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Intake of Fruits and Vegetables with Low-to-Moderate Pesticide Residues Is Positively Associated with Semen-Quality Parameters among Young Healthy Men[1–3]

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Abstract

Background: Numerous studies have shown that occupational or environmental pesticide exposure can affect male fertility. There is less evidence, however, regarding any potentially adverse effects of pesticide residues in foods on markers of male fertility potential.

Objectives: We examined the relations between fruit and vegetable intake, considering pesticide residue status, and semen quality and serum concentrations of reproductive hormones in healthy young men.

Methods: The Rochester Young Men's Study is a cross-sectional study that recruited men aged 18–22 y (n = 189) in Rochester, New York. Participants completed a questionnaire, provided a semen sample, had a blood sample drawn, and underwent a physical examination at enrollment. Semen samples were analyzed for total sperm count, sperm concentration, morphology, motility, ejaculate volume, total motile count, and total normal count. Dietary intake during the previous year was assessed by a validated food-frequency questionnaire. Fruit and vegetables were categorized as having high [Pesticide Residue Burden Score (PRBS) ≥4] or low-to-moderate (PRBS <4) pesticide residues on the basis of data from the USDA Pesticide Data Program. Linear regression models were used to analyze the associations of fruit and vegetable intake with semen variables and reproductive hormones while adjusting for potential confounding factors.

Results: The total intake of fruit and vegetables was unrelated to semen quality. However, the intake of fruit and vegetables with low-to-moderate pesticide residues was associated with a higher total sperm count and sperm concentration, whereas the intake of fruit and vegetables with high pesticide residues was unrelated to semen quality. On average, men in the highest quartile of low-to-moderate-pesticide fruit and vegetable intake (≥2.8 servings/d) had a 169% (95% CI: 45%, 400%) higher total sperm count and a 173% (95% CI: 57%, 375%) higher sperm concentration than did men in the lowest quartile (<1.1 servings/d; P-trend = 0.003 and 0.0005, respectively). The intake of fruit and vegetables, regardless of pesticide-residue status, was not associated with reproductive hormone concentrations.

Conclusions: The consumption of fruit and vegetables with low-to-moderate pesticide residues was positively related to sperm counts in young men unselected by fertility status. This suggests that pesticide residues may modify the beneficial effects of fruit and vegetable intake on semen quality. *J Nutr* 2016;146:1084–92.

Keywords: pesticides, semen quality, fruit and vegetables, diet, reproductive hormones

Introduction

The potentially deleterious effects of pesticides on the male reproductive system have received considerable attention. Animal studies have shown that pesticides can impair spermatogenesis by inducing oxidative stress (e.g., organophosphates, organochlorine, bipyridyl herbicide) (1), disrupting hormonal pathways (e.g., organophosphates, pyrethroids) (2, 3) or alkylating the chromatin structure of sperm cells (e.g., organophosphates, 2,4-Dichlorophenoxyacetic acid) (4, 5). Human data have also documented deleterious effects of occupational exposure to

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pesticides on semen quality (6, 7). However, whether less intense but more prevalent exposure to pesticides can also affect semen quality has received less attention.

Pesticide exposure occurs through a variety of routes, including inhalation of air or dust, ingestion of residues from foods, or dermal contact to pesticide-sprayed areas (8). In the general population, the primary exposure pathway is through dietary ingestion (9, 10), especially through the consumption of fruit and vegetables. The intake of conventionally grown fruit and vegetables was positively related to urinary metabolite concentrations of pyrethroid pesticides (11), whereas substituting organic produce for conventionally grown produce dramatically decreased the urinary metabolite concentrations of organophosphate pesticides (12). On the other hand, the consumption of fruit and vegetables may have a positive impact on testicular functions. Studies have shown that the Mediterranean or Prudent dietary patterns, which are characterized by high intakes of fruit and vegetables, fish, and whole grains, were associated with higher semen quality (13, 14). A case-control study in an infertility clinic in Iran also found that high intakes of fruit and vegetables were related to a lower risk of asthenozoospermia (15). Little is known, however, whether the intake of pesticide residues modifies the effects of fruit and vegetables on semen-quality variables.

Therefore, we developed a novel approach to estimate dietary pesticide exposure by coupling information from the USDA Pesticide Data Program (PDP)[13] with diet assessed through an FFQ (16). Using this approach, we found that intakes of fruit and vegetables with higher pesticide residues were inversely associated with total sperm count and normal sperm morphology in men presenting to a fertility center (16). To further evaluate the hypothesis that pesticide residues in fruit and vegetables influence male reproductive variables, we examined the association of fruit and vegetable intake, considering their pesticide-residue status, with semen quality and serum reproductive hormone concentrations in healthy young men.

Methods

Study population. The Rochester Young Men's Study is a cross-sectional study in healthy young men (aged 18–22 y), with no knowledge of their fertility potential, who were recruited through flyers and newspapers at college campuses in the Rochester, New York, area between the spring of 2009 and the spring of 2010. Men were eligible if they were born in the United States after 31 December 1987, were able to read and speak English, and were able to have their mothers complete a questionnaire. Of 305 men who contacted the study and met all eligibility criteria, 222 (73%) men enrolled in the study. The remaining 83 men did not participate in the study due to lack of interest after learning the details of the study protocol or failure to arrange a study visit. Upon entry, participants completed a questionnaire concerning lifestyle habits (including use of any pesticides in a hobby or other activities during the 3 mo preceding the semen sample collection), demographic characteristics, as well as medical and reproductive history. A validated diet assessment questionnaire was introduced in the fall of 2009 and was completed by all 194 men who joined the study thereafter. Of these, we excluded 3 men due to missing data on sperm morphology and 2 due to implausible caloric intake (>10,000 or <600 kcal/d), leaving 189 men for the final analysis. The study was approved by the University of Rochester Research Subjects Review Board, and informed consent was obtained from all subjects.

Physical examinations and semen analyses. Men underwent a physical examination, provided a semen sample, and had a blood sample drawn at enrollment. The physical examination included measurement of weight and height, assessment of testes location and volume while participants were in the standing position, and presence of varicocele. Men were instructed to abstain from ejaculation for ≥48 h before the clinic visit and to report the abstinence period at the time of sample collection. Reported abstinence time was used in the analysis, with the exception of that for 7 men whose abstinence times reported (>240 h) were truncated at 240 h. One semen sample was collected from each participant by masturbation and processed within 30 min of collection. Ejaculate volume was estimated by specimen weight, assuming a semen density of 1.0 g/mL. Sperm concentration was evaluated by hemocytometer (Improved Neubauer; Hauser Scientific). For that analysis, samples were diluted in a solution of 0.6 M $NaHCO_3$ and 0.4% (vol:vol) formaldehyde in distilled water, and we used the mean of 2 chambers of hemocytometer in the analysis. Sperm motility was classified as progressive (17). Briefly, 10 μL semen was placed on a glass slide on a heating stage of a microscope at 37°C at 400× magnification. Morphology was assessed by using strict criteria (18). Total sperm count was calculated as sperm concentration × volume. Total normal count (in millions) was defined as concentration × volume × percentage of morphologically normal spermatozoa, and total motile count (in millions) was defined as concentration × volume × percentage of total motility. As a quality-control measure, 6 sets of duplicate semen samples were sent during the study to the University of Copenhagen's Department of Growth and Reproduction from the Andrology Laboratory (University of Rochester).

