

Dietary patterns and semen quality in young men

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STUDY QUESTION: Are different dietary patterns associated with semen parameters in young men?

STUDY ANSWER: The consumption of a Prudent dietary pattern was significantly associated with higher progressive sperm motility and unrelated to sperm concentration and morphology. The consumption of a Western dietary pattern was unrelated to conventional semen quality parameters.

WHAT IS KNOWN ALREADY: Over the past decades there has been evidence of a concomitant decline in sperm and diet quality. Yet whether diet composition influences semen quality remains largely unexplored.

STUDY DESIGN, SIZE, DURATION: The Rochester Young Men's Study ($n = 188$) was a cross-sectional study conducted between 2009 and 2010 at the University of Rochester.

PARTICIPANTS, SETTING, METHODS: Men aged 18–22 years were included in this analysis. Diet was assessed via food frequency questionnaire and dietary patterns were identified by factor analysis. Linear regression was used to analyze the relation between diet patterns and conventional semen quality parameters (sperm concentration, progressive motility and morphology) adjusting for abstinence time, multi-vitamin use, race, smoking status, BMI, recruitment period, moderate-to-intense exercise and total calorie intake.

RESULTS: Two dietary patterns were identified by factor analysis. The 'Western' pattern was characterized by high intake of red and processed meat, refined grains, pizza, snacks, high-energy drinks and sweets. The 'Prudent' pattern was characterized by high intake of fish, chicken, fruit, vegetables, legumes and whole grains. The Prudent pattern was positively associated with percent progressively motile sperm in multivariate models (P -trend = 0.04). Men in the highest quartile of the Prudent diet had 11.3% (95% CI 1.3, 21.3) higher % progressively motile sperm compared with men in the lowest quartile. The Prudent pattern was unrelated to sperm concentration and morphology. The Western pattern was not associated with any semen parameter.

LIMITATIONS: This was a cross-sectional and observational study, which limited our ability to determine causality of diet on semen quality parameters.

WIDER IMPLICATIONS OF THE FINDINGS: Our findings support the suggestion that a diet rich in fruits, vegetables, chicken, fish and whole grains may be an inexpensive and safe way to improve at least one measure of semen quality.

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Key words: diet / dietary patterns / semen quality / male fertility

Introduction

During the past decades there has been much discussion about changes in semen quality with some studies finding significant declines

(Carlsen *et al.*, 1992; Auger *et al.*, 1995; Irvine *et al.*, 1996; Zou *et al.*, 2011) and others finding no change (Bujan *et al.*, 1996; Fisch *et al.*, 1996; Paulsen *et al.*, 1996). Despite the heterogeneity in study populations, overall, the data support a decline in sperm concentration in

most Western countries (Swan et al., 2000). While genetic, endocrine, congenital, intrauterine (e.g. maternal smoking) and demographic factors such as age, smoking and heavy alcohol use are risk factors for decreased sperm quality, there is increasing evidence that nutrition could play an important role (Wong et al., 2000; Attaman et al., 2012). Over the past 50 years, the average Western diet has changed dramatically. For example, compared with diets in the 1950s, today's US diet is characterized by higher intakes of total calories, meat, cheese, added fats, refined grains and added sugars, which typically reflect a poorer diet quality (USDA, 2003). While many nutritional studies of semen quality have focused on isolated micronutrients such as folate, zinc and various antioxidants, very few studies have focused on food groups or dietary patterns.

Unlike some risk factors for subfertility that cannot be reversed, diet poses an opportunity for intervention, thus making it important to consider in the counseling of subfertile men. Therefore, the objective of the present analysis is to investigate the associations between dietary patterns and semen quality parameters in a population of young, unselected, healthy men. To our knowledge, no previous studies have attempted to explore these associations in this kind of population.

Materials and methods

Study population

The men in this study were participants in the Rochester Young Men's Study (RYMS), a cross-sectional study conducted during 2009–2010 at the University of Rochester (Rochester, NY, USA). RYMS is part of a multi-center (Finland, USA, Spain and Denmark) international study aimed at evaluating the role of environmental contaminants on semen quality. Men were recruited into RYMS through flyers and newspapers at college campuses in the Rochester area. Subjects were eligible if they were born in the USA after 31 December 1987 (18–22 years), able to read and speak English and able to contact their mother and ask her to complete a questionnaire. A total of 389 potential participants contacted the study. Of these, 305 (78.4%) met all eligibility criteria from which 222 (72.8%) men participated in the study. Our analysis only includes men who completed a food frequency questionnaire (FFQ), which was introduced in the spring of 2009 ($n = 194$). Men were further excluded if they were missing information on calorie intake ($n = 3$), reported a calorie intake of <600 or $>15\,000$ kilocalories (kcal) per day ($n = 2$), or were missing information on an entire food group ($n = 1$). The total sample size used in this analysis consisted of 188 men. Subjects received \$75 upon completion of all study components. The University of Rochester Research Subjects Review Board approved the study and written informed consent was obtained from all subjects before their participation.

