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Trans fatty acid levels in sperm are associated with sperm concentration among men from an infertility clinic

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Abstract

We measured the sperm fatty acid composition using gas chromatography in anonymized semen samples of 33 men undergoing infertility evaluation at an academic medical center. *Trans* fatty acids were present in human sperm and were inversely related to sperm concentration ($r = -0.44$).

Keywords

fatty acids; diet; sperm; semen analysis; infertility

Trans fatty acids are unsaturated fatty acids with at least one double bond in the *trans*, instead of the physiologic *cis*, configuration. There are two sources of *trans* fats in the diet. Most are found in foods containing partially hydrogenated vegetable oils used in margarines and commercially prepared foods (1). Smaller amounts are found in meats and dairy products from ruminants (e.g. cattle, goats and sheep), as a result of bacterial action in the animal's rumen (1–2). Little is known about the reproductive health effects of *trans* fats. Their intake has been related to a higher risk of fetal loss (3) and infertility due to anovulation (4). In addition, rodent models have shown that *trans* fatty acid intake impairs spermatogenesis (5–7) but it is unknown whether the same relation exists in humans.

We collected semen samples as part of a pilot study to determine the feasibility of measuring sperm fatty acid composition in large scale studies. Between September 2008 and February 2009 we collected 33 de-identified semen samples from the same number of men presenting for evaluation of infertility at the Massachusetts General Hospital (MGH) Fertility Center. Samples from men presenting for post-vasectomy semen analysis were not included. Semen

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samples were produced on-site by masturbation into a sterile plastic specimen cup. Men were instructed to abstain from ejaculation for 48 hours before producing the semen sample. After collection, the sample was liquefied at 37ºC for 20 minutes before analysis. All semen samples were analyzed using Computer-Assisted Semen Analysis (CASA; Hamilton-Thorn Version 10HTM-IVOS) as previously described (8–9). Following the CASA assessment, study samples were chosen based on their sperm concentration value by over-selecting samples with concentrations below 20×10^6 sperm/mL in order to address the goals of the pilot study. Samples from azoospermic men were not included in the study. For each man we collected up to four 250μL aliquots in cryotubes from a single left-over unprocessed clinical semen sample scheduled to be discarded. Semen samples were then stored at −80°C until analysis. All clinically identifiable data, with the exception of sperm concentration, were de-linked from study samples.

Aliquots were thawed, mixed with 500μL of phosphate-buffered saline, and centrifuged at 600g for 12 minutes to pellet the sperm. After centrifugation, the supernatant seminal plasma was removed and replaced with 500μL normal saline to wash seminal fluid from cells. Fatty acids were extracted from sperm into isopropanol and hexane containing 50 mg of 2.6-di-tert-butyl-*p*-cresol as an antioxidant and transmethylated with methanol and sulfuric acid, as previously described (10–12). After esterification, the samples were evaporated and the fatty acids were redissolved in iso-octane and quantified by gas-liquid chromatography on a fused silica capillary *cis/trans* column (SP2560, Supelco, Belafonte, PA). Peak retention times were identified by injecting known standards (NuCheck Prep, Elysium, MN) and analyzed with the ChemStation A.08.03 software (Agilent Technologies). The fatty acid levels in each sample were expressed as the percentage of total fatty acids. Coefficients of variation ranged between 29.1% for 18:2 *trans* isomers and 43.7% for 18:1 *trans* isomers. The large assay variability may be due to heterogeneity in sperm concentration throughout a single semen sample since the variability of the same method is substantially lower in blood products and adipose tissue (12–13).

We calculated the median *trans* fatty acid level from all of the available aliquots (up to 4) from a single semen sample for each man and assumed the median to be the best estimate of fatty acid level for each man (14). We calculated Spearman correlation coefficients between sperm *trans* fatty acid levels and sperm concentration. We divided men into quartiles according to their levels of individual sperm *trans* fatty acids and calculated the median (25^{th}–75^{th} percentile) sperm concentration in each group. We also calculated the relative difference in sperm concentration across quartiles of sperm *trans* fat levels using linear regression models where the outcome was log-transformed sperm concentration.

The median sperm concentration was 14×10^6/mL, ranging from 0.01×10^6/mL to 400×10^6/mL. SFAs were the predominant fatty acid type in sperm representing 63.8% of all fatty acids, followed by PUFAs (19.6% of total) and MUFAs (12.8% of total). The observed relative fatty acid composition of sperm is in line with previously observed values (15–19). Total *trans* fatty acids accounted, on average, for less than 1% of total sperm fatty acids but were detectable in all men (range 0.14% to 4.43%).

