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Dietary fat and semen quality among men attending a fertility clinic

Jill A. Attaman[1,*], Thomas L. Toth[1], Jeremy Furtado[2], Hannia Campos[2], Russ Hauser[1,3], and Jorge. E. Chavarro[2,4]

[1]Vincent Obstetrics and Gynecology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02115, USA [2]Department of Nutrition, Harvard School of Public Health, Boston, MA 02115, USA [3]Department of Environmental Health, Harvard School of Public Health, Boston, MA 02115, USA [4]Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, USA

*Correspondence address. Tel: +1-603-653-9240; Fax: +1-603-650-0906; E-mail: Jill.A.Attaman@hitchcock.org

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BACKGROUND: The objective of this study was to examine the relation between dietary fats and semen quality parameters.

METHODS: Data from 99 men with complete dietary and semen quality data were analyzed. Fatty acid levels in sperm and seminal plasma were measured using gas chromatography in a subgroup of men ($n = 23$). Linear regression was used to determine associations while adjusting for potential confounders.

RESULTS: Men were primarily Caucasian (89%) with a mean (SD) age of 36.4 (5.3) years; 71% were overweight or obese; and 67% were never smokers. Higher total fat intake was negatively related to total sperm count and concentration. Men in the highest third of total fat intake had 43% (95% confidence interval (CI): 62–14%) lower total sperm count and 38% (95% CI: 58–10%) lower sperm concentration than men in the lowest third ($P_{trend} = 0.01$). This association was driven by intake of saturated fats. Levels of saturated fatty acids in sperm were also negatively related to sperm concentration ($r = -0.53$), but saturated fat intake was unrelated to sperm levels ($r = 0.09$). Higher intake of omega-3 polyunsaturated fats was related to a more favorable sperm morphology. Men in the highest third of omega-3 fatty acids had 1.9% (0.4–3.5%) higher normal morphology than men in the lowest third ($P_{trend} = 0.02$).

CONCLUSIONS: In this preliminary cross-sectional study, high intake of saturated fats was negatively related to sperm concentration whereas higher intake of omega-3 fats was positively related to sperm morphology. Further, studies with larger samples are now required to confirm these findings.

Key words: male infertility / semen quality / diet / fat / gas chromatography

Introduction

Infertility affects ~10–15% of couples attempting to conceive (Hull *et al.*, 1985; Mosher and Pratt, 1991) during their reproductive lifespan. A male factor is identifiable in 40–60% of couples and is the sole etiology in ~20% of all couples seeking assistance for infertility (Thonneau *et al.*, 1991; Wang *et al.*, 1997). Few risk factors for male infertility are known. Smoking, heavy use of marijuana, alcohol intake, cocaine use and exposure of testes to heat have all been implicated but with conflicting results (Smith and Asch, 1987; Bracken *et al.*, 1990; Wang *et al.*, 1997; Kunzle *et al.*, 2003; Jung and Schuppe, 2007; Practice Committee of American Society for Reproductive Medicine, 2008; Practice Committee of the American Society for Reproductive Medicine in collaboration with the Society for Reproductive Endocrinology and Infertility, 2008; Anderson *et al.*, 2010). Moreover, the role of other modifiable factors on male fertility remains largely unexplored.

Little is known of how diet may influence male reproductive potential. Emerging literature supports the hypothesis that specific nutritional factors can affect semen quality (Chavarro *et al.*, 2008; Mendiola *et al.*, 2009, 2010; Vujkovic *et al.*, 2009). Furthermore, animal data suggest that dietary fats may affect male fertility (Blesbois *et al.*, 2004; Mitre *et al.*, 2004; Estienne *et al.*, 2008; Stoffel *et al.*, 2008; Bongalhardo *et al.*, 2009; Roqueta-Rivera *et al.*, 2010). Human and animal studies have also correlated a specific fatty acid composition of sperm with semen quality (Zalata *et al.*, 1998; Conquer *et al.*, 1999; Gulaya *et al.*, 2001; Aksoy *et al.*, 2006; Tavilani *et al.*, 2006, 2007; Safarinejad *et al.*, 2010). Yet, the potential role of dietary fat intake on semen quality in humans has received little attention. The objective of this study was to investigate the relation between dietary fats and semen quality parameters among men attending a fertility clinic.