Dietary assessment

Diet was assessed using a previously validated 131-item FFQ (Rimm et al., 1992). Men were asked to report how often, on average, they consumed specified amounts of each food, beverage and supplement included in the questionnaire during the previous year. Options for frequency of food intake included nine categories ranging from never to six or more times per day. The selected frequency category for each food item was converted to a daily intake. For example, a response of '2–4/week' was converted to 0.43 servings/day. Nutrient intakes were estimated by summing the nutrient contribution of all items. The nutrient content and portion size of each item was obtained from a nutrient database derived from

the US Department of Agriculture and additional information obtained from manufacturers. The individual food items were collapsed into 40 pre-defined food groups based on the similarity of nutrient profiles or culinary usage. These 40 food groups were similar to those used in other studies of Western men (Hu et al., 2000).

Semen collection and analysis

Semen samples were collected by masturbation at the clinic where upon arrival men were asked to report the time of their previous ejaculation. The men were asked to abstain from ejaculation for at least 48 h before sample collection; however, they were not excluded if this was not the case. Abstinence times reported to be >240 h ($n = 3$) were truncated at 240 h. Sample processing was initiated within 30 min of collection. Ejaculate volumes were estimated by specimen weight, assuming a semen density of 1.0 g/ml. Sperm concentration was evaluated by hemocytometer (Improved Neubauer; Hauser Scientific, Inc., Horsham, PA, USA). Two chambers of the hemocytometer were counted, and the average was used in this analysis. Sperm count was calculated by multiplying sperm concentration by volume. Motility was analyzed using World Health Organization, 1999 criteria (Organization, 1999) and classified as both progressive (A + B) and total (A + B + C). Smears for morphology were air dried, fixed and shipped to the University Department of Growth and Reproduction at the Rigshospitalet (Copenhagen, Denmark). The slides were Papanicolaou stained and assessed using strict criteria (Merkvold et al., 1990). To increase consistency and comparability of methods over the course of the study, six sets of duplicate semen samples were sent during the study from the University of Copenhagen's Department of Growth and Reproduction to the Andrology Laboratory (University of Rochester), which is Clinical Laboratory Improvement Amendments certified.

Covariate assessment

A physical examination of each participant was performed on the same day as semen sampling in which weight and height were assessed to determine body mass index (BMI) and the presence of varicocele or other abnormalities was noted. Men also completed questionnaires concerning demographics, psychological stress, medical and reproductive history, physical activity, medication use and smoking habits. A history of genital disease was defined as self-reporting a history of infection of the testes, gonorrhoea, genital warts or herpes, or chlamydia. All covariates were 100% complete.

Statistical analysis

Factor analysis was used to derive food patterns based on 40 pre-defined food groups. Orthogonal transformations were used to achieve uncorrelated factors (dietary patterns) with simpler structures with greater interpretability. In determining the number of factors to retain, we considered eigenvalues (>1) (the amount of variance explained by the factor), the Scree plot (a plot of all the eigenvalues for the derived factors in descending order) and the interpretability of the factors. The substantive meanings of the rotated factors were considered in conjunction with the above empirical criteria and the derived factors were labeled on the basis of our interpretation of the data and prior literature (Hu et al., 2000). For every subject we calculated factor scores on each of the two retained factors by summing the frequency of consumption multiplied by factor loadings across all food items. Thus, each participant was given a score for the 'Prudent' and 'Western' patterns according to their consumption of items from each. The factor loadings that are >0.4 for either dietary pattern are presented in Table 1.

Men were classified into four groups according to quartiles of each dietary pattern for the main analysis. Quintiles and tertiles were also

Table 1 Food group loadings for two dietary patterns identified from food-frequency questionnaire data of 188 men.

