

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

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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* doi: 10.3945/ajcn.112.042432.

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.

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SUBJECTS AND METHODS

Because of the military drafting system in Denmark, all 18-year-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 \approx 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 non-participants, and they did not differ from participants with regard to age, but the participants were better educated than the non-participants (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 μ L well-mixed semen was placed on a clean glass slide, kept at 37°C, and covered with a 22 \times 22 mm² coverslip. The preparation was placed on the heated stage of a microscope at 37°C and was immediately examined at \times 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 NaHCO₃/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, gonorrhoea, *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 $>38^{\circ}\text{C}$ (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” (gonorrhoea 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

TABLE 1

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

Variables	Subjects (n = 701)	Quartile of percentage energy intake from saturated fat				P ¹
		<11.20 (n = 174)	11.21–13.27 (n = 179)	13.28–15.19 (n = 170)	>15.19 (n = 178)	
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)]						
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
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
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).

into deciles to test the dose-response association. Finally, we compared the percentage of energy from fat for men with semen 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/m²) 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

TABLE 2
Semen quality among 701 Danish men according to quartiles of total fats and major fatty acid intake¹

Quartile of dietary intake of macronutrients	No. of subjects	Semen volume	Sperm concentration	Total sperm count	Motile sperm	Morphologically normal forms
		<i>mL</i>	<i>millions/mL</i>	<i>millions</i>	<i>%</i>	<i>%</i>
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)
<i>P</i> -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)
<i>P</i> -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)
<i>P</i> -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)
<i>P</i> -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)
<i>P</i> -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)
<i>P</i> -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 fat³								
<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
<i>P</i> -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
<i>P</i> -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
<i>P</i> -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
<i>P</i> -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
<i>P</i> -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
<i>P</i> -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.

increasing percentage of energy from saturated fat was observed (Table 3). The highest quartile intake of saturated fat was 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 reference 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 $\sim 60\%$ 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 reference 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

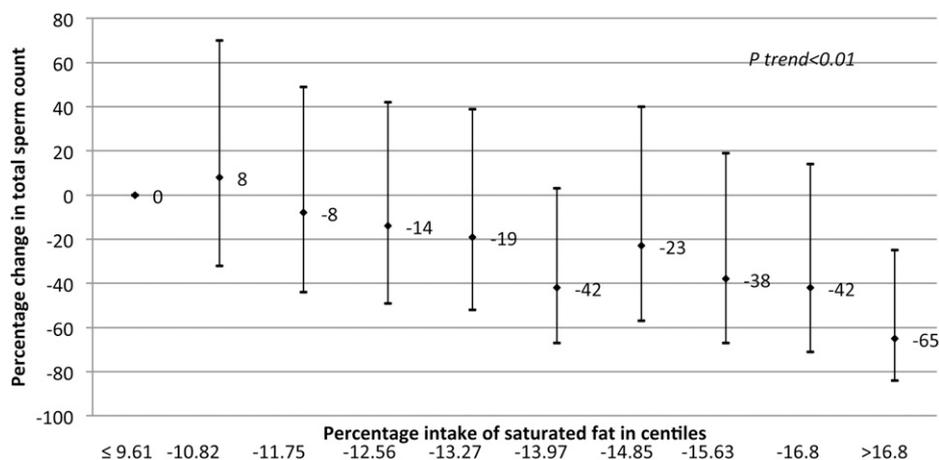


FIGURE 1. Adjusted percentage reduction in total sperm count (and 95% CIs) with increasing intakes of saturated fat (as a percentage of total energy, divided into deciles). Adjusted for period of abstinence (transformed by the natural logarithm), BMI, alcohol consumption, smoking, cryptorchidism, total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

have been due to multiple testing, but they were nevertheless in 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 unhealthy 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 AMA: designed the research; UNJ, MPL, PC, MJB, and THL: conducted the research; TKJ, BLH, TIH, and CD: analyzed the data and wrote the manuscript; and TKJ, BLH, CD, and TIH: interpreted the dietary data. 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.

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