Materials and Methods

Study population

Couples presenting for evaluation at the Massachusetts General Hospital Fertility Center, MA, USA, were invited to participate in an ongoing study of environmental factors and fertility (Hauser *et al.*, 2006). Male partners, aged 18–55 years and without a history of vasectomy, from couples using their own gametes for intrauterine insemination or assisted reproduction technologies were eligible for the present analysis. Of the 173 men recruited between December 2006 and August 2010 (~60% of the men were invited to participate), 109 completed a dietary assessment questionnaire. We excluded from the analysis men with incomplete semen analysis data ($n = 8$), azoospermia ($n = 1$) or missing data on critical covariates ($n = 1$), leaving 99 men for this analysis. The study was approved by the Institutional Review Boards of the Harvard School of Public Health and Massachusetts General Hospital, and informed consent was obtained from all participants. Upon entry, all subjects were administered a brief lifestyle and medical questionnaire by a trained research nurse and were asked to complete a full detailed questionnaire addressing lifestyle and medical history at home. Height and weight were measured on site by a research nurse.

Dietary exposure assessment

Diet was assessed using a previously validated 131-item food frequency questionnaire (FFQ) (Rimm *et al.*, 1992). Men were asked to report how often, on average, during the previous year they had consumed specified amounts of each food, beverage and supplement included in the questionnaire. Options for frequency of food intake included nine categories ranging from never to six or more times per day. Nutrient intakes were estimated by summing the nutrient contribution of all items after taking into consideration the brand and types of fats used in cooking and baking. The nutrient content of each item on the questionnaire and the specified portion size or dose were obtained from a nutrient database derived from the US Department of Agriculture (United States Department of Agriculture and Agricultural Research Service, 2008) and additional information was obtained from the manufacturers. Intakes of total fat and major types of fat were expressed as the percentage of energy consumed.

Semen analysis

A semen sample was produced on site by masturbation and collected into a sterile container. Subjects were asked to refrain from ejaculation for 48 h prior to sample production. At the time of production, the specific length of abstinence was obtained. After collection, the sample was liquefied at 37°C for 20 min before analysis and maintained at 37°C during the assessment. All specimens were analyzed within 45 min of collection. Sperm counts and percentage motility were determined with the use of computer-aided semen analysis (CASA) (Hamilton-Thorne Version 10HTM-IVOS) using 2 Chamber Leja® slides (Leja, The Netherlands). A 5 μl well-mixed, homogeneous sample was applied to each chamber of the Leja slide. Two known concentrations of Accu-bead®+ (Hamilton-Thorne, Inc., Beverly, MA, USA) were used each day for quality control and to confirm accuracy of the CASA counting. The Leja slide was placed on the warm CASA stage and analyzed. A manual count of the same sample was also performed and discrepancies >10% required a repeat count. On manual count, sperm concentration was obtained by averaging the total number of sperm in both chambers on the Leja slide. At least 100 sperm cells were counted for motility assessment. Reference values from the World Health Organization (WHO) were used to assess sperm concentration and motility (World Health Organization, 1999). Sperm morphology was quantified using strict Kruger criteria (Kruger *et al.*, 1988) after seminal smears were made on a glass slide and stained with Diff-Quik (Siemens Healthcare Diagnostics, Inc., Newark, DE, USA). A total of 200 sperm per sample were analyzed per laboratory protocol.

Fatty acid analysis

Fatty acid levels in sperm and seminal plasma were measured at the Department of Nutrition, Harvard School of Public Health, Boston, MA, USA, in a subsample of men ($n = 23$), chosen based on the availability of stored semen. Fatty acid content was analyzed using gas chromatography by modifying a protocol previously developed for the measurement of fatty acids in blood products and adipose tissue. Briefly, semen was separated into 250 μl aliquots and stored at −80°C until analysis. Immediately before analysis, semen was thawed, mixed with 500 μl of phosphate-buffered saline and centrifuged at 600 *g* for 12 min to pellet the sperm. After centrifugation, the supernatant seminal plasma was removed and replaced with 500 μl normal saline to wash seminal fluid from cells. This process was repeated two times, combining the supernatants into a single sample. Fatty acids from sperm or seminal plasma were extracted in a mixture of isopropanol and hexane containing 50 mg of 2,6-di-tert-butyl-*P*-cresol as an antioxidant. Fatty acids were then transmethylated with methanol and sulfuric acid, as previously described (Zock *et al.*, 1996, 1997; Baylin *et al.*, 2005). After esterification, samples were evaporated, redissolved in iso-octane and quantified by gas-liquid chromatography using a fused silica capillary *cis/trans* column (SP2560, Supelco, Belafonte, PA, USA). Peak retention times were identified by comparison with known fatty acid standards (NuCheck Prep, Elysium, MN, USA). The area of each peak was analyzed with ChemStation A.08.03 software (Agilent Technologies) and expressed as a percentage of total fatty acids.

