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SEMEN QUALITY IN RELATION TO ANTIOXIDANT INTAKE IN A HEALTHY MALE POPULATION

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Abstract

Objective—To assess the relationship between dietary antioxidant intake and semen quality in young healthy males

Design—Cross-sectional study

Setting—University and college campuses in the Rochester, New York, area

Patients—189 university-aged men

Interventions—None

Main Outcome Measures—Semen volume, total sperm count, concentration, motility, total motile count, and morphology

Results—Progressive motility was 6.5 (95% CI 0.6, 12.3) percentage units higher among men in the highest quartile of α -carotene intake compared to men in the lowest quartile. Similar results

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were observed for lutein intake. Lycopene intake was positively related to sperm morphology. The adjusted percentages (95% CI) of morphologically normal sperm in increasing quartiles of lycopene intake were 8.0 (6.7, 9.3), 7.7 (6.4, 9.0), 9.2 (7.9, 10.5) and 9.7 (8.4, 11.0). There was a non-linear relationship between vitamin C intake and sperm concentration, with men in the second quartile of intake having, on average, the highest sperm concentrations and men in the top quartile of intake having the lowest concentrations.

Conclusions—In a population of healthy young men, carotenoid intake was associated with higher sperm motility and, in the case of lycopene, better sperm morphology. Our data suggest that dietary carotenoids may have a positive impact on semen quality.

Keywords

Infertility; sperm quality parameters; vitamins; antioxidants; diet

INTRODUCTION

Accumulating evidence suggests that oxidative stress plays an important role in the pathophysiology of male factor infertility (1–3). In 1943, MacLeod demonstrated the rapid loss of motility that occurs when spermatozoa are exposed to oxygen, a phenomenon that he reversed by adding the antioxidant enzyme catalase to the seminal fluid (4). More recently, *in vivo* studies have correlated high levels of reactive oxygen species and low levels of antioxidants in human seminal plasma with low sperm motility and concentration (5–8).

Multiple randomized trials have investigated antioxidant supplementation for treatment of male infertility (9–14), with several demonstrating a positive effect on semen quality, especially sperm motility (10, 12, 14). However, almost all used combinations of multiple antioxidants, and many used supra-physiologic doses despite evidence that these may have detrimental effects on fertility (15–18). In fact, little is known about how usual levels of intake of various antioxidants and how antioxidant intake from food sources relate to sperm production and function. Only three observational studies have previously assessed the relationship between dietary antioxidant intake and semen quality (19–21). Of these, two studied healthy males (19, 20) and none assessed intake from food sources alone. The objective of our study was therefore to determine the relationships between intakes of vitamins A, C, E, and carotenoids from food and supplements on semen quality in a healthy male population.

MATERIALS AND METHODS

Study Population

The Rochester Young Men's Study (RYMS) is a cross-sectional study on male reproductive health incorporating measures of dietary intake and semen quality. Eligible subjects were English-speaking males born in the United States on or after January 1, 1988. Men who were unable or unwilling to contact their mothers for the purpose of filling out a questionnaire were excluded. This was a volunteer sample recruited by way of advertisements posted on college and university campuses and printed in newspapers in the Rochester, New York, area between 2009 and 2010. A total of 389 men responded, 305 (78%) of whom met the eligibility criteria. Our analysis only includes men who completed a food frequency questionnaire (n=194), which was introduced after the start of the study in the spring of 2009. The food frequency questionnaire was completed by all 194 men who were recruited after its introduction. Men were further excluded if they were missing information on calorie intake (n=3) or reported a calorie intake of less than 600 or greater

than 15,000 kilocalories (kcal) per day ($n=2$). Thus, the total sample size used in this analysis consisted of 189 men.

The study was conducted according to the ethical principles outlined in the Declaration of Helsinki. Approval was obtained from the University of Rochester Research Subjects Review Board, and written informed consent was obtained from all participants. Subjects were paid \$75.