Reproductive hormone measurement. Blood serum was frozen at –80°C and then shipped to Copenhagen, Denmark, on dry ice and stored at −20°C until hormone analysis was performed at the University Department of Growth and Reproduction at Rigshospitalet. Serum concentrations of follicle-stimulating hormone (FSH), luteinizing hormone, and sex hormone–binding globulin (SHBG) were assessed by using time-resolved immunofluorometric assays (DELFIA; PerkinElmer). The intra-assay CVs were all <5.0% for the FSH, luteinizing hormone, and SHBG assay. Serum testosterone concentrations were determined by a time-resolved fluoroimmunoassay (DELFIA) with intra- and interassay CVs <8%. Estradiol (E2) was measured by using RIA (Pantex) with an intra-assay CV <8% and an interassay CV <13%. Inhibin B concentrations were determined by a specific 2-sided enzyme immunometric assay (Oxford Bio-Innovation Ltd.), with intra- and interassay CVs of 13% and 18%, respectively. Free testosterone concentration was calculated by using the equation of Vermeulen et al., assuming a fixed albumin concentration of 43.8 g/L (19).

Dietary assessment. Diet was assessed by using a previously validated FFQ (20). Men were asked to report how often, on average, they had consumed specified amounts of 131 foods, beverages, and supplements in the questionnaire during the past year. One serving of fruit or vegetables was defined as 1 piece of fruit (e.g., 1 apple or 1 orange) or 0.5 cup of fruit or vegetables (e.g., 0.5 cup kale or 0.5 cup blueberries). The gram-equivalent serving size for each food is shown in **Supplemental Table 1**. In a previous validation study, the de-attenuated correlation (i.e., observed correlation corrected for random within-person variability) between two 1-wk diet records and reported intakes of fruit and vegetable in the FFQ ranged from 0.27 for spinach to 0.95 for bananas

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[3] Supplemental Tables 1–5 and Supplemental Figure 1 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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[13] Abbreviations used: E2, estradiol; FSH, follicle-stimulating hormone; MTHFR, 5, 10-methylenetetrahydrofolate reductase; PDP, Pesticide Data Program; PRBS, Pesticide Residue Burden Score; SHBG, sex hormone–binding globulin.

(21). Two previously described dietary patterns, the "Prudent" and "Western" patterns, were calculated to characterize overall food choices (14). Nutrient intakes were estimated by using a nutrient database derived from the USDA with additional information obtained from manufacturers (22).

Pesticide Residue Burden Score. Fruit and vegetables included in the FFQ were classified according to their pesticide-residue status by using data from the USDA's PDP. The PDP is a national program started in 1991 that annually tests agricultural commodities in the United States for the presence of ~450 different pesticide residues (23). To best represent the pesticide residues in the food supply, the PDP collects samples from 10 participating states comprising 50% of the nation's population. Before testing, the produce is either washed or peeled to mimic consumer practices, allowing for realistic estimates of exposure to these residues. To determine the mean pesticide-residue status of fruit and vegetables in this analysis, we used the USDA PDP annual reports for 2008–2010 corresponding to the periods in which the diet history of the participants was captured by the FFQ (24). When pesticide data for a specific food were not available for 2008–2010, we used the data from the most recent year for which they were available. Of 38 fruit and vegetables included in the FFQ, 34 were included in PDP reports. Four items (avocadoes, apricots, Brussels sprouts, and mixed vegetables, accounting for 5% of total fruit and vegetables) did not have corresponding pesticide data and were thus excluded from the pesticide-residue classification. We then classified fruit and vegetables as having high or low-to-moderate pesticide residues as previously described (16). Briefly, we considered 3 contamination measures from the PDP to classify fruit and vegetables: *1*) the percentage of samples tested with any detectable pesticides, *2*) the percentage of samples tested with pesticides exceeding the tolerances, and *3*) the percentage of samples with ≥3 types of detectable pesticides. We ranked the 34 fruit and vegetables according to each of the 3 PDP contamination measures, divided them into tertiles for each of these 3 measures, and assigned each food a score of 0, 1, or 2 corresponding to the bottom, middle, and top tertiles, respectively. The tertile cutoffs for the percentage of samples with detectable pesticides were 60% and 85%, for the percentage of samples exceeding tolerances were 0% and 0.4%, and for the percentage with ≥3 types of detectable pesticide types were 16% and 46%. The final Pesticide Residue Burden Score (PRBS) for each food was the sum of tertile scores across the 3 PDP contamination measures. Foods with a PRBS ≥4 were defined as high-pesticide-residue foods and those with a PRBS <4 were low-to-moderate-pesticide-residue foods (Supplemental Table 1). For example, an item with a PRBS equal to 6 (e.g., strawberries) indicates that ≥85% of the samples had detectable pesticides, ≥0.4% of the samples had pesticide residues exceeding the tolerances, and ≥46% of the samples had multiple pesticide residues.

Statistical analyses. Men were classified into quartiles of total intake of fruit and vegetables, intake of fruit and vegetables with high pesticide residues, and intake of low-to-moderate-pesticide-residue fruit and vegetables. To evaluate the distribution of baseline characteristics across quartiles of intake, we used the Kruskal-Wallis test for continuous measures and Fisher's exact tests for categorical variables. Spearman correlation coefficients were used to assess the relations between high- and low-to-moderate-pesticide-residue fruit and vegetable intake. Linear regression models were used to evaluate the relation of fruit and vegetable intake (total fruit and vegetable intake, high-pesticide-residue fruit and vegetable intake, and low-to-moderate-pesticide-residue fruit and vegetable intake) with semen-quality variables and reproductive hormone concentrations. Semen-quality variables (sperm concentration, total sperm count, total motile count, total normal count) and reproductive hormones (FSH, testosterone, E2, inhibin B) showing skewed distributions were natural log-transformed to meet normality assumptions of linear regression. Results for these variables were back-transformed to improve interpretability. Population marginal means were used to present marginal population means adjusted for the covariates in the model (25). We fitted regression models for the median (as opposed to the mean) using quantile regression (26) as a sensitivity analysis of the variables with skewed distributions. Tests for linear trend were performed by using median intakes of fruit and vegetables in each quartile as a continuous variable in linear regression and quantile regression models. We also evaluated whether the effect of high-pesticide-residue fruit and vegetable intake on the outcomes differs significantly from that of the low-to-moderate-pesticide-residue fruit and vegetable intake by testing the differences of the slopes (i.e., regression coefficients) in a multivariable linear regression model. To assess for nonlinearity, we used a likelihood ratio test comparing the model with the linear and cubic spline terms selected by a stepwise regression procedure with the model with the linear term only, controlling for covariates.

Potential confounding factors of the relation between fruit and vegetable intake and semen quality were identified by using directed acyclic graphs on the basis of previous literature and biological plausibility (14, 27–32). These factors were selected as covariates if they were associated with high or low-to-moderate-pesticide-residue fruit and vegetable intake at a P value <0.05 or if they changed the exposure coefficients in the multivariable model by >15%. In addition, we also decided a priori that certain factors would be included on the basis of previous literature and regardless of statistical significance. Specifically, abstinence time, smoking, and BMI were included regardless of significance because they are well-known correlates of most semen-quality variables, and thus help reduce the amount of unexplained random variability in the model (29, 33–35). Furthermore, dietary pattern scores were included in the models to allow us to distinguish relations between fruit and vegetable intake from those reflecting patterns of food choices. On the basis of these criteria, models were adjusted for age, BMI (kg/m^2), race (white or nonwhite), smoking status (never or ever), abstinence time (<2, 2–3, 3–4, or >4 d; missing), television viewing hours (h/wk), moderate to vigorous physical activity (h/wk), total energy intake (kcal/d), Prudent as well as Western dietary pattern scores (continuous), and season of sample collection (spring, summer, fall, or winter). The model for high-pesticide-residue fruit and vegetable intake was additionally adjusted for low-to-moderate, pesticide-residue fruit and vegetable intake, and vice versa. Analyses for sperm motility were additionally adjusted for time between ejaculation and start of semen analysis (36). Models for reproductive hormones were adjusted for time of blood sampling to account for circadian variation in hormone concentrations and for the same set of covariates as semen variables, with the exception of abstinence time and time between ejaculation and start of semen analysis.