Statistical analysis

There were 99 men with complete semen quality and dietary data. These men were divided into three groups according to tertiles of intake of total fat and major fat categories. We first calculated the mean levels and 95% confidence intervals (CIs) of semen quality parameters (total sperm count, sperm concentration, motility and morphology) for each tertile intake. We then used linear regression to estimate the mean difference in semen quality parameters for categories of increasing fat intake while accounting for differences in total energy intake, age, abstinence time, BMI, smoking and intake of alcohol and caffeine. Additional terms for intakes of protein and the remaining types of fatty acids to simulate the isocaloric substitution of fat for carbohydrates were included (Willett and Stampfer, 1998). In addition, we also fit a second set of regression models where fat intake was modeled as a continuous variable. Total sperm count and sperm concentration were log-transformed to meet the distributional assumptions of linear regression. Tests for linear trends (Rosner, 2000) were conducted using the median values of each tertile intake of total fat and major fatty acid group as a continuous variable, and semen quality parameters as the outcome of interest.

In the subgroup of men for whom sperm and seminal plasma fatty acid composition was available ($n = 23$), we estimated Pearson correlation coefficients of sperm and seminal plasma fatty acid levels with intakes of the respective fatty acid, adjusting for age and total energy intake. Likewise, we estimated Pearson correlation coefficients of sperm and seminal plasma fatty acid levels with semen quality parameters, adjusting for age, abstinence time, BMI, smoking status and intake of alcohol and caffeine. Lastly, we evaluated whether any association identified was modified by the primary infertility diagnosis or previous infertility exam by introducing cross-product terms between fat intake and the potential modifier and evaluating the statistical significance of this interaction term. Analyses

were conducted using Statistical Analysis Software (SAS) version 9.2 (SAS Institute, Inc., Cary, NC, USA). Results were considered significant when $P < 0.05$.

Results

Men were primarily Caucasian (89%) with a mean (SD) age of 36.4 (5.3) years; 71% were overweight (BMI: 25.0–29.9 kg/m^2) or obese (BMI of 30.0 kg/m^2 or greater); and 67% were never smokers. There were no appreciable differences in demographic or clinical characteristics of patients across categories of total fat intake (Table I). Overall, 41 men (41%) had a normal semen analysis, while 12 (12%) had a sperm concentration <20 million/ml, 52 (53%) had <50% motility and 32 (32%) had abnormal sperm morphology.

In crude analysis, total fat intake was negatively related to the total count and to sperm concentration (Table II). When modeling fat intake as a continuous variable, increasing total fat intake by 5% of total calories was associated with an 18% (95% CI: −4% to −30%) lower total sperm count (data not shown). The associations of total fat intake with lower sperm count and concentration appeared to be primarily driven by intake of saturated fat (data not shown). Increasing saturated fat intake by 5% of total calories at the expense of carbohydrates was associated with a 38% (−63 to 5%) lower total count, whereas the same 5% intake increase from monounsaturated fat (11% (−30 to 76%)) or polyunsaturated fat (−12% (−54 to 66%)) at the expense of carbohydrates was unrelated to the total sperm count (data not shown). In addition, in crude analysis higher intake of omega-3 polyunsaturated fatty acids (PUFAs) was associated with higher normal sperm morphology. Men in the highest tertile of omega-3 fatty acid intake had a 2.2 (95% CI: 0.7 to 3.4) higher percentage of morphologically normal sperm than men in the lowest tertile of intake.

Table I Characteristics of men attending a fertility clinic according to pretreatment categories of total fat intake.

