alcohol intake was calculated as the sum of the daily reported unit intake. In addition, they were asked about physical activity, and total metabolic hours of activity per week were estimated as the sum of hours spent on various activities taking into account the intensity of that activity.

Dietary intake was assessed by using a validated 136-item food-frequency questionnaire (FFQ) covering the 3 mo before recruitment. Intake of total dietary fat, saturated fat, polyunsaturated fat, and monounsaturated fat as a percentage of total energy was calculated. The absolute daily intake of omega-3 and omega-6 fatty acids (n−3 and n−6 fatty acids) was calculated (g/d) as the sum of linolenic acids (18:3n–3), stearidonic acid (18:4n–3), eicosapentaenoic acid (20:5n–3) and cocosahexaenoic acid (22:6n–3), and linoleic acid (18:2n–6) and arachidonic acid (20:4n–6), respectively. The questionnaire was a modified version of the FFQ previously used and validated in the Danish National Birth Cohort and the Danish Diet, Cancer and Health Studies (24, 25) but not in young men. Portion sizes for individual food items were estimated with the help of photo series, and nutrients were quantified on the basis of the Danish food-composition tables (26), which may increase the accuracy of our intake estimates for the young males.

Statistics

The men were divided into quartiles according to percentage intake of total energy from total fat and from saturated fat, polyunsaturated fat, and monounsaturated fat. In addition, quartiles of total daily n−3 and n−6 fatty acid intake (g/d) were calculated. We calculated median and 5th and 95th percentiles of semen quality variables for each quartile of the fat intakes and tested trends by inserting the different fat types as continuous variables in a univariate ANOVA, with semen variables as outcome variables (sperm concentration and total sperm count transformed by use of the natural logarithm). Then, we compared the distributions

of the variables from the questionnaires and physical examinations among men in the quartiles of saturated fat (% of energy) by chi-square test to identify potential confounders.

The data were analyzed by using multiple linear regression to estimate the difference in semen quality variables for increasing quartile of fat intake, taking into account the differences in confounders. 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 approximate normality and were then back-transformed to obtain the percentage change in these variables. Covariates initially included were possibly associated with semen variables or fat (intake of energy), and were excluded stepwise if they did not change the estimate by >10%. The same set of confounders was used for all analyses; period of abstinence (transformed by the natural logarithm), BMI, alcohol consumption, smoking, cryptorchidism (categorized as shown in **Table 1**), and for motility duration between time of ejaculation and analysis of the sample. In addition, we included the percentage of energy from protein intake and the remaining types of fatty acids to simulate the isocaloric substitution of fat and carbohydrates (27). We did not include maternal education level in the final models because it was missing among 10% of the sample, but we repeated the analyses including maternal education level. In addition, we included physical activity; however, neither of these 2 factors changed the associations. We evaluated whether the association between percentage of energy from fat and semen quality was the same in different categories of BMI, smoking, maternal education, and alcohol intake to test for effect modification. Additionally, we performed a second set of models in which nutrients were modeled as continuous variables and percentage of energy from saturated fat intake was divided into deciles to test the dose-response association. Finally, we compared the percentage of energy from fat for men with semen

TABLE 1
Information from questionnaires and physical examination among 701 Danish men according to percentage energy intake from saturated fat in quartiles

		Quartile of percentage energy intake from saturated fat				
Variables	Subjects (n = 701)	<11.20 (n = 174)	11.21–13.27 (n = 179)	13.28–15.19 (n = 170)	>15.19 (n = 178)	P^1
Information obtained at physical examination [% (n)]						
Examined between October and March	53 (374)	51	50	55	58	—
Period of abstinence >96 h	13 (89)	18	10	11	13	—
Varicocele stage 2 or 3	8 (53)	8	8	7	8	—
BMI						<0.05
<20 kg/m^2	15 (95)	10	15	11	23	—
20–24.99 kg/m^2	66 (432)	70	60	68	66	—
≥25 kg/m^2	19 (127)	20	25	21	11	—
Information obtained from questionnaire [% (n)]						<0.05
Fever >38°C within past 3 mo	8 (51)	6	7	14	4	—
Age >20 y	24 (164)	24	22	21	28	—
Alcohol intake >21 units/wk[2]	27 (190)	23	25	32	29	—
Total caffeine intake >300 mg/d	22 (151)	22	19	23	23	—
Physical activity >400 watt/wk	49 (341)	45	52	49	45	—
Maternal education level						—
<9 y	4 (31)	13	3	5	6	—
9–10 y	20 (141)	17	19	18	26	—
>10 y	65 (458)	66	71	70	55	—
Missing	110 (71)	5	7	8	13	—
Weekly current smoking [% (n)]	47 (332)	43	45	49	52	—
Exposure to mother's smoking in utero [% (n)]	27 (173)	26	29	27	27	—
Self-reported genital conditions [% (n)][3]	7 (47)	7	5	8	8	—
Sexually transmitted diseases [% (n)][4]	11 (77)	7	11	9	19	<0.05
Born with cryptorchidism [% (n)]	5 (32)	5	3	6	5	
Daily intake of macronutrients						
Total energy intake (MJ)	9.6 ± 4.5[5]	8.6 ± 3.2	9.4 ± 5.4	9.8 ± 4.0	10.6 ± 4.7	<0.05
Total fat (% of energy)	31 ± 5.9	24 ± 3.7	29 ± 2.2	33 ± 2.2	38 ± 3.4	<0.05
Monounsaturated fat (% of energy)	11.4 ± 2.4	4.2 ± 1.0	4.8 ± 0.7	5.2 ± 0.8	5.5 ± 0.9	<0.05
Polyunsaturated fat (% of energy)	4.9 ± 1.0	8.6 ± 1.7	10.8 ± 1.1	12.3 ± 1.3	13.8 ± 1.6	<0.05
n−3 Fatty acid (g/d)	0.9 ± 0.3	0.7 ± 0.3	0.9 ± 0.3	1.0 ± 0.3	1.1 ± 0.3	<0.05
n−6 Fatty acid (g/d)	3.7 ± 0.7	3.1 ± 0.6	3.5 ± 0.5	3.9 ± 0.6	4.1 ± 0.7	<0.05
Protein (% of energy)	16.5 ± 3.2	16.4 ± 3.6	16.6 ± 3.5	16.7 ± 2.8	16.1 ± 3.0	—
Carbohydrate (% of energy)	56 ± 7.7	65 ± 5.9	58 ± 5.6	54 ± 4.3	49 ± 5.3	<0.05