Food group ^a	'Prudent' ^b	'Western'
Processed meat	—	0.54
Red meat	—	0.56
Fish	0.57	—
Chicken	0.54	—
Butter	—	0.50
High fat dairy	—	0.44
Fruit	0.78	—
Cruciferous vegetables	0.72	—
Yellow vegetables	0.48	—
Tomatoes	0.62	—
Leafy green vegetables	0.75	—
Legumes	0.64	—
Potatoes	—	0.56
French fries	—	0.62
Other vegetables	0.73	—
Whole grains	0.52	—
Refined grains	—	0.57
Pizza	—	0.56
Snacks	—	0.55
High-energy drinks	—	0.56
Mayonnaise	—	0.42
Sweets	—	0.57
Variance explained	16.6%	9.3%

^aFood groups not included in the table are organ meat, margarine, low-fat dairy, liquor, wine, beer, tea, coffee, fruit juice, cold breakfast cereal, nuts and nut butters, low-energy drinks, olive oil and vinegar, creams, condiments due to loading factors less than or equal to |0.4| for both dietary patterns.

^bPrincipal components analysis was used as an extraction method in which the factor loading of a food group represents the contribution of that food group to the factor identified.

explored to assess the sensitivity of the results. Descriptive statistics were calculated for demographic characteristics and dietary intake. Fisher's exact test, χ^2 test and analysis of variance were used to test for associations across quartiles. Multivariable linear regression was used to evaluate the associations between quartile of consumption of the dietary pattern and sperm parameters. Sperm concentration and sperm count were log-transformed to normalize distributions. Dietary patterns and sperm parameters were also evaluated as continuous linear and quadratic variables. Tests for non-linearity used the likelihood ratio test, comparing the model without any dietary pattern terms to the model with the linear and quadratic term. Tests for trend were conducted across quartiles using a variable with the median dietary factor score in each quartile as a continuous variable in the linear regression models. The analysis of specific foods was done by centering the food at the mean intake and dividing by the standard deviation. Effect estimates from these analyses represent the effect of a 1 standard deviation increase in food intake.

The presence of confounding was evaluated using a hybrid approach combining prior knowledge using directed acyclic graphs (DAGs) and a statistical approach based on change in point estimates (Weng *et al.*, 2009). A set of variables was determined by a review of the prior literature

and a detailed DAGs was created identifying variables that should be included in the models. An exploratory confounding evaluation was also used with covariates being included in the model if they changed the exposure coefficient by >15% and were significant at the $P = 0.10$ level. Factors that were found to have an impact on the point estimates were abstinence time (h), multivitamin use (yes/no), race (white/other), smoking status (current/former or never), BMI (kg/m^2), recruitment period (2009/2010), moderate-to-vigorous exercise (≤ 5 h/week, 5.1–14 h/week, >14.1 h/week) and total energy intake (kcal). Motility analyses were additionally adjusted for time from semen collection to start of semen analysis, as is the common practice in andrology research (Amann and Chapman, 2009).

Effect modification by BMI (<25 kg/m^2 and ≥ 25 kg/m^2), smoking status (current and never/former smokers) and physical activity (<8.25 moderate-to-vigorous activity h/week (median) versus ≥ 8.25 h/week) was tested using cross-product terms in the final multivariate model. A sensitivity analysis excluding men with an anatomical defect of the reproductive organs or genital disease was also performed. SAS version 9.1 (SAS Institute, Cary, NC, USA) was used for all statistical analyses.

Results

Of 305 eligible men, 222 (72.8%) participated in the study and 188 (61.6%) were included in this analysis. Men included in this analysis ($n = 188$) were not significantly different from the entire study sample ($n = 222$) with regard to basic demographic characteristics (data not shown). Overall, this cohort of men was young (mean age: 19.7 years, range: 18–22 years), Caucasian (82.5%), of healthy weight (58.7% BMI <25), highly physically active (mean hours of moderate-to-intense activity per week, 10.6), non-smoking (77.3%) and without history of relevant reproductive disease ($<6\%$). The median sperm concentration was $53.0 \times 10^6/\text{ml}$ (interquartile range (IQR) 20.5 – $95.5 \times 10^6/\text{ml}$), percent progressively motile sperm was 60.5% (IQR: 49.5–69.5%) and percent morphologically normal sperm was 8.5% (IQR: 5.0–12.0%).