There were large differences in sperm concentration between the lowest and highest quartiles of total sperm *trans* fatty acids and a statistically significant inverse association between total sperm *trans* fatty acids and sperm concentration (Table 1). Similar associations were observed for 16:1n-7*t*, 18:1n-9*t*, 18:2n-6*ct* and total 18:2*t* isomers whereas levels of 18:1n-12*t*, 18:1n-7*t*, 18:2n-6*tt* and 18:2n-6*tc* were unrelated to sperm concentration. The correlations between sperm *trans* fatty acids and sperm concentration were comparable when the median across all available aliquots or a single random aliquot was used to represent each man's sperm fatty acid levels.

We also examined whether the associations of other sperm fatty acid levels with sperm concentration were consistent with those previously reported in the literature. Sperm levels of total PUFAs ($r = 0.68$) and DHA ($r = 0.72$) were positively related to sperm concentration whereas sperm levels of oleic acid ($r = -0.18$), total SFAs ($r = -0.42$) and total MUFAs ($r = -0.11$) were inversely related to sperm concentration in agreement with previous reports (15–16,19).

To our knowledge, this is the first study demonstrating the presence of *trans* fatty acids in human sperm and a relation between sperm *trans* fatty acids and sperm concentration in humans. Because human fatty acid metabolism cannot introduce *trans* double bonds into a fatty acid chain, the presence of *trans* fatty acids in a human cell or tissue implies dietary intake and can serve as a biomarker of diet. Even though we could not evaluate the relation between *trans* fat intake and sperm levels, several studies have previously shown that *trans* fatty acids in blood, red blood cells, plasma and adipose tissue are adequate markers of intake (12,20–21). Therefore, our results suggest that higher intake of *trans* fatty acids is related to a lower sperm concentration. However, it is not possible to know from our data what dietary intake levels are necessary to achieve the sperm *trans* fat levels associated with reduced sperm concentration nor the timing between intake and any eventual effects on spermatogenesis. Nevertheless, this finding is in agreement with previous animal models of supplementation with *trans* fatty acids. Male rodents fed *trans* fats accumulate them in the testis (5,22), have decreased fertility, serum testosterone levels, sperm count, motility and normal morphology and, in extreme cases, spermatogenic arrest and testicular degeneration (5–7). Given the potential clinical and public health implications of our finding it is important that it is replicated in other studies specifically designed to examine this relation.

Our study has some limitations which should be considered when interpreting our results. First, this study was originally designed to develop a laboratory method. As a result we used de-identified samples that could not be linked to data on important covariates such as age, body mass index, diet or lifestyle factors. Therefore, we cannot discount the possibility that sperm *trans* fats are a marker of a negative nutritional or lifestyle factor affecting sperm concentration that we could not account for in the analysis. An additional limitation is the small sample size of this study. Nevertheless, the strength of the observed associations suggests that power was not an important issue and that a larger study would be able to detect smaller associations than we did. Lastly, the variability of fatty acid determinations in sperm, reflected in high CVs, could also have affected our results. However, high CVs usually lead to an attenuation of observed associations suggesting that the association between sperm *trans* fats and sperm concentration may be stronger than what we observed. Also, we obtained the median fatty acid level from all the available aliquots (from the same ejaculate) for each man in an attempt to minimize the measurement error due to assay variability. We also replicated previous findings regarding sperm fatty acid composition and sperm concentration lending support to the validity of our methods and of our findings for *trans* fatty acids.

In summary, we found that *trans* fatty acids were present in human sperm and were inversely related to sperm concentration. Our data is in agreement with experimental data in rodents showing that *trans* fatty acids can profoundly affect spermatogenesis. Nevertheless, given the limitations of this study and the potential clinical and public health implications of our findings, it is important that these hypothesis generating findings are re-evaluated in larger, better designed studies and that the relation between intake of *trans* fats and sperm levels of these fatty acids is closely examined.

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Abbreviations

DPA	docosapentaenoic acid
DHA	docosahexaenoic acid
MUFA	mono-unsaturated fatty acid
PUFA	poly-unsaturated fatty acid
SFA	Saturated fatty acid
CI	confidence interval
SD	standard deviation

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Table 1

Association between sperm *trans* fatty acid levels and sperm concentration (n = 33).