[1] Chi-square test.
[2] One unit = 12 g alcohol.
[3] Self-reported information about torsion of testes, epididymitis, or inguinal hernia.
[4] Sexually transmitted diseases, gonorrhea, and *Chlamydia*.
[5] Mean ± SD (all such values).

variables below WHO reference values (20) (semen volume <1.5 mL, sperm concentration <15 million/mL, total sperm count <39 million/mL, motile sperms <40%, and morphologically normal sperm <4%) by binary logistic regression analyses, adjusting for the same set of confounders. The results are presented as coefficients with 95% CIs. We evaluated the fit of the models by testing the residuals for normality and by visually inspecting the residual plots. The analyses were performed by using PASW GradPack V.18.0 (SPSS Inc).

RESULTS

A total of 701 men without azoospermia participated. Their median age was 19.1 (5th–95th percentiles: 18.4–22.9) y, and their median BMI (in kg/m^2) was 22.5 (18.8–28.9). Their mean total energy intake was 9.6 ± 4.5 MJ and their percentages of energy from total fat, protein, and carbohydrates were 31.2 ± 5.9, 16.5 ± 3.2, and 56.4 ± 7.7, respectively.

Overall, demographic and clinical characteristics were the same across quartiles of percentage of energy intake from total fat (Table 1). Men in the highest quartile of total fat intake, however, had a lower BMI and more often reported having had sexually transmitted diseases.

Sperm concentration and total sperm count were lower among men with a high percentage of energy from saturated fat intake (**Table 2**). In addition, a high intake of n−3 fatty acids was associated with increased volume and n−6 fatty acids with higher percentages of motile and morphologic normal spermatozoa, although the latter were not statistically significant. A total of 13% and 18% of men in the first and fourth quartiles of percentages of energy from saturated fat, respectively, had a sperm concentration <15 million/mL (WHO manual, 2010).

After adjustment, a trend of decreasing sperm concentration (P = 0.04) and total sperm count (P = 0.02) associated with an increasing percentage of energy from saturated fat was observed (**Table 3**). The highest quartile intake of saturated fat was

TABLE 2
Semen quality among 701 Danish men according to quartiles of total fats and major fatty acid intake[1]