	Total fat intake			*P*-value*
	Low	Intermediate	High	
Median intake (range), % of calories	26 (19–29)	32 (30–33)	37 (34–44)	
N	32	34	33	
Age, years	36.4 (5.6)	36.0 (5.1)	36.9 (5.5)	0.80
BMI, kg/m^2	26.8 (3.9)	27.4 (3.8)	26.8 (4.5)	0.78
Caffeine intake, mg/day	161 (135)	204 (113)	159 (123)	0.25
Alcohol intake, g/day	14 (19)	18 (13)	14 (14)	0.50
Total energy intake, kcal/day	2043 (718)	2058 (568)	2154 (585)	0.74
Smoking status, %				
Never smoker	59	74	67	0.70
Past smoker	34	21	30	
Current smoker	6	6	3	
Race/ethnicity, %				
White/Caucasian	78	94	94	0.16
African American	6	0	3	
Asian	6	6	3	
Other	9	0	0	
Primary infertility diagnosis, %				
Ovulatory disorder	10	18	10	0.91
Other female factor	34	23	33	
Male factor	22	21	24	
Unexplained	34	38	33	
Previous infertility exam, %	66	76	79	0.49
Abstinence time, days	5.1 (10.3)	8.1 (30.9)	3.4 (2.4)	0.60
Semen analysis				
Total sperm count <40 million/ml, %	0	12	3	0.12
Concentration <20 million/ml, %	3	18	15	0.14
Motility <50% motile, %	59	53	45	0.55
Morphology <4% normal, %	34	32	30	0.96
Normal semen analysis[a], %	38	41	45	0.82

[a]Above World Health Organization reference values for concentration and motility and Kruger criteria for morphology.
*P-value from the analysis of variance for continuous variables and Fisher's exact test for categorical variables.

Table II Semen quality parameters (mean (95% CI)) of men by intake of total fat and major fatty acid groups.

Intake median, % of calories	*n*	Total sperm count (millions)	Sperm concentration (millions/ml)	Sperm motility (% motile)	Sperm morphology (% normal)
Total fat					
26	32	211 (158–283)	81 (61–107)	47 (39–55)	6.0 (5.0–7.1)
32	34	113 (85–150)*	49 (38–65)*	48 (41–56)	5.9 (5.0–7.0)
37	33	125 (94–166)*	51 (39–67)*	46 (38–54)	5.9 (4.8–6.9)
P_{trend}**		0.01	0.01	0.86	0.84
Saturated					
8	32	209 (156–280)	85 (65–112)	52 (44–59)	6.6 (5.5–7.6)
10	35	116 (88–155)*	48 (37–63)*	46 (38–53)	5.9 (4.9–6.9)
13	32	122 (91–164)*	50 (38–65)*	44 (36–51)	5.4 (4.4–6.5)
P_{trend}**		0.02	0.01	0.15	0.12
Monounsaturated					
10	32	201 (150–270)	84 (64–111)	47 (39–55)	5.5 (4.5–6.6)
12	33	119 (89–159)*	46 (35–60)*	48 (41–56)	6.4 (5.4–7.4)
15	34	124 (93–165)*	52 (40–68)*	46 (39–54)	5.9 (4.9–6.7)
P_{trend}**		0.03	0.02	0.83	0.62
Polyunsaturated					
4.2	33	165 (122–222)	73 (55–95)	45 (38–53)	5.4 (4.4–6.4)
5.6	33	135 (100–183)	54 (41–72)	47 (39–54)	5.8 (4.8–6.9)
7.3	33	132 (98–178)	50 (38–67)	49 (42–57)	6.6 (5.6–7.7)
P_{trend}**		0.31	0.07	0.50	0.09
Omega-6					
3.8	32	164 (121–222)	73 (55–97)	45 (37–53)	5.1 (4.1–6.1)
4.8	34	134 (100–180)	53 (41–71)	46 (39–54)	6.2 (5.2–7.2)
6.0	33	134 (100–182)	52 (39–68)	50 (42–58)	6.5 (5.5–7.6)
P_{trend}**		0.37	0.09	0.32	0.05
Omega-3					
0.4	34	132 (99–178)	62 (47–82)	45 (37–52)	5.0 (4.0–6.0)
0.5	35	163 (122–218)	60 (46–79)	50 (24–57)	5.8 (4.9–6.8)
0.8	30	135 (98–185)	53 (39–71)	47 (39–55)	7.2 (6.1–8.2)*
P_{trend}**		0.93	0.41	0.77	0.003

*Significantly different from men in the lowest tertile of intake at $P < 0.05$.
**P for trend from a linear regression model where the semen quality parameter was the outcome of interest and a variable with the median intake in each intake category was the predictor.