Semen Collection and Analysis

One semen sample was collected from each study participant. Semen samples were obtained by masturbation and processed within 30 minutes of collection. Although men were instructed to abstain from ejaculation for at least 48 hours, abstinence times ranged from 8 hours to 25 days. Semen volume was assessed by specimen weight, and sperm concentration was assessed using a hemocytometer (Improved Neubauer, Hauser Scientific, Horsham, PA, USA). Total sperm count was calculated as the product of semen volume and sperm concentration.

Motility was assessed by microscopy according to the World Health Organization (WHO) criteria (1999 edition) and reported as the percentage of sperm with rapid or slow progressive (A+B) motility. Total motile sperm count was calculated by multiplying semen volume, sperm concentration, and progressive (A+B) motility. For assessment of morphology, smears were air-dried, fixed, and shipped to the University Department of Growth and Reproduction at the Rigshospitalet (Copenhagen, Denmark), where the slides were Papanicolaou stained and assessed for morphology using strict criteria (22).

Diet Assessment

Participants completed a validated 131-item food frequency questionnaire (FFQ) (23). Men were asked to report how often, on average, they consumed specified amounts of foods, beverages, and supplements during the previous year. Nutrient intakes were derived by summing specific nutrient contribution across all items in the questionnaire using the U.S. Department of Agriculture nutrient database and additional information obtained from manufacturers. Micronutrients considered in this analysis were (preformed) vitamin A, vitamin C, vitamin E, and the carotenoids α -carotene, β -carotene, β -cryptoxanthin, lutein, and lycopene.

Covariate Assessment

Men completed questionnaires concerning demographics, medical and reproductive history, medication use, physical activity, and smoking habits. A physical examination was performed on the day of semen collection.

Statistical Analysis

Daily micronutrient intakes were adjusted for total energy intake using the nutrient residual method (24). Tests of association between subject characteristics and micronutrient intakes were performed using the Cochran-Armitage test for trend for categorical variables and general linear models for continuous variables.

Linear regression was used to model semen quality parameters as a function of quartile of intake of each micronutrient. Semen volume, sperm concentration, total count, and total motile count were log-transformed to account for their non-normal distributions. Prior subject-matter knowledge was used to identify potential confounders. We included variables previously associated with semen quality (age, body mass index, physical activity, caffeine intake, and alcohol intake as continuous variables, and current smoking as a binary

variable). Abstinence time was positively related to semen volume, total sperm count, sperm concentration, and total motile count. While it was not associated with intake of antioxidants and was, therefore, not a confounder (25) of the relation between antioxidant intake and semen quality, we nevertheless included a term for abstinence time as a continuous variable in all regression models to increase the precision of the effect estimates (26). We also adjusted for total energy intake as we expected it to be associated with intake of individual micronutrients as well as certain behavioral factors, such as physical activity, that affect semen quality, and because adjustment was also expected to improve the precision of the effect estimates for nutrient intakes (24, 26). We defined *a priori* a significant association between antioxidant intake and semen quality to be a linear trend between intake and the semen quality parameter of interest. Tests of linear trend were performed by assigning each subject the median intake within their quartile and including intake as a continuous variable in the model. Tests of non-linear trend were performed by inserting an additional quadratic term for intake. Adjusted means were calculated using the least-squares method (27).

To determine whether the relationships between antioxidant intakes and semen quality parameters were modified by smoking status or BMI, interaction terms between intake and either smoking status or BMI were inserted into the model. To determine the effect of supplement intake on the results, we repeated the analyses described above with micronutrient intake from food sources alone.

All data was complete with the exception of a single subject who had missing data for exercise. This subject was subsequently assigned the median value for exercise (8.6 hours) for inclusion in the multivariate analysis.

All tests of association were performed at a level of significance of 0.05. Statistical analyses were performed using Statistical Analysis Software (SAS), version 9.2 (SAS Institute Inc., Cary, NC).

RESULTS

One hundred and eighty-nine men with a mean (SD) age of 19.7 (1.0) years were included in the analysis. Most (83%) were Caucasian, 23% were current smokers, and 41% were either overweight or obese (BMI ≥ 25). Subjects were highly active, spending a median (IQR) of 8.3 (5.0–14.0) hours on moderate or vigorous physical activity each week.