Two dietary patterns were identified using factor analysis. The Prudent pattern was characterized by high intakes of fish, chicken, fruit, cruciferous vegetables, tomatoes, leafy green vegetables, legumes and whole grains, while the Western pattern was characterized by high intakes of red and processed meat, butter, high fat dairy, refined grains, pizza, snacks, high energy drinks, mayonnaise and sweets (Table 1). General participant background information is presented stratified by categories of the dietary patterns in Table 2. Men with higher consumption of the Prudent pattern were more physically active ($P < 0.001$) (Table 2). Men with higher consumption of the Western pattern more likely to have lower abstinence times before semen collection ($P = 0.02$). Consumption of both dietary patterns was positively associated with total calorie intake ($P < 0.001$). Caffeine and alcohol intake were positively associated with consumption of the Western pattern ($P = 0.002$ and 0.001 , respectively); however, alcohol and caffeine had no significant associations with the Prudent pattern.

The Western pattern was unrelated to sperm concentration, motility or morphology, ejaculate volume or total sperm count in both the calorie adjusted and fully adjusted models (Table 3). There was a suggestion of a curvilinear relationship between consumption of a Western pattern and sperm concentration (P -value for squared term, 0.01), but the addition of the quadratic terms did not contribute

Table II Demographic and dietary characteristics of participants in the Rochester Young Men's Study according to consumption of dietary patterns.

n	Prudent pattern				P-value ^a	Western pattern				P-value ^a
	Low 47	Low-Mod 47	Mod-High 47	High 47		Low 47	Low-Mod 47	Mod-High 47	High 47	
Age (years) ^b	19.8 (1.0)	19.6 (1.1)	19.9 (1.0)	19.6 (0.9)	0.43	19.9 (1.0)	19.8 (1.0)	19.6 (0.9)	19.6 (1.0)	0.31
Caucasian [n (%)]	38 (80.9)	41 (87.2)	39 (83.0)	37 (78.7)	0.73	36 (76.6)	40 (85.1)	40 (85.1)	39 (83.0)	0.66
BMI (kg/m ²)	25.7 (5.2)	25.2 (4.0)	24.9 (3.2)	25.3 (3.6)	0.83	25.1 (3.5)	24.9 (3.4)	26.0 (5.0)	25.3 (4.1)	0.65
Moderate-to-strenuous exercise (h/week)	7.7 (5.6)	9.5 (7.0)	10.2 (6.3)	15.2 (11.6)	<0.001	10.0 (8.5)	10.9 (9.1)	9.4 (7.1)	12.1 (8.7)	0.49
Current smoker [n (%)]	14 (29.8)	12 (25.5)	11 (23.4)	6 (12.8)	0.24	5 (10.6)	12 (25.5)	16 (34.0)	10 (21.3)	0.06
Abstinence time (h)	80.9 (42.9)	86.6 (50.3)	86.1 (40.7)	94.5 (62.2)	0.62	104.0 (62.2)	84.1 (45.8)	72.1 (26.9)	87.7 (52.7)	0.02
Undescended testes [n (%)]	1 (2.1)	1 (2.1)	1 (2.1)	2 (4.3)	0.89	1 (2.1)	2 (4.3)	1 (2.1)	1 (2.1)	0.89
Varicocele [n (%)]	2 (4.3)	1 (2.1)	0 (0.0)	2 (4.3)	0.76	3 (6.4)	1 (2.1)	0 (0/0)	1 (2.1)	0.40
Hydrocele [n (%)]	0 (0.0)	0 (0.0)	3 (6.5)	0 (0.0)	0.01	0 (0.0)	0 (0.0)	0 (0.0)	3 (6.4)	0.02
Inguinal hernia repair [n (%)]	2 (4.3)	5 (10.6)	2 (4.3)	1 (2.1)	0.41	1 (2.1)	5 (10.6)	2 (4.3)	2 (4.3)	0.41
History of genital disease [n (%)]	0 (0.0)	2 (4.3)	2 (4.3)	1 (2.1)	0.76	2 (4.3)	0 (0.0)	1 (2.1)	2 (4.3)	0.58
Multivitamin user [n (%)]	8 (17.0)	13 (27.7)	14 (29.8)	17 (36.2)	0.22	19 (40.4)	14 (29.8)	11 (23.4)	8 (17.0)	0.07
Calories (kcal/day)	2305.5 (820.6)	2947.6 (1078.3)	3146.7 (844.1)	3850.3 (1476.3)	<0.001	2165.3 (870.8)	2628.4 (598.1)	3053.7 (704.1)	4402.6 (1227.5)	<0.001
Carbohydrates (% energy)	48.9 (7.6)	51.3 (6.7)	48.9 (5.6)	51.9 (6.3)	0.05	53.0 (7.0)	48.9 (7.4)	48.8 (6.0)	50.3 (5.5)	0.006
Protein (% energy)	14.9 (2.9)	15.5 (2.6)	17.3 (2.9)	18.1 (2.9)	<0.001	17.8 (3.8)	16.2 (3.1)	16.0 (2.6)	15.7 (2.4)	0.005
Total fat (% energy)	31.6 (5.0)	29.7 (4.4)	31.2 (4.8)	29.2 (4.6)	0.04	28.4 (4.9)	30.5 (5.0)	31.4 (4.6)	31.3 (4.1)	0.01
Alcohol (g/day)	20.1 (21.3)	21.4 (23.8)	18.6 (20.7)	15.7 (22.5)	0.63	8.5 (10.3)	21.8 (22.9)	21.7 (21.3)	23.6 (27.2)	0.002
Caffeine (mg/day)	90.2 (88.6)	111.6 (101.4)	124.0 (261.3)	88.4 (121.7)	0.65	46.4 (54.4)	72.6 (76.5)	152.9 (258.3)	142.3 (135.3)	0.001
Sugar (g/day)	133.1 (66.7)	188.6 (93.8)	170.6 (64.5)	233.2 (116.5)	<0.001	134.2 (75.0)	152.0 (54.0)	177.4 (65.2)	261.9 (117.1)	<0.001
Folate (μg/day)	415.7 (135.3)	605.1 (218.8)	693.6 (180.2)	1036.4 (323.9)	<0.001	662.1 (408.7)	604.8 (269.0)	629.8 (210.5)	854.0 (295.4)	<0.001
Zinc (mg/day)	12.4 (4.6)	16.6 (6.5)	19.3 (5.6)	24.6 (10.1)	<0.001	15.0 (8.4)	15.1 (5.1)	17.8 (6.2)	25.0 (8.6)	<0.001