Adjustment for total energy intake, age, abstinence time, BMI, caffeine intake, alcohol consumption and smoking status increased the strength of the associations of saturated fat intake with lower sperm count and sperm concentration. Men in the highest third of saturated fat intake had a 41% (−14 to −60%) lower sperm concentration than men in the lowest third ($P_{trend} = 0.01$). Higher intake of omega-3 PUFA was related to a more favorable sperm morphology ($P_{trend} = 0.01$) (data not shown).

Further adjustment for protein intake and the remaining types of fat attenuated the associations of monounsaturated and saturated fats with sperm count and sperm concentration (Table III). In these models, monounsaturated fat intake was no longer related to the sperm count or concentration. However, men in the highest tertile of saturated fat intake had a 38% lower sperm concentration than men in the lowest tertile, even though the test for the linear trend was no longer significant. Moreover, total fat intake remained inversely associated with the sperm count and concentration in these models. Likewise, intake of omega-3 PUFAs remained positively associated with normal sperm morphology. There was a suggestion that omega-3 PUFA intake was related to a lower percentage of sperm with head defects: men in the upper two tertiles of omega-3 PUFA intake had 2.6% (−6.2 to 0.9%) fewer sperm with head defects than men in the lowest tertile of intake (data not shown). However, this relation failed to reach statistical significance ($P = 0.15$). Omega-3 PUFA intake was unrelated to the percentage of sperm with mid-piece or tail defects (data not shown). The results of models using fat intakes as continuous variables closely mirrored the results based on tertiles of intake (data not shown).

We examined the correlation of dietary fatty acids with sperm and seminal plasma levels of the respective fatty acid in a subgroup of men

Table III Adjusted[a] difference (95% CI) in semen quality parameters of men by intake of total fat and major fatty acid groups.

Intake median, % of calories	*n*	Total sperm count (% difference)	Sperm concentration (% difference)	Sperm motility (% motile)	Sperm morphology (% normal)
Total fat					
26	29	Ref.	Ref.	Ref.	Ref.
32	31	−46 (−64, −12)*	−37 (−57, −8)*	3 (−8, 13)	0.4 (−1.0, 1.8)
36	31	−43 (−62, −14)*	−38 (−58, −10)*	0 (−11, 10)	0 (−1.3, 1.4)
P_{trend}		0.01	0.01	0.99	0.90
Saturated					
8	29	Ref.	Ref.	Ref.	Ref.
10	32	−40 (−66, −4)*	−38 (−60, −4)*	−8 (−20, 4)	−1.1 (−2.7, 0.4)
13	30	−35 (−61, 9)	−38 (−62, 0)*	−11 (−24, 2)	−1.6 (−3.3, 0.1)
P_{trend}		0.20	0.10	0.13	0.08
Monounsaturated					
10	29	Ref.	Ref.	Ref.	Ref.
12	30	−36 (−59, −1)*	−43 (−62, −14)*	1 (−11, 13)	1.1 (−0.4, 2.7)
15	32	−29 (−58, 22)	−29 (−57, 16)	−1 (−15, 13)	0.7 (−1.1, 2.6)
P_{trend}		0.28	0.26	0.83	0.50
Polyunsaturated					
4.2	30	Ref.	Ref.	Ref.	Ref.
5.6	30	−11 (−42, 34)	−20 (−47, 20)	3 (−8, 14)	0.2 (−1.2, 1.7)
7.3	31	−13 (−47, 43)	−35 (−60, 4)	3 (−9, 15)	0.6 (−1.1, 2.2)
P_{trend}		0.58	0.07	0.62	0.50
Omega-6					
3.8	29	Ref.	Ref.	Ref.	Ref.
4.9	31	−7 (−41, 46)	−22 (−49, 18)	2 (−10, 13)	0.6 (−0.9, 2.1)
6.0	31	−15 (−49, 43)	−31 (−57, 13)	7 (−6, 20)	0.7 (−1.0, 2.4)
P_{trend}		0.55	0.15	0.27	0.43
Omega-3					
0.4	31	Ref.	Ref.	Ref.	Ref.
0.5	32	48 (−2, 124)	6 (−29, 57)	4 (−6, 15)	0.8 (−0.5, 2.2)
0.7	29	33 (−18, 113)	−2 (−62, 53)	5 (−7, 18)	1.9 (0.4, 3.5)*
P_{trend}		0.30	0.89	0.42	0.02

*Significantly different from men in the lowest tertile of intake at $P < 0.05$.
[a]Adjusted for protein intake, remaining fatty acids, total energy intake, age, abstinence time, BMI, caffeine intake, alcohol consumption and smoking status.