	Quartiles of sperm fatty acid levels						
	1 (n = 8)	**2 (n = 8)**	**3 (n = 9)**	**4 (n = 8)**	**P, trend**	r_1*	r_2**
Total *trans*							
Median fatty acid level, %	0.25	0.43	0.61	1.77			
Sperm concentration (×10^6/mL)	100 [50 – 141]	14 [3 – 18]	12 [3 – 49]	3 [0.5 – 51]			
Relative Difference (95% CI)	Ref.	−89 (34, −99)	−91 (0, −99)	−96 (−53, −99)	0.05	−0.44	−0.50
16:1 trans							
16:1 n−7 t							
Median fatty acid level, %	0.02	0.05	0.07	0.12			
Sperm concentration (×10^6/mL)	59 [14 – 119]	13 [5 – 70]	15 [12 – 86]	1 [0.3 – 5]			
Relative Difference (95% CI)	Ref.	−52 (433, −96)	−72 (193, −97)	−97 (−66, −99)	0.03	−0.44	−0.31
18:1 trans							
Total 18:1 *trans*							
Median fatty acid level, %	0.10	0.18	0.24	0.78			
Sperm concentration (×10^6/mL)	8 [0.4 – 108]	34 [12 – 89]	12 [4 – 15]	9 [0.7 – 79]			
Relative Difference (95% CI)	Ref.	212 (4359, −78)	12 (1386, −92)	−30 (907, −95)	0.52	−0.16	−0.01
18:1 n−12 t							
Median fatty acid level, %	0.02	0.04	0.06	0.24			
Sperm concentration (×10^6/mL)	16 [0.6 – 57]	13 [6 – 50]	6 [3 – 93]	33 [4 – 79]			
Relative Difference (95% CI)	Ref.	−37 (847, −96)	−6 (1306, −93)	9 (1526, −93)	0.83	−0.01	−0.01
18:1 n−9 t							
Median fatty acid level, %	0.10	0.18	0.24	0.78			
Sperm concentration (×10^6/mL)	79 [6 – 135]	16 [2 – 52]	12 [6 – 15]	4 [0.1 – 59]			
Relative Difference (95% CI)	Ref.	−57 (453, −97)	−60 (374, −97)	−93 (−10, −99)	0.04	−0.32	−0.29
18:1 n−7 t							
Median fatty acid level, %	0.02	0.04	0.07	0.27			
Sperm concentration (×10^6/mL)	32 [3 – 98]	18 [2 – 89]	12 [4 – 15]	9 [0.7 – 79]			
Relative Difference (95% CI)	Ref.	−30 (899, −95)	−71 (289, −98)	−73 (285, −98)	0.40	−0.10	−0.14
18:2 trans							

	Quartiles of sperm fatty acid levels						
	1 (n = 8)	**2 (n = 8)**	**3 (n = 9)**	**4 (n = 8)**	**P, trend**	**r_1***	**r_2****
Total 18:2 *trans*							
Median fatty acid level, %	0.10	0.18	0.24	0.78			
Sperm concentration (×10^6/mL)	75 [59 – 100]	16 [3 – 75]	3 [0.2 – 10]	9 [0.5 – 72]			
Relative Difference (95% CI)	Ref.	−87 (24, −99)	−98 (−85, −99)	−95 (−54, −99)	0.04	−0.49	−0.50
18:2 n−6 t,t							
Median fatty acid level, %	0.00	0.04	0.07	0.59			
Sperm concentration (×10^6/mL)	35 [5 – 75]	107 [56 – 141]	3 [0.3 – 12]	5 [0.5 – 15]			
Relative Difference (95% CI)	Ref.	567 (5669, −33)	−91 (−23, −99)	−84 (36, −98)	0.10	−0.48	−0.08
18:2 n−6 c,t							
Median fatty acid level, %	0.00	0.03	0.06	0.18			
Sperm concentration (×10^6/mL)	75 [35 – 101]	17 [5 – 78]	12 [3 – 15]	1 [0.3 – 29]			
Relative Difference (95% CI)	Ref.	−59 (350, −94)	−93 (−28, −99)	−96 (−54, −99)	0.01	−0.45	−0.38
18:2 n−6 t,c							
Median fatty acid level, %	0.00	0.01	0.02	0.06			
Sperm concentration (×10^6/mL)	75 [59 – 100]	9 [0.4 – 15]	14 [10 – 62]	2 [0.1 – 34]			
Relative Difference (95% CI)	Ref.	−92 (−11, −99)	−19 (778, −93)	−91 (8, −99)	0.19	−0.29	−0.30

CI: Confidence Interval

*Spearman correlation coefficient between the sperm levels of the specified fatty acid and sperm concentration. Sperm fatty acid level is the median concentration across all aliquots from the same sample for an individual. All $r \geq |0.35|$ are statically significant at $p<0.05$.

**Spearman correlation coefficient between the sperm levels of the specified fatty acid and sperm concentration. Sperm fatty acid level is a random aliquot from all available aliquots for each individual. All $r \geq |0.35|$ are statically significant at $p<0.05$.