^aFor continuous variables, the analyses of variance were used to test for associations between levels of diet score. For categorical variables, χ^2 tests and fisher exact tests (when one or more cell counts were ≤ 5) were used to test the associations between levels of diet score.

^bValues presented are mean (standard deviation) unless otherwise indicated.

Table III The association between consumption of a Western and Prudent dietary pattern and semen quality parameters.

Adjusted means (95% CI)	Western pattern				P-value ^a	Prudent pattern				P-value ^a
	Low	Low-Mod	Mod-High	High		Low	Low-Mod	Mod-High	High	
<i>n</i>	47	47	47	47		47	47	47	47	
Sperm concentration (10 ⁶ /ml) ^b										
Energy adjusted	40.7 (29.7, 55.8)	45.0 (33.8, 60.0)	46.8 (35.5, 34.1)	48.4 (34.1, 68.8)	0.54	39.5 (29.6, 52.7)	42.3 (32.0, 55.9)	52.1 (39.6, 68.5)	47.7 (35.5, 64.1)	0.35
Adjusted ^c	43.9 (29.9, 64.7)	53.3 (36.3, 78.2)	52.5 (36.4, 75.8)	50.7 (33.1, 77.5)	0.55	44.3 (30.3, 64.9)	46.2 (32.3, 66.1)	56.1 (39.2, 80.3)	51.2 (34.9, 75.2)	0.66
% Progressively motile sperm ^d										
Energy adjusted	55.4 (50.7, 60.0)	60.7 (56.5, 65.0)	60.1 (56.0, 64.1)	57.4 (52.2, 62.6)	0.80	56.2 (51.9, 60.6)	58.0 (53.9, 62.2)	59.5 (55.4, 63.6)	59.7 (55.3, 64.2)	0.30
Adjusted	59.5 (53.6, 65.4)	62.6 (56.8, 68.4)	60.8 (55.2, 66.3)	58.4 (51.9, 64.9)	0.48	57.1 (51.4, 62.9)	59.0 (53.7, 64.4)	61.2 (55.8, 66.6)	63.7 (57.8, 69.7)	0.04
% Morphologically normal										
Energy adjusted	7.9 (6.4, 9.3)	8.8 (7.5, 10.2)	8.27 (7.0, 9.6)	9.6 (7.9, 11.2)	0.26	8.6 (7.2, 10.0)	9.3 (8.0, 10.6)	8.7 (7.4, 10.0)	7.9 (6.6, 9.3)	0.35
Adjusted	7.9 (6.1, 9.8)	8.9 (7.0, 10.7)	8.4 (6.6, 10.1)	9.6 (7.5, 11.6)	0.59	8.7 (6.9, 10.6)	9.2 (7.5, 10.9)	8.6 (6.9, 10.3)	7.8 (6.0, 9.7)	0.27

^aP-value for linear trend across quartiles.