($n = 23$) (Table IV). Overall, dietary fatty acids were unrelated to the sperm or seminal plasma levels of the same fatty acid. Modest correlations were observed for dietary oleic acid with levels in seminal plasma ($r = 0.54$); dietary eicosapentanoic acid (EPA) with seminal plasma levels ($r = 0.32$); dietary docosahexanoic acid (DHA) with seminal plasma levels ($r = 0.44$) and total dietary omega-6 PUFAs with seminal plasma levels ($r = 0.26$).

We also examined the correlation of sperm and seminal plasma fatty acids with semen quality parameters in this subgroup of 23 men (Table V). Sperm levels, and to a lesser extent seminal plasma levels, of saturated fatty acids were inversely related to sperm concentration and motility. Correlation between total saturated fatty acids and sperm concentration reached statistical significance. Conversely, sperm and seminal plasma levels of omega-3 PUFAs (primarily sperm DHA and seminal plasma EPA) were positively related to sperm concentration and motility, with seminal plasma EPA and sperm concentration reaching statistical significance. Lastly, we examined whether the associations observed between total fat intake and saturated fat intake with sperm count and concentration, as well as the relation between omega-3 fatty acids and sperm morphology, were modified by a history of previous infertility exam or the presence of abnormalities in the semen analysis. There was no evidence that any of the observed associations was significantly different in groups of men defined by a previous infertility exam or having a concurrently abnormal semen analysis.

Discussion

In this study, our results showed moderate associations between dietary fats and semen quality, some of which reached statistical

Table IV Adjusted[a] Pearson correlation coefficients (*r*) between dietary fatty acid intake and fatty acid levels in sperm and seminal plasma in men attending a fertility clinic (*n* = 23).

Fatty acid	Sperm fatty acids	Seminal plasma fatty acids
Total saturated	0.09	0.00
Palmitic	−0.07	−0.04
Stearic	0.18	0.18
Total monounsaturated	−0.04	0.32
Palmitoleic	0.10	−0.24
Oleic	0.04	0.54
Total polyunsaturated	0.05	0.16
Omega-3	0.10	0.26
α-Linolenic	−0.10	−0.25
EPA	−0.12	0.32
DHA	0.10	0.44
Omega-6	0.05	0.26
Linoleic	0.04	0.19
Arachidonic	−0.02	−0.02

EPA, eicosapentanoic acid; DHA, docosahexanoic acid.
For this sample size, all correlation coefficients $\geq|0.43|$ are significant at $P < 0.05$.
[a]Adjusted for age and total energy intake.

significance. Total fat intake was negatively related to total sperm count and sperm concentration. These associations appeared to be driven primarily by intake of saturated fat. Conversely, intake of omega-3 PUFAs was positively correlated to normal sperm morphology. Although dietary fats were not strongly associated with sperm or seminal plasma fatty acid levels, fatty acid composition of sperm and seminal plasma had modest correlations with semen quality.

To our knowledge, no previous study has evaluated the relation between habitual dietary fat intake and semen quality parameters. However, our results can be compared to some extent with previous cross-sectional analyses of diet and semen quality. Our data are in agreement with the results of a Spanish study showing intake of processed meat, an important source of saturated fats, to be associated with poorer semen quality (Mendiola *et al.*, 2009). In contrast, men adhering to a traditional Dutch dietary pattern (high in meat products) had higher sperm concentration when compared with men who consumed a diet with high intake of fruits, vegetables and fish (Vujkovic *et al.*, 2009). Clearly, further research on the relation between dietary fats and semen quality is warranted.

We also found that higher intake of omega-3 PUFAs was significantly related to a more favorable sperm morphology as well as a suggestion of a lower frequency of head defects. Our data are in agreement with two clinical trials examining the effect of omega-3 fatty acids on semen quality. Conquer *et al.* randomized 28 Canadian asthenozoospermic men to placebo, or 400 or 800 mg of DHA for 3 months, and DHA supplementation failed to improve sperm motility or concentration, in agreement with our null findings for these parameters, while results on sperm morphology were not reported (Conquer *et al.*, 2000). Safarinejad randomized 238 Iranian oligoasthenoteratospermic men to receive a supplement containing 1840 mg EPA and DHA or placebo for 32 weeks (Safarinejad, 2011) and found that omega-3 fatty acid supplementation increased the total percentage of sperm with normal morphology, in agreement with our findings. In addition, supplementation also improved total sperm count, concentration and motility, and, in agreement with our data, supplementation significantly increased sperm and seminal plasma EPA and DHA. Differences in the patient population, omega-3 fatty acid dose and duration of use, background omega-3 fatty acid intake and involvement of unspecified amounts of omega-6 fatty acid (corn oil) in placebo may account for differences across studies. Furthermore, our results are also supported by animal data. Dietary supplementation of omega-3 fatty acid in the boar improved sperm morphology in specific breeds, while results regarding the total sperm number and motility have been inconsistent (Mitre *et al.*, 2004; Estienne *et al.*, 2008; Yeste *et al.*, 2011). Despite the consistency of our findings with previous human and animal data, there is currently no known biological mechanism to account for the relation between omega-3 fatty acid intake and improved normal sperm morphology or specific morphological defects.