Quartile of dietary intake of macronutrients	No. of subjects	Semen volume	Sperm concentration	Total sperm count	Motile sperm	Morphologically normal forms
		mL	*millions/mL*	*millions*	%	%
Total fat						
<27.30% of energy	174	3.1 (1.1–6.5)	52 (5–225)	164 (13–649)	69 (36–86)	7.5 (1.0–17.2)
27.30–31.40% of energy	179	3.3 (1.2–5.8)	50 (4–169)	161 (16–505)	71 (42–86)	7.5 (0.5–16.0)
31.50–35.0% of energy	170	3.2 (1.5–6.0)	44 (3–144)	140 (8–506)	67 (35–86)	6.5 (0.5–15.5)
>35.0% of energy	178	3.1 (1.3–6.7)	44 (3–156)	125 (11–479)	70 (35–88)	7.0 (0.0–16.5)
P-trend[2]		0.87	0.16	0.17	0.90	0.73
SFA						
<11.20% of energy	172	3.1 (1.2–6.3)	50 (8–215)	163 (19–651)	68 (36–87)	7.5 (1.0–16.0)
11.20–13.27% of energy	179	3.4 (1.1–6.2)	53 (6–171)	174 (13–539)	71 (42–85)	8.0 (1.0–18.0)
13.28–15.19% of energy	176	3.1 (1.4–6.1)	44 (2–147)	130 (9–504)	69 (36–86)	6.0 (0.5–15.0)
>15.19% of energy	174	3.1 (1.3–6.4)	45 (3–151)	128 (10–459)	69 (31–88)	6.5 (0.0–16.2)
P-trend[2]		0.49	0.03	0.02	0.49	0.32
MUFA						
<9.80% of energy	174	3.1 (1.1–6.3)	52 (5–214)	164 (13–667)	69 (37–86)	8.0 (1.0–17.0)
9.80–11.39% of energy	179	3.2 (1.1–5.8)	50 (4–168)	158 (14–442)	70 (35–85)	7.0 (0.5–16.0)
11.40–12.90% of energy	173	3.2 (1.5–6.9)	41 (3–149)	132 (9–530)	69 (31–86)	6.5 (0.0–15.5)
>12.90% of energy	175	3.2 (1.3–6.1)	48 (5–151)	139 (15–483)	69 (43–88)	7.5 (1.3–16.5)
P-trend[2]		0.84	0.38	0.53	0.94	0.85
PUFA						
<4.31% of energy	177	3.0 (1.0–6.3)	52 (2–212)	141 (8–635)	70 (35–85)	7.3 (0.4–15.6)
4.31–4.90% of energy	180	3.2 (1.3–6.3)	43 (3–176)	140 (13–531)	67 (36–86)	6.5 (1.0–17.1)
4.91–5.49% of energy	173	3.2 (1.3–6.2)	48 (5–166)	168 (9–535)	70 (35–85)	7.0 (0.5–16.5)
>5.49% of energy	171	3.2 (1.4–6.2)	48 (8–148)	148 (16–503)	71 (43–89)	7.5 (1.0–16.0)
P-trend[2]		0.23	0.39	0.18	0.07	0.11
Sum of n−3 fatty acids						
<0.72 g/d	183	3.1 (1.0–6.3)	50 (3–197)	143 (11–639)	70 (36–86)	7.5 (0.5–17.5)
0.72–0.88 g/d	174	3.1 (1.3–5.7)	44 (2–178)	138 (5–448)	68 (33–85)	7.5 (0.0–16.2)
0.89–1.09 g/d	179	3.1 (1.2–6.2)	52 (5–166)	149 (18–523)	70 (37–87)	6.5 (1.0–15.5)
>12.90 g/d	165	3.4 (1.5–7.3)	44 (4–148)	159 (11–507)	72 (39–88)	7.5 (0.6–16.4)
P-trend[2]		0.04	0.33	0.11	0.19	0.19
Sum of n−6 fatty acids						
<3.21 g/d	180	3.1 (1.1–6.3)	48 (2–199)	139 (5–634)	68 (35–86)	7.0 (0.5–15.5)
3.21–3.62 g/d	171	3.2 (1.2–6.8)	44 (4–180)	141 (13–480)	68 (34–85)	6.5 (0.5–18.5)
3.63–4.07 g/d	176	3.2 (1.3–6.1)	52 (4–166)	158 (10–570)	70 (35–86)	7.3 (0.4–15.2)
>4.07 g/d	174	3.2 (1.4–6.1)	48 (7–151)	149 (16–509)	71 (43–88)	7.5 (1.0–16.7)
P-trend[2]		0.47	0.62	0.37	0.07	0.07

[1] All values are medians; 5th–95th percentiles in parentheses.
[2] Trend tested by inserting continuous variables of fat intake into unadjusted univariate analyses of variance with semen variables (sperm concentration and total sperm count transformed by the natural logarithm).

TABLE 3
Adjusted differences in semen quality by percentage intake of total fats and major fatty acid groups from a multiple linear regression analysis