Last, to evaluate the robustness of the pesticide-residue classification, we conducted sensitivity analyses in which we modified the criteria for pesticide-residue classification using the median as the cutoff for the 3 measures of contamination, excluding pesticides in the top and bottom 2.5% of the limit of detection distribution, and limiting the score to organophosphates and pyrethroid pesticides. We also performed additional analyses by adjusting for variables in the final multivariable model that were not initially selected as final covariates. Additional sensitivity analyses were conducted excluding men with a history of pesticide use within 3 mo and by using a quantile regression model. Statistical analyses were performed with SAS version 9.4 (SAS Institute). Two-sided P values <0.05 were considered significant.

Results

The 189 men included in this analysis were predominantly white, nonsmokers, and physically active (**Table 1**). Men with higher intakes of total fruit and vegetables were more physically active and had higher total caloric, total carbohydrate, and protein intakes. No other baseline characteristics were significantly associated with total fruit and vegetable intake. Intakes of high- and low-to-moderate-pesticide-residue fruit and vegetables were positively related to each other (ρ = 0.70).

Total fruit and vegetable intake was unrelated to semen quality (**Table 2**). Excluding potatoes from total fruit and vegetables did not change the results (data not shown). When fruit and vegetables were classified as high or low-to-moderate

TABLE 1 Baseline characteristics of the 189 men according to quartile of high- and low-to-moderate-pesticide-residue FV intake[1]

	Overall	Intake of high-pesticide FVs Quartile 1	Intake of high-pesticide FVs Quartile 4	Intake of low-to-moderate-pesticide FVs Quartile 1	Intake of low-to-moderate-pesticide FVs Quartile 4
n	189	47	47	47	47
Intake,[2] servings/d	3.8 (0–23.9)	0.6 (0–1.0)	3.8 (2.7–12.7)	0.7 (0–1.1)	4.2 (2.8–13.0)
Demographic characteristics					
Age, y	19.6 (18.9, 20.5)	19.6 (18.9, 20.7)	19.3 (18.6, 20.0)	19.7 (18.9, 20.7)	19.6 (18.7, 20.5)
BMI, kg/m^2	24.6 (22.7, 26.4)	24.2 (21.6, 25.9)	24.4 (22.6, 26.4)	24.0 (22.5, 27.3)	24.6 (23.3, 27.3)
Never-smokers, *n* (%)	146 (77.3)	36 (76.6)	39 (83.0)	32 (68.1)	39 (83.0)
TV viewing, h/wk	14 (4, 20)	14 (4, 14)	14 (4, 20)	14 (4, 14)	14 (4, 20)
Moderate-vigorous exercise, h/wk	8 (5, 14)	7 (4, 10]	10 (7, 16)*	7 (4, 10)	12 (7, 20)*
White, *n* (%)	156 (82.5)	34 (72.3)	35 (74.5)*	38 (80.9)	34 (72.3)*
Abstinence time, d	2.9 (2.2, 4.1)	2.9 (2.2, 4.7)	3.0 (2.2, 3.7)	2.7 (2.1, 4.0)	3.0 (2.5, 4.1)
Season of semen collection, *n* (%)					
Spring	94 (49.7)	25 (53.2)	18 (38.3)	29 (61.7)	17 (36.2)
Summer	16 (8.47)	5 (10.6)	3 (6.38)	4 (8.51)	4 (8.51)
Fall	45 (23.8)	12 (25.5)	15 (31.9)	8 (17.0)	15 (31.9)
Winter	34 (18.0)	5 (10.6)	11 (23.4)	6 (12.8)	11 (23.4)
Diet					
Alcohol, g/d	13 (3.1, 26)	12 (2.0, 23)	9.1 (3.1, 24)	17 (3.5, 27)	10 (3.1, 20)
Caffeine, g/d	58.7 (27.3, 124)	42.8 (18.0, 109)	62.9 (34.6, 129)	65.6 (26.3, 142)	51 (27.3, 113)
Total carbohydrate, % of energy	49.1 (45.6, 54.4)	47.4 (43.8, 53.4)	50.6 (45.9, 54.4)	47.9 (44.8, 53.3)	49.9 (45.6, 55.8)
Total protein, % of energy	16.1 (14.3, 18.0)	15.4 (13.5, 17.8)	16.9 (15.2, 18.2)	15.0 (12.6, 17.5)	17.4 (15.0, 18.5)*
Total fat, % of energy	30.0 (27.7, 33.4)	31.1 (28.8, 34.9)	30.1 (28.1, 33.1)	31.1 (29.0, 34.4)	30.2 (26.9, 33.0)
Total energy intake, kcal/d	2939 (2275, 3678)	2086 (1609, 2446)	3712 (3047, 5077)*	2327 (1834, 2968)	3352 (2939, 4784)*
Prudent pattern score	−0.2 (−0.6, 0.3)	−0.7 (−1.0, −0.5)	0.9 (0.3, 1.8)*	−0.9 (−1.0, −0.6)	1.0 (0.4, 1.8)*
Western pattern score	−0.2 (−0.7, 0.6)	−0.6 (−0.9, −0.2)	0.1 (−0.6, 1.5)*	−0.2 (−0.6, 0.3)	−0.3 (−0.8, 0)
Multivitamin use, *n* (%)	53 (28.0)	33 (70.2)	30 (63.8)	36 (76.6)	31 (66.0)
Reproductive history, *n* (%)					
Testes low in scrotum	174 (92.1)	44 (93.6)	41 (87.2)	43 (91.5)	40 (85.1)
Varicocele	8 (4.23)	2 (4.26)	3 (6.38)	3 (6.38)	3 (6.38)
Hydrocele	4 (2.12)	0 (0.00)	2 (4.26)	0 (0.00)	1 (2.13)
Genital disease[3]	11 (5.82)	3 (6.38)	5 (10.6)	1 (2.13)	1 (2.13)

[1] Values are medians (IQRs) unless indicated otherwise. *$P < 0.05$ across quartiles on the basis of Kruskal-Wallis test (for continuous variables) or extended Fisher's exact tests (for categorical variables). FV, fruit and vegetable; TV, television.
[2] Values are medians; ranges in parentheses.
[3] Self-report of any of the following: infection of epididymis, testicle, or prostate; urinary tract infection; gonorrhea; genital warts or herpes; chlamydia; or other diseases of the penis, testicles, urinary tract, or scrotum.

in pesticide residues on the basis of their PRBS, diverging patterns of association were identified between intake of these foods and semen quality. The relations of low-to-moderate and high-pesticide-residue fruit and vegetable intakes were significantly different from each other for total sperm count (P = 0.006) and sperm concentration (P = 0.005). Specifically, the intake of low-to-moderate-pesticide-residue fruit and vegetables was positively related to total sperm count (P-trend = 0.003) and sperm concentration (P-trend = 0.0005) (Table 2). On average, men in the top quartile of low-to-moderate-pesticide-residue fruit and vegetables intake had a 169% (95% CI: 45%, 400%) higher total sperm count and a 173% (95% CI: 57%, 375%) higher sperm concentration than did men in the lowest quartile of intake. Furthermore, increasing intake of low-to-moderate-pesticide-residue fruit and vegetables was associated with a significantly higher total normal count (P = 0.03) and total motile count (P = 0.006) (**Figure 1**). In contrast, the intake of high-pesticide-residue fruit and vegetables was not related to any of the semen-quality variables evaluated (P > 0.05). Excluding men with a history of recent pesticide usage (n = 2) did not change the findings (data not shown). The results were similar when using quantile regression models (data not shown).