We examined the correlation between dietary fatty acids and levels of each fatty acid in sperm and seminal plasma in a subgroup of 23 men for whom sperm and seminal fatty acid composition was available. Overall, dietary fatty acids were unrelated with the sperm or seminal plasma levels of the same fatty acid. This is not an unexpected finding, especially for saturated fat intake, as saturated fatty acids can be synthesized endogenously from acetyl CoA. Therefore, the presence of saturated fatty acids at a cellular level may serve as a marker for *de novo* lipogenesis and enzymatic activity. In contrast, PUFAs are essential fats as they are not synthesized endogenously by mammals and must be obtained from foods, such as cold water fish, flax seeds and nuts, or in supplements. Nonetheless, only modest correlations were observed between dietary intake of omega-3 PUFA and fatty acid levels in sperm or seminal plasma. This may be related to imperfect measures of dietary intake or may reflect the increased local metabolism at the level of the testes, as expression of enzymes involved in PUFA metabolism and those involved in *de novo* lipogenesis are elevated in the testes, specifically in Sertoli cells, when compared with other body tissues (Leonard *et al.*, 2000; Tvrdik *et al.*, 2000; Zhang *et al.*, 2001; Saether *et al.*, 2003; Mandal *et al.*, 2004). Peripheral (i.e. Sertoli cell) conversion rates of PUFAs in the testis may also be substantially higher. Thus, fatty acid composition of sperm and seminal plasma may reflect both diet and local metabolism of both unsaturated and saturated fats.

Sperm levels, and to a lesser extent seminal plasma levels, of saturated fatty acids were negatively related to sperm concentration and motility. This is in agreement with previous work showing higher saturated fatty acid concentrations in sperm of asthenozoopermic (Aksoy *et al.*, 2006; Tavilani *et al.*, 2006) and oligospermic males when compared with normozoospermic subjects (Aksoy *et al.*, 2006). Similarly, sperm and seminal plasma levels of omega-3 PUFAs, specifically sperm DHA and seminal plasma EPA, were positively related to sperm concentration and motility. These findings are consistent with those presented in the literature. Levels of omega-3 PUFAs in human sperm, specifically DHA, have been positively correlated to sperm concentration, motility and morphology (Zalata *et al.*, 1998; Conquer *et al.*, 1999; Gulaya *et al.*, 2001; Aksoy *et al.*, 2006; Tavilani *et al.*, 2006, 2007; Safarinejad *et al.*, 2009; Safarinejad, 2011).

Table V Adjusted[a] Pearson correlation coefficients of sperm and seminal plasma fatty acid levels with semen quality parameters in men attending a fertility clinic ($n = 23$).

	Sperm count	Sperm concentration	Sperm motility	Sperm morphology
Sperm fatty acids				
Total saturated	−0.35	−0.56	−0.26	0.03
Palmitic	−0.31	−0.45	−0.33	−0.11
Stearic	−0.00	−0.21	−0.28	−0.17
Total monounsaturated	0.10	0.09	−0.21	−0.09
Palmitoleic	0.16	0.31	0.00	0.05
Oleic	0.10	0.01	−0.20	−0.08
Total polyunsaturated	0.25	0.34	0.30	0.07
Omega-3	0.28	0.36	0.32	0.07
α-Linolenic	0.15	0.23	0.15	0.27
EPA	0.15	0.24	0.15	0.27
DHA	0.29	0.38	0.32	0.07
Omega-6	0.23	0.31	0.26	0.07
Linoleic	0.22	0.27	0.22	0.07
Arachidonic	0.23	0.31	0.17	0.25
Seminal plasma fatty acids				
Total saturated	−0.15	−0.34	−0.27	−0.04
Palmitic	−0.08	−0.23	−0.31	0.09
Stearic	−0.13	−0.33	−0.52	0.05
Total monounsaturated	−0.07	0.10	0.23	−0.09
Palmitoleic	0.01	0.13	−0.04	−0.31
Oleic	0.03	−0.04	0.26	0.12
Total polyunsaturated	0.10	0.04	0.30	0.17
Omega-3	0.12	0.08	0.33	0.17
α-Linolenic	−0.03	0.14	0.14	−0.34
EPA	0.39	0.43	0.38	0.19
DHA	0.09	0.04	0.29	0.17
Omega-6	0.09	0.04	0.28	0.16
Linoleic	0.07	0.02	0.22	0.16
Arachidonic	0.07	0.00	0.29	0.15