Quartile of dietary intake of macronutrients	Semen volume[1]		Sperm concentration[1,2]		Total sperm count[1,2]		Morphologically normal forms[1]	
	β Coefficient	95% CI	β Coefficient	95% CI	β Coefficient	95% CI	β Coefficient	95% CI
	mL		*millions/mL*		*millions*		*%*	
Total fat[3]								
<27.30% of energy	Reference		Reference		Reference		Reference	
27.30–31.40% of energy	0.0	−0.3, 0.4	3	−21, 34	5	−20, 38	−0.6	−1.5, 0.7
31.5–35.0% of energy	−0.1	−0.4, 0.3	−8	−30, 20	8	−30, 22	−1.0	−2.0, 0.2
>35.0% of energy	0.0	−0.4, 0.3	−9	−30, 20	−9	−31, 21	−0.4	−1.5, 0.7
P-trend[4]	0.89		0.37		0.36		0.40	
Continuous[5]	0.0	−0.02, 0.02	−0.4	−2.0, 1.2	−0.5	−2.1, 1.2	−0.01	−0.07, 0.06
Saturated fat								
<11.20% of energy	Reference		Reference		Reference		Reference	
11.20–13.27% of energy	0.1	−0.3, 0.5	−13	−36, 18	−13	−37, 19	0.6	−0.7, 1.8
13.28–15.19% of energy	−0.1	−0.6, 0.4	−27	−50, 6	−31	−53, 0.0	−0.9	−2.4, 0.6
>15.19% of energy	0.1	−0.5, 0.7	−38	−61, −0.1	−41	−64, −4	−0.5	−2.5, 1.4
P-trend[4]	0.91		0.04		0.02		0.22	
Continuous[5]	0.0	−0.1, 0.1	−7.6	−14.3, −0.4	−10.1	−16.7, −2.9	−0.03	−0.3, 0.3
Monounsaturated fat								
<9.8% of energy	Reference		Reference		Reference		Reference	
9.8–11.39% of energy	0.0	−0.4, 0.4	8	−22, 49	13	−19, 58	−1.7	−3.0, −0.4
11.40–12.9% of energy	0.2	−0.4, 0.7	−8	−38, 38	2	−33, 53	−2.1	−3.7, −0.4
>12.9% of energy	0.1	−0.6, 0.8	12	−36, 94	23	−30, 216	−2.4	−4.7, −0.2
P-trend[4]	0.61		0.99		0.70		0.05	
Continuous[5]	0.0	−0.2, 0.2	5.6	−7.4, 20.3	8.3	−5.3, 23.9	−0.4	−0.9, 0.2
Polyunsaturated fat								
<4.31% of energy	Reference		Reference		Reference		Reference	
4.31–4.90% of energy	0.2	−0.2, 0.6	−8	−33, 26	−4	−31, 33	−0.4	−1.7, 0.9
4.91–5.49% of energy	0.1	−0.4, 0.6	2	−31, 50	7	−28, 59	−0.8	−2.4, 0.8
>5.49% of energy	0.1	−0.6, 0.8	−12	−49, 52	−10	−49, 58	−1.1	−3.4, 1.1
P-trend[4]	0.96		0.89		0.97		0.31	
Continuous[5]	0.0	−0.4, 0.4	3.4	−22.9, 38.5	−0.3	−26.1, 34.5	0.1	−1.1, 1.3
Sum of n−3 fatty acids								
<0.72 g/d	Reference		Reference		Reference		Reference	
0.72–0.88 g/d	0.1	−0.3, 0.5	−10	−32, 20	−7	−30, 24	−0.1	−1.0, 1.7
0.89–1.09 g/d	0.2	−0.2, 0.6	12	−18, 51	20	−12, 65	−0.5	−1.8, 0.7
>12.9 g/d	0.5	−0.2, 1.0	−8	−36, 33	6	−28, 55	0.2	−1.3, 1.8
P-trend[4]	0.05		0.94		0.40		0.99	
Continuous[5]	0.7	0.03, 1.3	1.1	−31, 84.0	35.8	−17.6, 123	0.9	−1.1, 2.9
Sum of n−6 fatty acids								
<3.21 g/d	Reference		Reference		Reference		Reference	
3.21–3.62 g/d	0.2	−0.2, 0.6	4	−23, 39	9.5	−19, 48	−0.1	−1.3, 1.1
3.63–4.07 g/d	0.1	−0.4, 0.5	21	−14, 71	27.1	−11, 81	−0.1	−1.5, 1.4
>4.07 g/d	0.2	−0.5, 0.8	1	−36, 61	8.8	−32, 75	−0.1	−2.0, 1.8
P-trend[4]	0.78		0.65		0.49		0.94	
Continuous[5]	0.02	−0.4, 0.5	2.6	−27.8, 45.9	4.1	−28, 49	1.3	−0.2, 2.8

[1] Adjusted for period of abstinence (transformed by the natural logarithm), BMI, alcohol consumption, smoking, and cryptorchidism categorized according to Table 2 and total energy intake, protein intake, and remaining fatty acids.
[2] The sperm concentration and total sperm count were transformed by using the natural logarithm and were back-transformed to obtain the percentage change.
[3] Total fat intake was not adjusted for the intake of remaining fatty acids.
[4] Trend in values.
[5] The estimate provides the change in semen quality with an increase of 1% of energy as fat.

associated with a 38% (95% CI: 0.1%, 61%) lower sperm concentration and a 41% (4%, 64%) lower total sperm count compared with an intake of saturated fat in the lowest quartile (Table 3).

Results modeling the percentage of energy from saturated fat as a continuous variable showed similar results: an 8% (0.4%, 14%) lower sperm concentration and a 10% (3%, 17%) lower total sperm count for each percentage of energy increase in saturated fat (Table 3). Grouping percentage of energy from saturated fat into deciles showed similarly significant trends in sperm concentration and total sperm count with higher saturated fat intake (**Figure 1**). Those with the highest decile intake of saturated fat had 59% (14%, 80%) and 65% (25%, 84%) lower sperm concentrations and a lower total sperm count, respectively, than did those with

the lowest decile intake. A higher percentage of energy from monounsaturated fat was associated with a lower percentage of sperm with normal morphology ($P = 0.05$), and a higher intake of n−3 fatty acids was associated with a higher semen volume ($P = 0.05$) (Table 3). No association between the percentage of motile sperm and any particular fat was detected (data not shown). When semen variables were categorized according to WHO 2010 reference values, the adjusted OR of having semen variables below references values increased with an increasing percentage of energy from saturated fat, although not statistically significantly so (data not shown).