We also assessed the possibility of nonlinearity in the association of low-to-moderate-pesticide-residue fruit and vegetable intake with total sperm count and sperm concentration. Analyses in which the intake of low-to-moderate-pesticide-residue fruit and vegetables was modeled as a cubic spline for total sperm count and sperm concentration had a better fit than when modeled as a linear term (P-nonlinearity = 0.03 and 0.06, respectively). However, nonlinearity was driven by the men in the right tail of the low-to-moderate-pesticide-residue fruit and vegetable intake distribution (**Supplemental Figure 1**). In analyses excluding men in the upper 10% of the low-to-moderate-pesticide-residue fruit and vegetable intake distribution, there was no longer evidence of nonlinearity (P-nonlinearity = 0.91 and 0.92, respectively), but there was still a strong linear relation (P-linearity = 0.001 and < 0.0001, respectively; Supplemental Figure 1).

Results of the sensitivity analyses were consistent with those of the primary analysis. When rankings of each of the 3 contamination measures were dichotomized (rather than divided into tertiles), similar positive trends, albeit weaker, were observed between increased intake of low-pesticide-residue fruit and vegetables and sperm count as well as sperm concentration (**Supplemental Table 2**). Likewise, the associations between the

TABLE 2 Adjusted semen-quality variables according to intakes of high- or low-to-moderate-pesticide-residue vegetables and fruit in 189 men[1]

Quartile range, servings/d	*n*	Total sperm count, millions	Sperm concentration, millions/mL	Total motility,[2] % total motile	Progressive motility,[2] % progressive motile	Sperm morphology, % normal	Ejaculate volume, mL
Total FV intake							
Q1 (0.00–2.14)	47	105 (73.0, 150)	34.2 (24.7, 47.2)	61.1 (56.6, 65.6)	57.6 (52.8, 62.4)	8.1 (6.5, 9.7)	3.5 (3.0, 4.0)
Q2 (2.15–3.81)	47	164 (121, 224)*	48.3 (36.6, 63.7)	64.7 (60.9, 68.6)	60.9 (56.8, 65.0)	9.1 (7.7, 10)	3.7 (3.3, 4.1)
Q3 (3.82–5.62)	48	130 (96.9, 174)	41.8 (32.1, 54.3)	59.5 (55.8, 63.1)	54.8 (50.9, 58.6)	8.3 (7.1, 9.6)	3.6 (3.2, 4.0)
Q4 (5.63–23.9)	47	168 (111, 255)	60.1 (41.4, 87.4)	65.7 (60.5, 70.9)	60.4 (54.8, 65.9)	9.0 (7.2, 11)	3.1 (2.5, 3.7)
P-trend		0.34	0.13	0.60	0.96	0.71	0.33
High-pesticide-residue FV intake (defined as a PRBS of 4, 5, or 6)[3]							
Q1 (0.00–0.99)	47	138 (97.6, 196)	42.6 (31.2, 58.2)	61.6 (57.2, 66.0)	57.9 (53.3, 62.6)	7.9 (6.4, 9.3)	3.6 (3.1, 4.1)
Q2 (1.00–1.74)	47	144 (105, 197)	49.2 (37.1, 65.4)	64.2 (60.2, 68.2)	60.4 (56.1, 64.6)	9.7 (8.4, 11)	3.3 (2.8, 3.7)
Q3 (1.75–2.69)	48	156 (116, 209)	45.9 (35.3, 59.8)	60.7 (57.0, 64.4)	55.8 (51.9, 59.7)	8.4 (7.2, 9.7)	3.7 (3.3, 4.1)
Q4 (2.70–12.7)	47	121 (82.6, 178)	42.9 (30.4, 60.5)	64.4 (59.6, 69.2)	59.5 (54.4, 64.6)	8.5 (6.9, 10)	3.2 (2.7, 3.7)
P-trend		0.62	0.88	0.60	0.86	0.95	0.50
Low-to-moderate pesticide residue FV intake (defined as a PRBS of 0, 1, 2, or 3)[4]							
Q1 (0.00–1.09)	47	87.2 (61.7, 123)	30.1 (22.1, 40.9)	60.2 (55.8, 64.6)	55.8 (51.1, 60.4)	8.6 (7.1, 10)	3.3 (2.8, 3.8)
Q2 (1.10–1.89)	47	134 (99.3, 180)*	40.9 (31.3, 53.4)	63.5 (59.7, 67.3)	59.3 (55.2, 63.3)	7.9 (6.6, 9.2)	3.6 (3.2, 4.0)
Q3 (1.90–2.79)	48	137 (103, 183)*	41.1 (31.8, 53.1)	60.6 (56.9, 64.2)	55.9 (52.1, 59.8)	8.3 (7.1, 9.6)	3.7 (3.3, 4.1)
Q4 (2.80–13.0)	47	234 (158, 349)*	82.0 (57.5, 117)*	66.7 (61.6, 71.8)	62.6 (57.2, 68.0)	9.7 (8.0, 11)	3.2 (2.6, 3.7)
P-trend		0.003	0.0005	0.16	0.16	0.32	0.63
P[5]		0.006	0.005	0.59	0.40	0.49	0.80

[1] Values are means (95% CIs). Semen-quality variables were adjusted for total energy intake, abstinence time, age, BMI, race, television viewing hours, moderate-vigorous physical activity, Prudent and Western dietary patterns, smoking status, and seasonality. *$P < 0.05$ compared with men in the lowest quartile of intake. FV, fruit and vegetable; PRBS, Pesticide Residue Burden Score; Q, quartile.
[2] Motility was additionally adjusted for time from current ejaculation to start of semen analysis.
[3] Additionally adjusted for low-to-moderate-pesticide-residue FV intake.
[4] Additionally adjusted for high-pesticide-residue FV intake.
[5] *P* value of test comparing the difference in the slopes of the linear trend terms of the low-pesticide-residue and the moderate-to-high-pesticide-residue FV intake modeled simultaneously in a single regression model.

intake of low-pesticide produce and semen quality were similar on the basis of the median cutoff (**Supplemental Table 3**). Moreover, additional adjustment for other variables (i.e., intake of high-fat dairy, low-fat dairy, processed meat, fish, alcohol, and sugar-sweetened beverages or use of multivitamins) that were not initially included in the final multivariable models did not have an impact on our conclusions (data not shown). In addition, when the score was restricted to organophosphates and pyrethroid pesticides (**Supplemental Table 4**) and when pesticides with very low or very high limits of detection in the PDP were excluded (**Supplemental Table 5**), the results were consistent with the primary analysis, although the association between the intake of low-to-moderate-pesticide-residue fruit and vegetables was also related to total motility in these analyses.

The relations between fruit and vegetable intake, with and without higher pesticide residues, and reproductive hormone concentrations are summarized in **Tables 3** and **4**. There was a positive association between high-pesticide-residue fruit and vegetable intake and concentrations of E2 and free testosterone with borderline significance (*P*-trend = 0.08 and 0.08, respectively). However, when modeling the median E2 and free testosterone concentrations by using quantile regression models, the association was no longer observed (*P*-trend = 0.43 and 0.26, respectively). We did not find any evidence of other associations between fruit and vegetable intake, regardless of their pesticide-residue status, and concentrations of reproductive hormones.