^bParameter was natural log-transformed for normality.

^cAdjusted means are presented for the median abstinence time (70.5 h), multivitamin use (no), race (white), smoking status (former or never), BMI (24.6 kg/m²), recruitment period (2009), moderate-to-intense exercise (5.1–14 h/week) and total calorie intake (2939 kcals).

^dAdditionally adjusted for time from semen collection to start of semen analysis (10 h).

Table IV The association between high loading factors in the prudent dietary pattern and sperm motility.

	Mean intake (SD) ^a	β^b	95% CI	P-value ^c
Fish ^d	0.30 (0.35)	1.28	(-0.86, 3.42)	0.24
Canned tuna (3–4 oz)	0.10 (0.17)	1.35	(-0.67, 3.36)	0.19
Cooked shrimp, lobster, scallops or clams (1 serving)	0.06 (0.11)	0.22	(-1.84, 2.27)	0.84
Chicken or Turkey	0.82 (0.77)	0.49	(-1.97, 2.95)	0.70
Chicken/Turkey sandwich or frozen dinner (1 serving)	0.32 (0.43)	0.66	(-1.64, 2.96)	0.57
Chicken/Turkey with skin (3 oz)	0.20 (0.22)	1.96	(-0.30, 4.21)	0.09
Chicken/turkey without skin (3 oz)	0.30 (0.37)	1.08	(-3.01, 1.21)	0.40
Fruit	1.36 (1.23)	1.70	(-0.70, 4.10)	0.17
Bananas (1)	0.32 (0.46)	0.16	(-1.99, 2.30)	0.89
Cantaloupe ($\frac{1}{4}$ melon)	0.07 (0.11)	2.30	(0.19, 4.40)	0.03
Fresh apples or pears (1)	0.32 (0.38)	1.02	(-1.14, 3.18)	0.35
Blueberries ($\frac{1}{2}$ cup)	0.11 (0.25)	0.61	(-1.68, 2.91)	0.60
Cruciferous vegetables	0.32 (0.45)	0.80	(-1.47, 3.06)	0.49
Broccoli ($\frac{1}{2}$ cup)	0.18 (0.23)	0.24	(-1.94, 2.43)	0.83
Tomatoes	0.74 (0.75)	1.30	(-0.86, 3.46)	0.24
Tomatoes (2 slices)	0.38 (0.53)	1.04	(-1.05, 3.13)	0.33
Tomato sauce ($\frac{1}{2}$ cup)	0.26 (0.28)	-0.11	(-2.23, 2.02)	0.92
Leafy green vegetables	0.59 (0.74)	1.42	(-0.75, 3.60)	0.20
Iceberg lettuce (1 cup)	0.32 (0.41)	0.18	(-1.99, 2.34)	0.87
Legumes	0.44 (0.66)	1.40	(-0.78, 3.57)	0.21
String beans ($\frac{1}{2}$ cup)	0.08 (0.13)	0.08	(-2.18, 2.34)	0.94
Beans ($\frac{1}{2}$ cup)	0.17 (0.28)	0.52	(-1.61, 2.65)	0.63
Other vegetables	0.71 (0.70)	0.55	(-1.87, 2.97)	0.66
Celery (2–3 sticks)	0.10 (0.22)	0.19	(-2.11, 2.48)	0.87
Peppers (3 slices)	0.24 (0.33)	-0.64	(-2.81, 1.54)	0.57
Corn ($\frac{1}{2}$ cup)	0.17 (0.18)	1.44	(-0.67, 3.55)	0.18
Mixed vegetables ($\frac{1}{2}$ cup)	0.15 (0.21)	0.59	(-1.70, 2.89)	0.61
Whole grains	1.10 (1.14)	1.61	(-0.53, 3.74)	0.14
Dark bread (1 slice) ^e	0.73 (0.88)	1.94	(-0.09, 3.97)	0.06
Brown rice (1 cup)	0.16 (0.32)	-0.57	(-2.71, 1.57)	0.60

^aAll foods groups are in units of 1 serving/day.

^bFood were centered at the mean intake and divided by the standard deviation for all analyses. Therefore, betas represent the difference in % progressive sperm motility per 1 standard deviation increase in food intake.