We repeated the analyses including maternal education level and physical activity in the models; the size of the associations was essentially similar before and after adjustment, except for slightly inflated CIs. We also excluded total energy intake from the models, which generally did not affect the estimates. Stratified analyses were performed, and similar associations were observed between saturated fat intake and sperm concentration, total sperm count, and percentage of morphologically normal sperm among smokers and nonsmokers, among men drinking > or <21 units of alcohol/wk, among men with different BMI categories (Table 1), and among men with differences in maternal education.

DISCUSSION

In this cross-sectional study of 701 young Danish men, we detected a lower sperm concentration and a lower total sperm count among men with a high percentage of saturated fat. A significant dose-response association was found, ie, saturated fat intake above the recommended 10% was associated with a reduced sperm count and concentration. Men with the highest decile percentage of energy from saturated fat had an ~60% lower sperm concentration and total sperm count compared with men with the lowest decile intake. In addition, the percentage of spermatozoa with normal morphology was lower among men with a high percentage of energy from monounsaturated fat, and semen volume was higher among men with a high intake of n−3 fatty acids. The lower semen quality among men with a higher percentage of energy from saturated fat was not due to a higher total energy intake, because adjustment for total energy did not change the findings. The cross-sectional nature of our study made it difficult to draw firm conclusions about this association, and we could not exclude the possibility that men with a high saturated fat intake generally had an unhealthier lifestyle and health behavior, which may also affect their semen quality. However, all analyses were adjusted for other macronutrients, physical activity, BMI, smoking, alcohol consumption, and maternal socioeconomic status, which made this possibility less likely.

Adjusted ORs of having semen variables below WHO 2010 references values were increased with an increasing percentage of energy from saturated fat, although not statistically significantly so. However, among these normal men, few had semen quality below clinically relevant cutoff values, and the power of these dichotomized were therefore limited.

To our knowledge, no previous studies have examined the association between fat intake and semen quality among men from the general population. However, our results agree with and are of the same magnitude as those in a newly published study among 99 US men attending an infertility clinic, which found that men in the highest third of total fat intake had a 43% lower total sperm count and a 38% lower concentration than did men in the lowest third of total fat intake (6). A Spanish study among infertile men showed a similar association between intake of processed meat as a source of saturated fat and poor semen quality (4). This finding is in contrast with the findings among infertile men with a traditional Dutch dietary pattern (high in meat content), who had a higher sperm concentration than did men with high fruit, vegetable, and fish intakes (5). Likewise, in a subpopulation of 33 of the 99 US infertile men, associations between *trans* fatty acids and saturated fat in seminal plasma and semen quality were found, but these results were not adjusted for confounders (28). The association between the serum and seminal plasma concentrations of fatty acids, however, is not clear. Reverse causality may be a problem in many of these studies, because the men may have changed their lifestyles as a consequence of infertility.

The associations between a high intake of n−3 fatty acids and high semen volume and a high intake of monounsaturated fat and percentages of spermatozoa with normal morphology may have been due to multiple testing, but they were nevertheless in

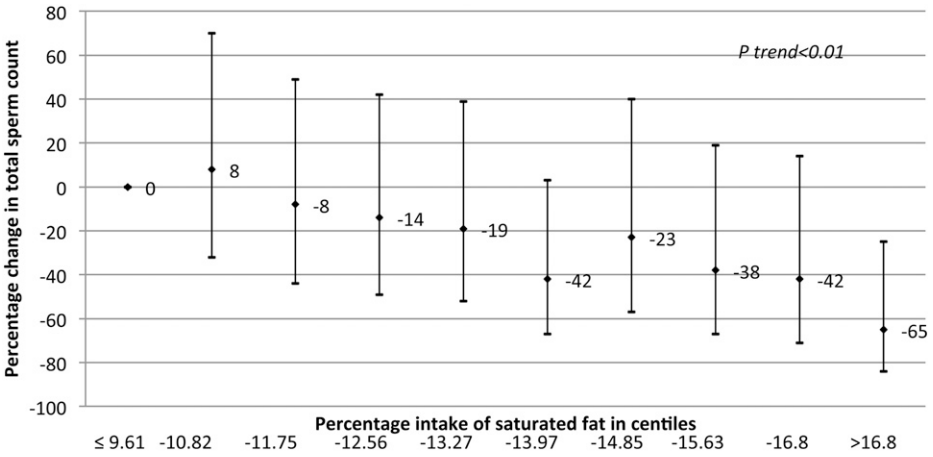

FIGURE 1. Adjusted percentage reduction in total sperm count (and 95% CIs) with increasing intakes of saturated fat (as a percentage of total energy, divided into deciles). Adjusted for period of abstinence (transformed by the natural logarithm), BMI, alcohol consumption, smoking, cryptorchidism, total energy intake, protein intake, and remaining fatty acids in multiple linear regression.

line with results from a clinical trial among 238 Iranian oligospermic men and a recent study among 99 US infertile men (6, 29) but contradictory to the findings of a Canadian study (30). Our findings are also supported by animal studies in which dietary supplementation of n−3 fatty acids in boars improved sperm morphology, whereas the results for total sperm count and motility are inconsistent (31–33).