Discussion

We evaluated the relation between the intake of fruit and vegetables, with regard to their pesticide-residue status, with markers of male reproductive variables in healthy young men. We found that the intake of low-to-moderate-pesticide-residue fruit and vegetables was associated with higher total sperm count and sperm concentration. This association was linear within the range of fruit and vegetable intakes observed in the general US population (37). However, the overall intake of high-pesticide-residue fruit and vegetables was unrelated to semen-quality variables or reproductive hormone concentrations. No clear associations between fruit and vegetable intake, regardless of pesticide-residue status, were observed with reproductive hormone concentrations.

Fruit and vegetables are known to have multiple effects that benefit health in general (38) and could potentially benefit semen quality. For example, antioxidants in fruit and vegetables could scavenge reactive oxygen species, thereby preventing damage to spermatozoa (39). Studies have shown that intakes of lycopene, cryptoxanthin, vitamin C, and β-carotene, antioxidants commonly found in fruit and vegetables, were associated with higher semen quality (40–42). Greater adherence to dietary patterns rich in fruit and vegetables has also been related to better semen quality (14). In addition, high-fiber diets, especially those rich in soluble fibers from fruit and vegetables, were shown to improve insulin resistance, which may, in turn, influence semen quality

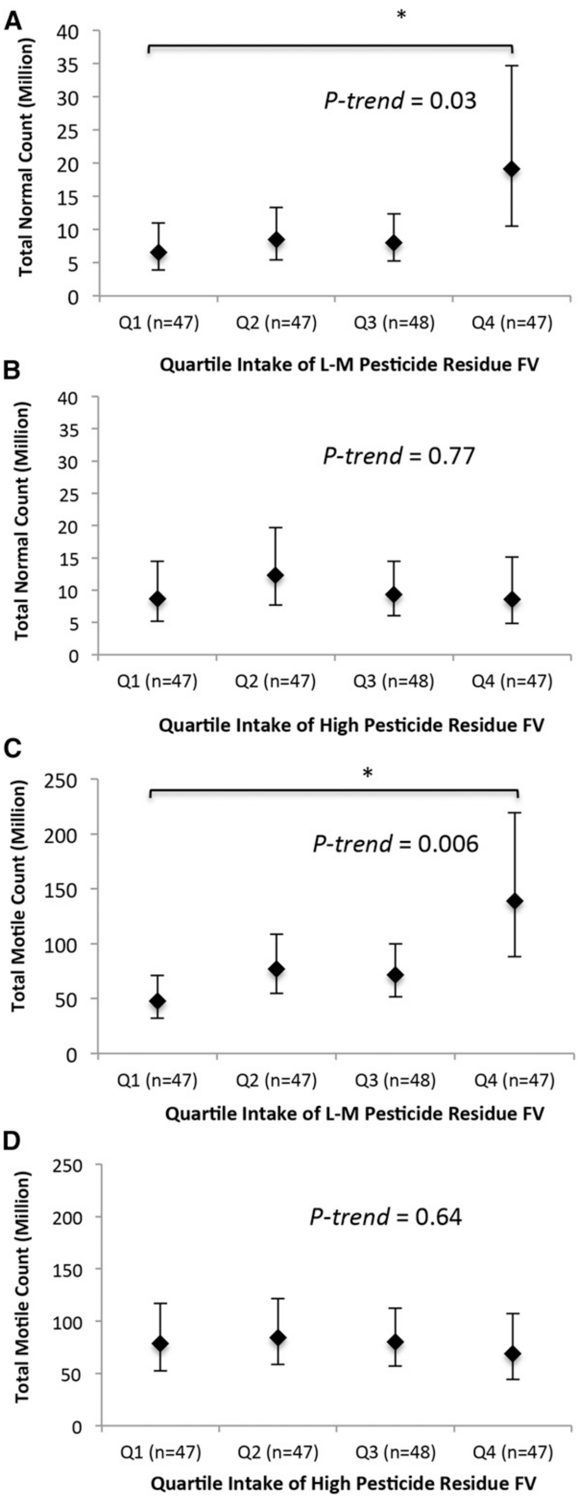

FIGURE 1 Adjusted total normal count and total motile count according to quartile intake of high- or L-M-pesticide-residue FVs in 189 men. Values are means (95% CIs) of total normal count across quartile intakes of L-M-pesticide-residue FVs (A) and intakes of high-pesticide-residue FVs (B) and total motile count across quartile intakes of L-M-pesticide-residue FVs (C) and intakes of high-pesticide-residue FVs (D). Results were adjusted for total energy intake, abstinence time, age, BMI, television viewing hours, physical activity, race, Prudent and Western dietary patterns, smoking status, and seasonality. Panels A and C were additionally adjusted for high-pesticide-residue FV intake. Panels B and D were additionally adjusted for L-M-pesticide-residue FV intake. Tests for trend were conducted across quartiles by using the median value of a variable in each quartile. *$P < 0.05$ compared with men in the lowest quartile of intake. FV, fruit and vegetable; L-M, low-to-moderate; Q, quartile.

(43, 44). Moreover, the literature suggests that folate, another important nutrient in fruit and vegetables, is essential for spermatogenesis. For example, observational studies have found that low folate concentrations in seminal plasma were associated with increased sperm DNA damage (45) and lower sperm density (46). A meta-analysis also showed that the 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C→T polymorphism, which reduced folate metabolism by 70% in the homozygote (TT) state, was associated with a pooled OR of 1.39 (95% CI: 1.15, 1.69) for male factor infertility (47). These findings were supported by 1 trial, which found that 15 mg folic acid supplementation/d for 90 d led to a 53% increase in sperm concentration and a doubling in the proportion of motile sperm (48). On the other hand, the widespread use of pesticides in modern conventional agriculture has raised public concern. According to the USDA, 60% of all produce tested in the United States contains residues of ≥1 pesticide and more than one-third of produce has multiple pesticides in a single produce sample (24). Although pesticide exposure through diet intake is prevalent, the evaluation of its impact on reproductive health has been limited.

To our knowledge, the only previous study to evaluate dietary pesticide exposure and male reproductive health is a similar study we conducted in men attending a fertility clinic. In that study we found an inverse association between the intake of high-pesticide-residue fruit and vegetables and total sperm count and normal sperm morphology, as well as a positive association between the intake of low-to-moderate-pesticide-residue produce and morphologically normal sperm (16).

The results of both these studies agree on a general hypothesis that pesticide residues in fruit and vegetables may modify how antioxidants and other constituents of these foods relate to semen quality. Specifically, these data are consistent with a scenario in which beneficial effects of antioxidants and other nutrients concentrated in fruit and vegetables (40, 41, 49) are offset by xenobiotic chemicals such as pesticides (50). Additional support for this hypothesis comes from a study in China that reported an interaction between 1,1,1-trichloro-2,2,bis(*p*-chlorophenyl)ethane (DDT) and vitamin B concentrations on clinical pregnancy (51) and from a rodent model in which lycopene was found to protect against deltamethrin-induced testicular damage (52). Although we did not observe the deleterious effects of high-pesticide-residue produce on semen quality in the present study, the divergence between these 2 studies could be explained by the differences in study design and participant characteristics, including differences in age (median: 19 compared with 35 y), physical activity (median: 8 compared with 3 h/wk), season of sample collection (50% compared with 23% in spring), and untested fertility in the present report relative to men from an infertility clinic. Clearly, additional research is needed to clarify whether pesticide residues in food can harm the human reproductive system and the interplay between dietary factors and environmental chemical exposures on this relation.