^cModels are adjusted for abstinence time (h), multivitamin use (yes/no), race (white/other), smoking status (current/former or never), BMI (kg/m²), recruitment period (2009/2010), exercise (≤ 5 , 5.1–14 and > 14.1 h/week of moderate or intense exercise), total calorie intake (kilocalories) and time from semen collection to start of semen analysis.

^dOnly high loading factors ($>|0.4|$) in the Prudent pattern were investigated and only foods with a median intake of >0 servings/day contributing to those loading factors were investigated.

^eIncludes whole wheat, oatmeal, other whole grain, rye and Pumpnickel bread.

significantly to the model for sperm concentration (P -value for likelihood ratio test = 0.20). The Prudent pattern was unrelated to semen quality in energy-adjusted models. However, after adjustment for abstinence time and physical activity, higher consumption of the Prudent pattern was associated with higher percent progressively motile sperm. This association persisted with further adjustment for additional potential confounders (Table III). Specifically, men in second, third and fourth quartiles had adjusted mean progressively motile sperm percentages that were 2.1% (95% CI -7.1, 11.3), 5.9% (95% CI: -3.5, 15.4) and 11.3% (95% CI: 1.3, 21.3) higher than men in the lowest quartile. We also saw similar associations

with the Prudent pattern and total sperm motility and after adjustment for the Western dietary pattern (data not shown). The Prudent pattern was not associated with sperm concentration, morphology, volume or sperm count.

Higher consumption of any single food group did not account for the association between the Prudent pattern and motility, as no single group was statistically significant. When individual foods were considered, only intake of cantaloupes was significantly related to sperm motility (Table IV).

No significant differences in effect estimates were seen when assessing consumption of either diet pattern and semen parameters in

overweight or non-overweight men, in current or never/former smokers and more physically active men or less physically active men. There was also no difference in the results when men with an anatomical defect of the reproductive organs or genital disease were excluded. Additionally, when dietary patterns were categorized in tertiles or quintiles, results were consistent with those presented above.

Discussion

In a cohort of young healthy men, higher consumption of the Prudent diet pattern characterized by high intakes of fish, chicken, fruit, vegetables, legumes and whole grains was significantly and positively associated with progressive sperm motility. This pattern was unrelated to other semen quality parameters. The Prudent pattern has also been related to lower risk of cardiovascular disease (Hu *et al.*, 2000), diabetes (van Dam *et al.*, 2002) and colorectal cancer in other cohorts of men (Magalhaes *et al.*, 2012). Consumption of the Western diet pattern was not significantly associated with sperm concentration, motility or morphology. This association suggests the possibility of using dietary intervention as a potential approach to increase sperm motility in reproductive aged men.

Studies on the association between dietary patterns and semen quality among young, apparently healthy men are sparse. The findings presented here are not entirely consistent with a similar study performed among subfertile men attending an *in vitro* fertilization clinic in the Netherlands (Vujkovic *et al.*, 2009). In the latter study, the authors found a positive association between adherence to a 'Traditional Dutch' diet and sperm concentration and an inverse association between adherence to a 'Health Conscious' diet and DNA fragmentation index. Comparing their two dietary patterns to ours, the 'Traditional Dutch' diet had higher intakes of whole grains and lower intakes of beverages and sweets, while the Western diet had higher intakes of refined grains, high-energy drinks and sweets. Similarly, the Prudent pattern had higher intakes of meat, alcohol and dairy than the 'Health Conscious' diet. Finally, over two-thirds of the men in the Netherlands study had been diagnosed with subfertility compared with our study of young men unaware of their fertility status. The diagnosis of disease can elicit both changes and differential reporting in diet which could also possibly explain the divergent results (Willett, 1998).

The potential positive effect of a diet similar to our Prudent pattern on sperm motility is supported in the literature and biologically. In a case-control study of 30 men with poor semen quality and 31 normospermic controls, controls had significantly higher intake of lettuce, tomatoes and fruits (Mendiola *et al.*, 2009). In an observational study of 250 men undergoing ICSI, sperm motility was positively influenced by consumption of fruits and cereals (Braga *et al.*, 2012). Furthermore, several observational and experimental studies have shown that higher antioxidant intake (such as vitamin E, vitamin C and β -carotene) which is found in fruits and vegetables was associated with improved sperm motility (and other semen parameters such as sperm counts and morphology), in both fertile and infertile men (Keskes-Ammar *et al.*, 2003; Eskenazi *et al.*, 2005; Akmal *et al.*, 2006; Mendiola *et al.*, 2010). A recent systematic review of 17 randomized trials of antioxidant supplementation showed that most trials reported an improvement in sperm motility with antioxidants compared with placebo (Ross *et al.*, 2010). If this is in fact true, this could in part explain why we saw an increase in sperm motility with