A high intake of saturated fat is generally considered unhealthy and has been associated with an increased risk of cardiovascular disease and prostate cancer (1, 2). It is recommended that saturated fat intake should be <10% (34), and, interestingly, we found a lower sperm count with a saturated fat intake >10%. In addition, the inverse association between the monounsaturated fat to saturated fat ratio and prostate cancer (35) suggests that the inflammatory effect of saturated fat may influence the endocrine system. We can only speculate on the potential mechanisms. A study in rabbits suggested that a diet rich in n−3 fatty acids improves sperm quality by modifying the sperm lipid composition also in the sperm subfractions (36). It could also be caused by altered lipid metabolism and increasing insulin production, resulting in secondary effects in various target organs. One study in rabbits fed a cholesterol-enriched diet found not only a marked increase in plasma cholesterol concentrations but also the same phenotype reported here with decreased semen volume and total motility and increased sperm abnormality (37). Sperm concentration was also decreased, although not significantly so. The authors speculated that the observed effects could have been due to changes in the cholesterol concentration of sperm membrane, which may affect membrane dynamics and sperm functionality.

More than 30% of the saturated fat intake among the men in this study was derived from dairy and cheese products. Residues of lipophilic environmental chemicals that bioaccumulate in fat have been found in high-fat dairy products (38), and some of these lipophilic chemicals may have endocrine-disrupting abilities (39), eg, polychlorinated biphenyls and polyfluorinated chemicals, both of which have been associated with poor semen quality (40, 41).

Our study was large, and the participation rate of 20% to 30% was higher than that of other population-based semen-quality studies (18, 42–44). Also, most (95%) of these young men had no knowledge of their own fertility potential, so it was unlikely to have affected their motivation to participate. However, the study was cross-sectional; thus, reverse causation was possible, although unlikely, because the men were unaware of their semen quality when they responded to the FFQ. In addition, they were unlikely to have changed their lifestyle as a consequence of infertility, which made reverse causation less possible and any reporting bias was therefore likely to have been nondifferential and to have underestimated rather than overestimated the associations with fat intake. The men were asked to report food intake 3 mo before the completion of the questionnaire. If this differed from their general food intake, misclassification of exposure may have occurred, but because they responded to the questionnaire before they delivered their semen sample, this nondifferential misclassification was likely to result in an underestimation of the association between saturated fat intake and semen quality. In addition, spermatozoa mature within 3 mo; therefore, estimated nutrient intake over the past 3 mo is a good choice for studying the association between semen quality and diet. Semen quality may not adequately address fertility potential, because it may decline considerably before the ability to conceive is affected (45), even though a sperm concentration <40 million/mL has been associated with a prolonged ability to conceive (46).

In this study, mean total energy intake and percentage intake of fat estimated from a quantitative FFQ were 9.6 MJ and 31.2%, respectively, which is lower than in previous studies among young Danish men (47). FFQs have been shown to have adequate validity and reproducibility for the use in epidemiologic studies (27), although not tested in young men, it is nevertheless prone to measurement error usually attenuating the associations of interest (27). We obtained only one semen sample from each man, and considerable intraindividual variation exists in semen quality. However, studies have suggested that it is more efficient to include a larger number of men with one sample than fewer men with more samples (48).

Men who had a high intake of saturated fat also had an unhealthier lifestyle. They had a lower BMI, which was also previously reported (47). BMI is not always related to fat mass, and men with a high saturated fat intake may possibly have a low BMI because of a lower muscle mass. Because these lifestyle and demographic factors may also be associated with semen quality, we tried to adjust for them to the extent possible in the analyses, and they did not appear to explain the associations between saturated fat intake and semen quality. However, we cannot exclude the possibility that a dietary pattern with a high intake of saturated fat is associated with residual or unmeasured confounding.

In conclusion, among 701 young men from the general population, we showed a dose-response association between increased intake of saturated fat and a lower sperm concentration and total sperm count. Our findings, if confirmed, may be of public health concern. It is possible that the high intake of saturated fat in the Western world may help explain the recently reported high frequency of subnormal sperm counts and the high rate of infertility. In any case, the current findings suggest that adapting dietary intake toward eating less saturated fat may be beneficial for both general and reproductive health.

The authors' responsibilities were as follows—NJ, TKJ, NES, and A-MA: designed the research; UNJ, MPL, PC, MBJ, and THL: conducted the research; and TKJ, BLH, TIH, and CD: analyzed the data, interpreted the dietary data, and wrote the manuscript. All authors had primary responsibility for the final content. None of the authors declared a conflict of interest. The sponsors of the study had no role in the study design, data collection, analysis, interpretation, or writing of the manuscript.

REFERENCES

1. Hooper L, Summerbell CD, Thompson R, Sills D, Roberts FG, Moore HJ. Davey Smith G. Reduced or modified dietary fat for preventing cardiovascular disease. Cochrane Database Syst Rev 2011;CD002137.
2. Escrich E, Moral R, Grau L, Costa I, Solanas M. Molecular mechanisms of the effects of olive oil and other dietary lipids on cancer. Mol Nutr Food Res 2007;51:1279–92.
3. Showell MG, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. Cochrane Database Syst Rev 2011;CD007411.
4. Mendiola J, Torres-Cantero AM, Moreno-Grau JM, Ten J, Roca M, Moreno-Grau S, Bernabeu R. Food intake and its relationship with semen quality: a case-control study. Fertil Steril 2009;91:812–8.
5. Vujkovic M, de Vries JH, Dohle GR, Bonsel GJ, Lindemans J, Macklon NS, van der Spek PJ, Steegers EA, Steegers-Theunissen RP. Associations between dietary patterns and semen quality in men undergoing IVF/ICSI treatment. Hum Reprod 2009;24:1304–12.