This study has some limitations. First, pesticide exposure was estimated by using surveillance data rather than individual-level biomarkers of pesticide exposure. Second, due to the lack of data on organic food consumption, we assumed all food items on the FFQ were conventionally grown. Third, our method assumes

TABLE 3 Adjusted serum reproductive hormone concentrations according to quartile of high- or low-to-moderate-pesticide-residue FV intake in 189 men[1]

Quartile range, servings/d	*n*	LH, IU/L	FSH, IU/L	E2, pmol/L	Testosterone, nmol/L	Free testosterone, nmol/L	Inhibin B, pg/mL
High-pesticide FV intake (PRBS of 4, 5, or 6)							
Q1 (0.00–0.99)	47	3.8 (3.3, 4.2)	2.4 (2.1, 2.9)	79.3 (72.5, 86.8)	18.1 (16.1, 20.3)	0.44 (0.39, 0.49)	200.9 (180.8, 223.3)
Q2 (1.00–1.74)	47	3.8 (3.4, 4.3)	2.4 (2.0, 2.7)	90.1 (83.0, 97.9)*	19.1 (17.2, 21.2)	0.46 (0.41, 0.50)	181.5 (164.9, 199.8)
Q3 (1.75–2.69)	48	3.7 (3.3, 4.1)	2.5 (2.2, 2.9)	88.6 (82.1, 95.6)	19.1 (17.3, 21.0)	0.45 (0.41, 0.49)	184.2 (168.4, 201.4)
Q4 (2.70–12.7)	47	3.5 (3.0, 4.1)	2.8 (2.4, 3.4)	93.8 (84.9, 104)*	20.8 (18.3, 23.7)	0.52 (0.46, 0.57)	174.9 (155.7, 196.6)
P-trend		0.52	0.21	0.08	0.46	0.08	0.21
Low-to-moderate-pesticide FV intake (PRBS of 0, 1, 2, or 3)							
Q1 (0.00–1.09)	47	3.8 (3.3, 4.3)	2.5 (2.1, 2.9)	88.2 (80.6, 96.5)	20.1 (17.9, 22.6)	0.49 (0.44, 0.54)	187.1 (168.2, 208.0)
Q2 (1.10–1.89)	47	3.5 (3.1, 3.9)	2.4 (2.0, 2.7)	81.1 (74.9, 87.7)	18.2 (16.5, 20.2)	0.44 (0.40, 0.49)	191.5 (174.6, 210.1)
Q3 (1.90–2.79)	48	4.0 (3.7, 4.4)	2.8 (2.5, 3.2)	95.6 (88.7, 103)	20.6 (18.7, 22.7)	0.48 (0.43, 0.52)	172.1 (157.5, 188.2)
Q4 (2.80–13.0)	47	3.5 (2.9, 4.0)	2.5 (2.1, 3.0)	86.7 (78.2, 96.2)	18.0 (15.8, 20.6)	0.46 (0.40, 0.52)	190.8 (168.9, 215.5)
P-trend		0.58	0.72	0.82	0.46	0.72	0.96

[1] Values are means (95% CIs) unless otherwise indicated. Serum hormone concentrations were adjusted for total energy intake, abstinence time, age, BMI, race, television viewing hours, moderate-vigorous physical activity, Prudent and Western dietary patterns, smoking status, hour of blood sampling, and seasonality. $^*P < 0.05$ compared with men in the lowest quartile of intake. E2, estradiol; FSH, follicle-stimulating hormone; FV, fruit and vegetable; LH, luteinizing hormone; PRBS, Pesticide Residue Burden Score; Q, quartile.

that the potential effect of fruit and vegetables on semen-quality variables is equal across all types of fruit and vegetables, which may not be the case in reality. For instance, if the fruit and vegetables that are rich in antioxidants are those that require the most pest management (e.g., blueberries, strawberries, and sweet peppers), our findings are likely an underestimate of the true relation between the dietary pesticide exposure and semen quality. Fourth, only a single semen sample was obtained from each subject. However, these limitations are likely to result in nondifferential misclassification, which would lead to an underestimation of the true association. Moreover, previous work suggests that there is limited gain in information from the use of multiple semen samples per man in research settings (53, 54). Another limitation is that the findings may not be generalizable to the US population because the intake of fruit and vegetables (median: 3.8 servings/d) in our cohort was nearly double the median intake in the US population (median: 2.0 servings/d) (55). In addition, as is the case with all observational studies, we cannot rule out the presence of unmeasured confounding despite our adjustment for a large number of potential confounding factors. Last, semen variables are not perfect proxies of fertility, and thus our findings might not directly translate into male fertility (56, 57). Nonetheless, current work has shown that semen quality is not only a sensitive biomarker for environmental exposure (58) but also predicts both mortality and longevity (59, 60), providing information on the effect of environmental exposure on overall male health.

A major strength of the current study is its population of men who were unselected by fertility status, which makes it unlikely that the results are explained by changes in diet made in response to fertility issues. Second, we used a hemocytometer for determination of sperm concentration and weighed the sample for the assessment of ejaculate volumes, which provides more accurate measures and was in accordance with WHO criteria

TABLE 4 Adjusted serum SHBG and reproductive hormone ratios according to quartile of high- or low-to-moderate-pesticide-residue FV intake in 189 men[1]

Quartile range, servings/d	*n*	SHBG, nmol/L	Testosterone:LH	FT:LH	E2:testosterone	Inhibin B:FSH
High-pesticide FV intake (PRBS of 4, 5, or 6)						
Q1 (0.00–0.99)	47	28.6 (25.1, 32.5)	5.4 (4.7, 6.3)	124.7 (107.8, 144.2)	4.4 (4.0, 4.8)	82.1 (64.8, 104)
Q2 (1.00–1.74)	47	28.1 (25.0, 31.6)	5.4 (4.7, 6.1)	124.6 (109.2, 142.3)	4.7 (4.3, 5.1)	77.1 (62.1, 95.7)
Q3 (1.75–2.69)	48	29.9 (26.9, 33.4)	5.6 (5.0, 6.4)	128.3 (113.4, 145.1)	4.7 (4.3, 5.0)	72.3 (59.1, 88.4)
Q4 (2.70–12.7)	47	26.2 (22.7, 30.2)	6.2 (5.2, 7.3)	152.5 (129.8, 179.1)	4.5 (4.1, 5.0)	61.7 (47.5, 80.2)
P-trend		0.43	0.25	0.09	0.93	0.16
Low-to-moderate-pesticide FV intake (PRBS of 0, 1, 2, or 3)						
Q1 (0.00–1.09)	47	28.4 (25.0, 32.3)	5.9 (5.0, 6.8)	137.6 (118.8, 159.3)	4.4 (4.0, 4.8)	75.6 (59.6, 95.9)
Q2 (1.10–1.89)	47	26.9 (24.1, 30.1)	5.6 (5.0, 6.4)	133.3 (117.3, 151.6)	4.4 (4.1, 4.8)	81.3 (66.1, 100)
Q3 (1.90–2.79)	48	31.5 (28.3, 35.1)	5.5 (4.9, 6.2)	123.9 (109.5, 140.2)	4.6 (4.3, 5.0)	61.1 (50.0, 74.6)
Q4 (2.80–13.0)	47	26.1 (22.6, 30.3)	5.5 (4.7, 6.6)	133.8 (113.1, 158.3)	4.8 (4.3, 5.3)	75.4 (57.4, 99.1)
P-trend		0.62	0.70	0.83	0.25	0.83

[1] Values are means (95% CIs) unless otherwise indicated. Serum hormone concentrations were adjusted for total energy intake, abstinence time, age, BMI, race, television viewing hours, moderate-vigorous physical activity, Prudent and Western dietary patterns, smoking status, hour of blood sampling, and seasonality. E2:testosterone, ratio of estradiol (pmol/L) to testosterone (nmol/L); FT:LH, ratio of calculated free testosterone (pmol/L) to luteinizing hormone (IU/L); FV, fruit and vegetable; inhibin B:FSH, ratio of inhibin B (pg/mL) to follicle-stimulating hormone (IU); PRBS, Pesticide Residue Burden Score; Q, quartile; SHBG, sex hormone–binding globulin; testosterone:LH, ratio of testosterone (nmol/L) to luteinizing hormone (IU/L).