[a]Adjusted for age, abstinence time, smoking status and BMI.
For this sample size, all correlation coefficients $\geq|0.43|$ are significant at $P < 0.05$.

It is important to consider the strengths and limitations of our study. To our knowledge, this is the largest study to date examining the influence of specific dietary fats on male fertility. A potential limitation of this study is the use of a FFQ to assess habitual dietary intake. Although the FFQ has been shown to have adequate validity and reproducibility for use in epidemiological studies (Willett and Lenart, 1998), it is nonetheless prone to measurement error usually leading to attenuation of the associations of interest. In addition, analysis of a single semen sample from each participant should be acknowledged, although it has been suggested that analysis of multiple semen samples per subject may not be superior in research studies (Carlsen *et al.*, 2005; Stokes-Riner *et al.*, 2007). Furthermore, use of disposable chambers for analyzing sperm concentration and motility may result in an underestimation of sperm concentration in men with oligospermia when compared with the method using the haemocytometer (Tomlinson *et al.*, 2001). However, consistent use of the Leja slides throughout our study allows relative comparisons to be made. In addition to the small sample size for fatty acid analysis, another important limitation of this study is the possibility that any of the observed associations is the result of reverse causation, specifically that men with abnormal semen quality changed their diet in response to knowing that they have semen quality abnormalities. We believe, however, that reverse causation is an unlikely explanation for our findings. First, the observed associations with dietary fat intake are not in the direction that would be expected in the case of reverse causation. If men had adjusted their diet in response to a diagnosis of abnormal semen quality, one would expect that men with abnormal semen parameters would improve the quality of their diet, resulting in spurious associations between intake of higher quality fats (e.g. *n*-3 fatty acids) and lower semen quality, as well as higher intake of low quality fats (e.g. saturated fat) and higher semen quality. Secondly, we would not expect that men with a low sperm concentration

would adjust their diet differently compared with men with low sperm motility or poor morphology. As a result, we would expect that spurious associations created by reverse causation would be observed for all semen quality parameters simultaneously. In contrast, we observed specific associations for total and saturated fat with sperm concentration (but not motility or morphology), and for *n*-3 fatty acids with morphology (but not concentration or motility). In addition, if knowledge of one's semen quality led to changes in diet resulting in a spurious association, we would expect that the associations would be limited to men with greater knowledge of their fertility status. However, our data suggest that the observed associations do not differ according to whether or not men had previous infertility exams or whether or not they had a concurrent abnormal analysis. Lastly, it is important to acknowledge that cross-sectional studies are weak in terms of their usefulness in establishing causality. It is therefore important that the relation between fat intake and semen quality be evaluated in other studies, ideally in prospective studies.

In summary, we observed significant relationships between dietary fats and specific measures of semen quality. Diets containing higher amounts of PUFAs and lower amounts of saturated fats were associated with more favorable semen quality parameters. Given the limitations of the current study, in particular the fact that it is a cross-sectional analysis and that it is the first report of a relation between dietary fat and semen quality, it is essential that these findings be reproduced in future work. However, adopting these lifestyle modifications may not only be beneficial for reproductive health but also for global general health.

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Authors' roles

This study was designed by J.A.A., T.L.T., R.H. and J.E.C. Data analysis was performed by J.A.A. and J.E.C. J.A.A drafted the manuscript. J.F. and H.C. adapted and developed the method to measure sperm and seminal plasma fatty acids using gas chromatography and performed these assays. All authors provided substantial intellectual contributions and approved the final version of the manuscript.

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Conflict of interest

None declared.

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