6. Attaman JA, Toth TL, Furtado J, Campos H, Hauser R, Chavarro JE. Dietary fat and semen quality among men attending a fertility clinic. Hum Reprod 2012;27:1466–74.
7. Hull MGR, Glazener CMA, Kelly NJ, Conway DI, Foster PA, Hinton RA, Coulson C, Lambert PA, Watt EM, Desai KM. Population study of causes, treatment, and outcome of infertility. Br Med J (Clin Res Ed) 1985;291:1693–7.
8. Ravnborg TL, Jensen TK, Andersson AM, Toppari J, Skakkebaek NE, Jorgensen N. Prenatal and adult exposures to smoking are associated with adverse effects on reproductive hormones, semen quality, final height and body mass index. Hum Reprod 2011;26:1000–11.
9. Jensen TK, Swan SH, Skakkebaek NE, Rasmussen S, Jorgensen N. Caffeine intake and semen quality in a population of 2,554 young Danish men. Am J Epidemiol 2010;171:883–91.
10. Jensen TK, Jorgensen N, Punab M, Haugen TB, Suominen J, Zilaitiene B, Horte A, Andersen AG, Carlsen E, Magnus Ø, et al. Association of in utero exposure to maternal smoking with reduced semen quality and testis size in adulthood: a cross-sectional study of 1,770 young men from the general population in five European countries. Am J Epidemiol 2004;159:49–58.
11. Gaur DS, Talekar MS, Pathak VP. Alcohol intake and cigarette smoking: impact of two major lifestyle factors on male fertility. Indian J Pathol Microbiol 2010;53:35–40.
12. Ramlau-Hansen CH, Thulstrup AM, Storgaard L, Toft G, Olsen J, Bonde JP. Is prenatal exposure to tobacco smoking a cause of poor semen quality? A follow-up study. Am J Epidemiol 2007;165:1372–9.
13. Ramlau-Hansen CH, Thulstrup AM, Aggerholm AS, Jensen MS, Toft G, Bonde JP. Is smoking a risk factor for decreased semen quality? A cross-sectional analysis. Hum Reprod 2007;22:188–96.
14. Martini AC, Molina RI, Estofan D, Senestrari D, Fiol de CM, Ruiz RD. Effects of alcohol and cigarette consumption on human seminal quality. Fertil Steril 2004;82:374–7.
15. Bracken MB, Eskenazi B, Sachse K, McSharry J-E, Hellenbrand K, Leo-Summers L. Association of cocaine use with sperm concentration, motility, and morphology. Fertil Steril 1990;53:315–22.
16. Sadeu JC, Hughes CL, Agarwal S, Foster WG. Alcohol, drugs, caffeine, tobacco, and environmental contaminant exposure: reproductive health consequences and clinical implications. Crit Rev Toxicol 2010;40:633–52.
17. Andersen AG, Jensen TK, Carlsen E, Jørgensen N, Andersson AM, Krarup T, Keiding N, Skakkebaek NE. High frequency of sub-optimal semen quality in an unselected population of young men. Hum Reprod 2000;15:366–72.
18. Jørgensen N, Joensen UN, Jensen TK, Jensen MB, Almstrup K, Olesen IA, Juul A, Andersson AM, Carlsen E, Petersen JH, et al. Human semen quality in the new millennium: a prospective cross-sectional population-based study of 4867 men. BMJ Open 2012;July;2.
19. World Health Organization. WHO laboratory manual for the examination of human semen and sperm-cervical mucus interaction. 4th ed. Cambridge, United Kingdom: Cambridge University Press, 1999.
20. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva, Switzerland: WHO, 2010.
21. Jørgensen N, Auger J, Giwercman A, Irvine DS, Jensen TK, Jouannet P, Keiding N, Le Bon C, MacDonald E, Pekuri AM, et al. Semen analysis performed by different laboratory teams: an intervariation study. Int J Androl 1997;20:201–8.
22. Menkveld R, Stander FS, Kotze TJ, Kruger TF, Van Zyl JA. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Hum Reprod 1990;5:586–92.
23. Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A, Feeley M. Effects of caffeine on human health. Food Addit Contam 2003;20:1–30.
24. Tjønneland A, Overvad K, Haraldsdottir J, Bang S, Ewertz M, Jensen OM. Validation of a semiquantitative food frequency questionnaire developed in Denmark. Int J Epidemiol 1991;20:906–12.
25. Mikkelsen TB, Osler M, Olsen SF. Validity of protein, retinol, folic acid and n−3 fatty acid intakes estimated from the food-frequency questionnaire used in the Danish National Birth Cohort. Public Health Nutr 2006;9:771–8.
26. National Food Institute, Technical University of Denmark. Danish food composition databank, edition 7.01. Available from: www.foodcomp.dk. 2012 (cited August 2011).
27. Willett WC, Lenart E. Nutritional epidemiology. 2nd ed. New York, NY: Oxford University Press, 1998.