(61). Third, we used a previously validated dietary questionnaire, which not only gives confidence to the validity of self-reported fruit and vegetable intake but also allowed us to assess and adjust for overall food choices, thereby reducing the likelihood of residual confounding by other dietary behaviors. Moreover, a study with a similar methodology (coupling USDA PDP surveillance data and FFQ data) showed that long-term estimated dietary exposure to organophosphate pesticide was consistent with urinary concentrations of diakylphosphate (62). Last, we had detailed information on a variety of lifestyle risk factors, reproductive history, and results of a physical examination, which allowed for the adjustment of potential confounding factors.

In conclusion, the findings suggest that a higher intake of low-to-moderate-pesticide-residue fruit and vegetables was positively related to semen quality in young men with untested fertility. Furthermore, the beneficial effects of fruit and vegetable intake on semen quality appeared to be offset by the presence of high amounts of pesticide residues. This interpretation of our data is consistent with our previous study of this question in men presenting to a fertility clinic (16). Given the paucity of literature on this topic coupled with the widespread use of pesticides in agriculture, future studies are urgently needed to confirm these findings and, ideally, interventional trials should be used to evaluate how environmental chemicals may be altering the effects of dietary factors on human health and reproduction.

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SHS was involved in the study concept and design; JM, NJ, SHS, and JEC contributed to the acquisition of data; Y-HC, AJG, PLW, JM, NJ, HL, RH, SHS, and JEC contributed to the analysis and interpretation of the data; Y-HC and JEC drafted the manuscript; Y-HC analyzed the data; AJG, PLW, JM, NJ, HL, RH, SHS, and JEC were involved in critical revision of the manuscript for important intellectual content; and Y-HC and JEC had primary responsibility for final content. All authors read and approved the final manuscript.

References

1. Abdollahi M, Ranjbar A, Shadnia S, Nikfar S, Rezaie A. Pesticides and oxidative stress: a review. Med Sci Monit 2004;10:RA141–7.
2. Chen H, Xiao J, Hu G, Zhou J, Xiao H, Wang X. Estrogenicity of organophosphorus and pyrethroid pesticides. J Toxicol Environ Health A 2002;65:1419–35.
3. He J, Chen J, Liu R, Wang S, Song L, Chang HC, Wang X. Alterations of FSH-stimulated progesterone production and calcium homeostasis in primarily cultured human luteinizing-granulosa cells induced by fenvalerate. Toxicology 2004;203:61–8.
4. Rahman MF, Mahboob M, Danadevi K, Saleha Banu B, Grover P. Assessment of genotoxic effects of chloropyriphos and acephate by the comet assay in mice leucocytes. Mutat Res 2002;516:139–47.
5. Amer SM, Aly FA. Genotoxic effect of 2,4-dichlorophenoxy acetic acid and its metabolite 2,4-dichlorophenol in mouse. Mutat Res 2001;494:1–12.
6. Perry MJ. Effects of environmental and occupational pesticide exposure on human sperm: a systematic review. Hum Reprod Update 2008;14:233–42.
7. Martenies SE, Perry MJ. Environmental and occupational pesticide exposure and human sperm parameters: a systematic review. Toxicology 2013;307:66–73.
8. Barr DB, Barr JR, Driskell WJ, Hill RH Jr., Ashley DL, Needham LL, Head SL, Sampson EJ. Strategies for biological monitoring of exposure for contemporary-use pesticides. Toxicol Ind Health 1999;15:168–79.
9. Xue J, Zartarian V, Tornero-Velez R, Tulve NS. EPA's SHEDS-multimedia model: children's cumulative pyrethroid exposure estimates and evaluation against NHANES biomarker data. Environ Int 2014;73:304–11.
10. Yu Y, Li C, Zhang X, Zhang X, Pang Y, Zhang S, Fu J. Route-specific daily uptake of organochlorine pesticides in food, dust, and air by Shanghai residents, China. Environ Int 2012;50:31–7.
11. Fortes C, Mastroeni S, Pilla MA, Antonelli G, Lunghini L, Aprea C. The relation between dietary habits and urinary levels of 3-phenoxybenzoic acid, a pyrethroid metabolite. Food Chem Toxicol 2013;52:91–6.
12. Lu C, Toepel K, Irish R, Fenske RA, Barr DB, Bravo R. Organic diets significantly lower children's dietary exposure to organophosphorus pesticides. Environ Health Perspect 2006;114:260–3.
13. Cutillas-Tolín A, Minguez-Alarcon L, Mendiola J, Lopez-Espin JJ, Jorgensen N, Navarrete-Munoz EM, Torres-Cantero AM, Chavarro JE. Mediterranean and Western dietary patterns are related to markers of testicular function among healthy men. Hum Reprod 2015;30:2945–55.
14. Gaskins AJ, Colaci DS, Mendiola J, Swan SH, Chavarro JE. Dietary patterns and semen quality in young men. Hum Reprod 2012;27:2899–907.
15. Eslamian G, Amirjannati N, Rashidkhani B, Sadeghi MR, Hekmatdoost A. Intake of food groups and idiopathic asthenozoospermia: a case-control study. Hum Reprod 2012;27:3328–36.
16. Chiu YH, Afeiche MC, Gaskins AJ, Williams PL, Petrozza JC, Tanrikut C, Hauser R, Chavarro JE. Fruit and vegetable intake and their pesticide residues in relation to semen quality among men from a fertility clinic. Hum Reprod 2015;30:1342–51.
17. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva (Switzerland): WHO Press; 2010.
18. Menkveld R, Stander FS, Kotze TJ, Kruger TF, van Zyl JA. The evaluation of morphological characteristics of human spermatozoa according to stricter criteria. Hum Reprod 1990;5:586–92.
19. Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol Metab 1999;84:3666–72.
20. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of a expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114–26.
21. Feskanich D, Rimm EB, Giovannucci EL, Colditz GA, Stampfer MJ, Litin LB, Willett WC. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. J Am Diet Assoc 1993;93:790–6.
22. Gebhardt S, Lemar L, Haytowitz D, Pehrsson P, Nickle M, Showell B, Thomas R, Exler J, Holden J. USDA national nutrient database for standard reference, release 21. USDA, Agricultural Research Service; 2008.
23. USDA. US Department of Agriculture pesticide data program (PDP): annual summary. Washington (DC): USDA, Agricultural Marketing Service; 2013. [cited 2015 Oct 10]. Available from: http://www.ams.usda.gov/AMSv1.0/PDP.
24. USDA. US Department of Agriculture pesticide data program (PDP): annual summary. USDA, Agricultural Marketing Service; 2008–2010; 2010 [cited 2015 Oct 10]. Available from: https://www.ams.usda.gov/datasets/pdp/pdpdata.
25. Searle SR, Speed FM, Milliken GA. Population marginal means in the linear model: an alternative to least squares means. Am Stat 1980;34:216–21.
26. Beyerlein A. Quantile regression—opportunities and challenges from a user's perspective. Am J Epidemiol 2014;180:330–1.
27. Afeiche MC, Bridges ND, Williams PL, Gaskins AJ, Tanrikut C, Petrozza JC, Hauser R, Chavarro JE. Dairy intake and semen quality among men attending a fertility clinic. Fertil Steril 2014;101:1280–7.
28. Afeiche MC, Gaskins AJ, Williams PL, Toth TL, Wright DL, Tanrikut C, Hauser R, Chavarro JE. Processed meat intake is unfavorably and fish intake favorably associated with semen quality indicators among men attending a fertility clinic. J Nutr 2014;144:1091–8.
29. Yu B, Qi Y, Liu D, Gao X, Chen H, Bai C, Huang Z. Cigarette smoking is associated with abnormal histone-to-protamine transition in human sperm. Fertil Steril 2014;101:51–7, e1.
30. Li Y, Lin H, Li Y, Cao J. Association between socio-psycho-behavioral factors and male semen quality: systematic review and meta-analyses. Fertil Steril 2011;95:116–23.
31. Jensen TK, Swan S, Jorgensen N, Toppari J, Redmon B, Punab M, Drobnis EZ, Haugen TB, Zilaitiene B, Sparks AE, et al. Alcohol and male reproductive health: a cross-sectional study of 8344 healthy men from Europe and the USA. Hum Reprod 2014;29:1801–9.
32. Chiu YH, Afeiche MC, Gaskins AJ, Williams PL, Mendiola J, Jorgensen N, Swan SH, Chavarro JE. Sugar-sweetened beverage intake in relation to semen quality and reproductive hormone levels in young men. Hum Reprod 2014;29:1575–84.