higher consumption of the Prudent pattern. The production of reactive oxygen species (ROS) has been associated with loss of motility and a decreased capacity for sperm-oocyte fusion (Aitken, 1989; Agarwal *et al.*, 2003). Thus, antioxidants which protect against ROS generation may play a critical role in protecting male germ cells against oxidative damage (Fraga *et al.*, 1991). Since multivitamins are an important source of micronutrients (specifically antioxidants), we controlled for this variable in the association between diet and semen quality parameters.

RYMS is, to our knowledge, the first study to look at the association between dietary patterns and semen quality in men from a non-clinical population and as such it had a number of strengths. First, the study population was composed of healthy volunteers from a relatively homogenous setting (young, college men) with no knowledge of their fertility potential. We also had detailed information on a variety of lifestyle risk factors which improved our ability to adjust for confounding. The study's relatively large sample size of 188 men gave us the opportunity to produce reliable dietary patterns which were comparable to previous studies conducted in the US population (Heidemann *et al.*, 2008; Deshmukh-Taskar *et al.*, 2009). Finally, the use of dietary pattern analysis as opposed to nutrient or whole food analysis more closely reflects the real world and allows for easier translation of these results to the public (Hu, 2002).

While this study expands on previous research, it does have several limitations. The most salient limitation is that we used conventional semen quality parameters as a measure of male fertility potential. Jedrzejczak *et al.* showed that the best prognostic value for male fertility status was progressive sperm motility with a value $>24\%$ successfully identifying a fertile man in 91% of cases, and a value below this identifying a man with subfertility in 78% of cases. Similarly, Sripada *et al.* showed that after adjusting for differences in male and female age, parity, year of first visit, and duration of infertility, sperm motility was still significantly associated with spontaneous pregnancy ($P = 0.007$). However, other research has shown a limited value of progressive sperm motility in pregnancy prediction (Bonde *et al.*, 1998). Therefore, although some semen quality parameters, including sperm motility, are known to predict spontaneous fertility (Jedrzejczak *et al.*, 2008; Sripada *et al.*, 2010), it is not possible to know whether the observed association of diet with sperm motility may translate in clinically relevant differences in fertility. As in all observational studies, misclassification of dietary intake is possible. The FFQ we used has been validated against dietary records in other populations; however, it has not been validated in this population (Rimm *et al.*, 1992). While we used the FFQ to determine typical daily intake for the previous year, the sensitive period for semen quality is the 3-month window prior to sample collection. Fortunately, diet is fairly consistent over time and participants generally 'telescope' their report so that it reflects more recent patterns of intake which would benefit this study (Willett, 1998). Additionally, we only had one semen sample from each man. While several semen samples collected over 1–2 weeks may have been preferable, there are limited advantages to using more than one semen sample per man in the setting of an epidemiologic study (Stokes-Riner *et al.*, 2007). While the homogeneity of our study population increased the internal validity of our study, it limits the generalizability of our finding to clinical groups and more diverse populations. It is also possible that there is still unmeasured confounding by factors such as illicit drug and other

medication use. Finally, this was a cross-sectional and observational study, which limited our ability to determine causality of diet on semen quality parameters.

In conclusion, higher consumption of a Prudent dietary pattern was associated with higher sperm motility in young healthy men while consumption of a Western dietary pattern was unrelated to semen quality. Our findings support the suggestion that a diet rich in fruits, vegetables, fish, chicken and whole grains may be an inexpensive and safe way to improve at least one measure of semen quality. Further research is needed to confirm these findings and extend these results to other populations.

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Authors' roles

S.H.S. was involved in study concept and design. J.E.C., J.M. and S.H.S. were involved in acquisition of data. A.J.G., J.E.C. and S.H.S. were involved in the analysis and interpretation of data. A.J.G., D.S.C. and J.E.C. were involved in drafting of the manuscript. A.J.C., J.M., S.H.S. and J.E.C. were involved in critical revision of the manuscript for important intellectual content. A.J.G. was involved in statistical analysis.

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Conflict of interest

None declared.

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