28. Chavarro JE, Furtado J, Toth TL, Ford J, Keller M, Campos H, Hauser R. Trans-fatty acid levels in sperm are associated with sperm concentration among men from an infertility clinic. Fertil Steril 2011;95:1794–7.
29. Safarinejad MR. Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic anti-oxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: a double-blind, placebo-controlled, randomised study. Andrologia 2011;43:38–47.
30. Conquer JA, Martin JB, Tummon I, Watson L, Tekpetey F. Effect of DHA supplementation on DHA status and sperm motility in asthenozoospermic males. Lipids 2000;35:149–54.
31. Estienne MJ, Harper AF, Crawford RJ. Dietary supplementation with a source of omega-3 fatty acids increases sperm number and the duration of ejaculation in boars. Theriogenology 2008;70:70–6.
32. Mitre R, Cheminade C, Allaume P, Legrand P, Legrand AB. Oral intake of shark liver oil modifies lipid composition and improves motility and velocity of boar sperm. Theriogenology 2004;62:1557–66.
33. Yeste M, Barrera X, Coll D, Bonet S. The effects on boar sperm quality of dietary supplementation with omega-3 polyunsaturated fatty acids differ among porcine breeds. Theriogenology 2011;76:184–96.
34. Kris-Etherton PM, Innis S, Ammerican DA. Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J Am Diet Assoc 2007;107:1599–611.
35. Stamatiou K, Delakas D, Sofras F. Mediterranean diet, monounsaturated: saturated fat ratio and low prostate cancer risk. A myth or a reality? Minerva Urol Nefrol 2007;59:59–66.
36. Mourvaki E, Cardinali R, Dal BA, Corazzi L, Castellini C. Effects of flaxseed dietary supplementation on sperm quality and on lipid composition of sperm subfractions and prostatic granules in rabbit. Theriogenology 2010;73:629–37.
37. Saez Lancellotti TE, Boarelli PV, Monclus MA, Cabrillana ME, Clementi MA, Espínola LS, Cid Barría JL, Vincenti AE, Santi AG, Fornés MW. Hypercholesterolemia impaired sperm functionality in rabbits. PLoS ONE 2010;5:e13457.
38. Alcock RE, Behnisch PA, Jones KC, Hagenmaier H. Dioxin-like PCBs in the environment-human exposure and the significance of sources. Chemosphere 1998;37:1457–72.
39. Norgil Damgaard I, Main KM, Toppari J, Skakkebaek NE. Impact of exposure to endocrine disrupters in utero and in childhood on adult reproduction. Best Pract Res Clin Endocrinol Metab 2002;16:289–309.
40. Joensen UN, Bossi R, Leffers H, Jensen AA, Skakkebaek NE, Jorgensen N. Do perfluoroalkyl compounds impair human semen quality? Environ Health Perspect 2009;117:923–7.
41. Richthoff J, Rylander L, Jonsson BA, Akesson H, Hagmar L, Nilsson-Ehle P, Stridsberg M, Giwercman A. Serum levels of 2,2',4,4',5,5′-hexachlorobiphenyl (CB-153) in relation to markers of reproductive function in young males from the general Swedish population. Environ Health Perspect 2003;111:409–13.
42. Paasch U, Salzbrunn A, Glander HJ, Plambeck K, Salzbrunn H, Grunewald S, Stucke J, Vierula M, Skakkebaek NE, Jørgensen N. Semen quality in sub-fertile range for a significant proportion of young men from the general German population: a co-ordinated, controlled study of 791 men from Hamburg and Leipzig. Int J Androl 2008;31:93–102.
43. Swan SH, Brazil C, Drobnis EZ, Liu F, Kruse RL, Hatch M, Redmon JB, Wang C, Overstreet JW. Geographic differences in semen quality of fertile U.S. males. Environ Health Perspect 2003;111:414–20.
44. Jørgensen N, Andersen AG, Eustache F, Irvine DS, Suominen J, Petersen JH, Andersen AN, Auger J, Cawood EH, Horte A, et al. Regional differences in semen quality in Europe. Hum Reprod 2001;16:1012–9.
45. Slama R, Kold-Jensen T, Scheike T, Ducot B, Spira A, Keiding N. How would a decline in sperm concentration over time influence the probability of pregnancy? Epidemiology 2004;15:458–65.
46. Bonde JP, Ernst E, Jensen TK, Hjollund NH, Kolstad H, Henriksen TB, Scheike T, Giwercman A, Olsen J, Skakkebaek NE. Relation between semen quality and fertility: a population-based study of 430 first-pregnancy planners. Lancet 1998;352:1172–7.
47. Available from: www.food.dtu.dk. 2012 (cited August 2011).
48. Carlsen E, Petersen JH, Andersson AM, Skakkebaek NE. Effects of ejaculatory frequency and season on variations in semen quality. Fertil Steril 2004;82:358–66.