33. Schisterman EF, Cole SR, Platt RW. Overadjustment bias and unnecessary adjustment in epidemiologic studies. Epidemiology 2009;20:488–95.
34. Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP, Jensen TK, Van Wely M, Cao J, Martini AC, Eskandar M, et al. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. Hum Reprod Update 2013;19:221–31.
35. Hamad MF, Shelko N, Kartarius S, Montenarh M, Hammadeh ME. Impact of cigarette smoking on histone (H2B) to protamine ratio in human spermatozoa and its relation to sperm parameters. Andrology 2014;2:666–77.
36. Amann RP, Chapman PL. Total sperm per ejaculate of men: obtaining a meaningful value or a mean value with appropriate precision. J Androl 2009;30:642–9.
37. Kimmons J, Gillespie C, Seymour J, Serdula M, Blanck HM. Fruit and vegetable intake among adolescents and adults in the United States: percentage meeting individualized recommendations. Medscape J Med 2009;11:26.
38. Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, Hu FB. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. BMJ 2014;349:g4490.
39. Gharagozloo P, Aitken RJ. The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy. Hum Reprod 2011;26:1628–40.
40. Eskenazi B, Kidd SA, Marks AR, Sloter E, Block G, Wyrobek AJ. Antioxidant intake is associated with semen quality in healthy men. Hum Reprod 2005;20:1006–12.
41. Zareba P, Colaci DS, Afeiche M, Gaskins AJ, Jorgensen N, Mendiola J, Swan SH, Chavarro JE. Semen quality in relation to antioxidant intake in a healthy male population. Fertil Steril 2013;100:1572–9.
42. Mínguez-Alarcón L, Mendiola J, Lopez-Espin JJ, Sarabia-Cos L, Vivero-Salmeron G, Vioque J, Navarrete-Munoz EM, Torres-Cantero AM. Dietary intake of antioxidant nutrients is associated with semen quality in young university students. Hum Reprod 2012;27:2807–14.
43. Chandalia M, Garg A, Lutjohann D, von Bergmann K, Grundy SM, Brinkley LJ. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. N Engl J Med 2000;342:1392–8.
44. La Vignera S, Condorelli R, Vicari E, D'Agata R, Calogero AE. Diabetes mellitus and sperm parameters. J Androl 2012;33:145–53.
45. Boxmeer JC, Smit M, Utomo E, Romijn JC, Eijkemans MJ, Lindemans J, Laven JS, Macklon NS, Steegers EA, Steegers-Theunissen RP. Low folate in seminal plasma is associated with increased sperm DNA damage. Fertil Steril 2009;92:548–56.
46. Wallock LM, Tamura T, Mayr CA, Johnston KE, Ames BN, Jacob RA. Low seminal plasma folate concentrations are associated with low sperm density and count in male smokers and nonsmokers. Fertil Steril 2001;75:252–9.
47. Tüttelmann F, Rajpert-De Meyts E, Nieschlag E, Simoni M. Gene polymorphisms and male infertility—a meta-analysis and literature review. Reprod Biomed Online 2007;15:643–58.
48. Bentivoglio G, Melica F, Cristoforoni P. Folinic acid in the treatment of human male infertility. Fertil Steril 1993;60:698–701.
49. Imamovic Kumalic S, Pinter B. Review of clinical trials on effects of oral antioxidants on basic semen and other parameters in idiopathic oligoasthenoteratozoospermia. Biomed Res Int 2014;2014:426951.
50. Bebe FN, Panemangalore M. Exposure to low doses of endosulfan and chlorpyrifos modifies endogenous antioxidants in tissues of rats. J Environ Sci Health B 2003;38:349–63.
51. Ouyang F, Longnecker MP, Venners SA, Johnson S, Korrick S, Zhang J, Xu X, Christian P, Wang MC, Wang X. Preconception serum 1,1,1-trichloro-2,2,bis(p-chlorophenyl)ethane and B-vitamin status: independent and joint effects on women's reproductive outcomes. Am J Clin Nutr 2014;100:1470–8.
52. Ismail MF, Mohamed HM. Modulatory effect of lycopene on deltamethrin-induced testicular injury in rats. Cell Biochem Biophys 2013;65:425–32.
53. Carlsen E, Swan SH, Petersen JH, Skakkebaek NE. Longitudinal changes in semen parameters in young Danish men from the Copenhagen area. Hum Reprod 2005;20:942–9.
54. Stokes-Riner A, Thurston SW, Brazil C, Guzick D, Liu F, Overstreet JW, Wang C, Sparks A, Redmon JB, Swan SH. One semen sample or 2? Insights from a study of fertile men. J Androl 2007;28:638–43.
55. Moore LV, Dodd KW, Thompson FE, Grimm KA, Kim SA, Scanlon KS. Using behavioral risk factor surveillance system data to estimate the percentage of the population meeting US Department of Agriculture food patterns fruit and vegetable intake recommendations. Am J Epidemiol 2015;181:979–88.
56. Guzick DS, Overstreet JW, Factor-Litvak P, Brazil CK, Nakajima ST, Coutifaris C, Carson SA, Cisneros P, Steinkampf MP, Hill JA, et al. Sperm morphology, motility, and concentration in fertile and infertile men. N Engl J Med 2001;345:1388–93.
57. Jedrzejczak P, Taszarek-Hauke G, Hauke J, Pawelczyk L, Duleba AJ. Prediction of spontaneous conception based on semen parameters. Int J Androl 2008;31:499–507.
58. Nordkap L, Joensen UN, Blomberg Jensen M, Jorgensen N. Regional differences and temporal trends in male reproductive health disorders: semen quality may be a sensitive marker of environmental exposures. Mol Cell Endocrinol 2012;355:221–30.
59. Jensen TK, Jacobsen R, Christensen K, Nielsen NC, Bostofte E. Good semen quality and life expectancy: a cohort study of 43,277 men. Am J Epidemiol 2009;170:559–65.
60. Eisenberg ML, Li S, Behr B, Cullen MR, Galusha D, Lamb DJ, Lipshultz LI. Semen quality, infertility and mortality in the USA. Hum Reprod 2014;29:1567–74.
61. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva (Switzerland): WHO Press; 2010.
62. Curl CL, Beresford SA, Fenske RA, Fitzpatrick AL, Lu C, Nettleton JA, Kaufman JD. Estimating pesticide exposure from dietary intake and organic food choices: the Multi-Ethnic Study of Atherosclerosis (MESA). Environ Health Perspect 2015;123:475–83.