Median crude daily intakes of vitamins A, C, and E were 2556 (1489–3789) IU, 167 (108–279) mg, and 8.8 (5.8–14.8) mg, respectively. Median total carotenoid intake was 7531 (3903–11621) IU. While supplemental antioxidants were consumed by 40% of men, the main intake source of these nutrients was food. The corresponding median daily intakes from food sources alone were 2053 (1269–2926) IU, 138 (95–204) mg, 7.2 (5.2–10.1) mg, and 7069 (3757–11136 IU), respectively.

Median semen volume, sperm concentration, and total count were 3.2 (2.2–4.4) mL, 53 (21–96) million/mL, and 159 (71–312) million, respectively. The median progressive motility and total progressive motile count were 61% (50–70%) and 95 (34–200) million, respectively. The median proportion of sperm with normal morphology was 9% (5–12%).

Subject characteristics by quartile of antioxidant intake are shown in Table 1. More physically active men reported higher intakes of vitamins A, C, E, and carotenoids. Smoking was most common among men in the lowest quartile of intake of each antioxidant. Alcohol intake was negatively associated with intake of each antioxidant, while caffeine intake was negatively associated with vitamin C intake only. Intake of antioxidants was also associated with the macronutrient composition of diet.

Adjusted least-squares means relating intake of carotenoids to semen quality are shown in Table 2. Carotenoid intake was positively related to sperm motility, with α -carotene and lutein showing the strongest association. The percentage of progressively motile sperm among men in the highest quartile of α -carotene intake was, on average, 6.5 (95% CI 0.6, 12.3) percentage units higher compared to men in the lowest quartile ($p_{\text{trend}} = 0.06$) (Figure 1A). Men in the highest quartile of lutein intake had 4.4 (95% CI -1.4, 10.2) percentage units higher progressive motility compared to men in the lowest quartile ($p_{\text{trend}} = 0.06$) (Figure 1B). Lycopene consumption was positively associated with sperm morphology. The percentage of morphologically normal sperm among men in the highest quartile of lycopene intake was, on average, 1.7 (95% CI -0.1, 3.6) percentage units higher than among men in the lowest quartile ($p_{\text{trend}} = 0.02$).

The association between total carotenoid and α -carotene intakes and sperm motility was stronger when limiting the analysis to intake from food sources alone ($p_{\text{trend}} = 0.05$ and 0.02, respectively). Additional adjustment for fat and protein intake attenuated the relation between lutein and sperm motility ($p_{\text{trend}} = 0.11$) but did not change the relations between total carotenoid and α -carotene intake and sperm motility ($p_{\text{trend}} = 0.07$ and 0.06, respectively) or the relation between lycopene intake and sperm morphology ($p_{\text{trend}} = 0.02$). Adjustment for folic acid did not change the associations between motility and carotenoid intake.

There was a non-linear relationship between α -carotene intake and sperm concentration, total count, and total motile count ($p_{\text{non-linearity}} = 0.04$, 0.03, and 0.04, respectively), with all three parameters being highest among men in the middle quartiles of intake.

Adjusted least-squares means relating intake of vitamins A, C, and E from all sources and food sources alone are presented in Table 3. High intake of vitamin C from food sources alone was associated with lower sperm concentration, count, and motile count. Men in the highest quartile of vitamin C from food sources had sperm concentrations that were 22% (95% CI -47%, 16%) lower than men in the lowest quartile ($p_{\text{trend}} = 0.04$). However, the linear association between vitamin C intake and sperm concentration disappeared after additional adjustment for folic acid ($p_{\text{trend}} = 0.10$). Furthermore, the relationships between vitamin C intake and the parameters described above were not clearly linear, as men in the second quartile of intake had the highest adjusted mean sperm concentration, count, and motile count. A quadratic model supported the existence of a non-linear association between vitamin C intake and sperm concentration ($p_{\text{non-linearity}} = 0.06$), an association that persisted even after further adjustment for folic acid intake.

No significant associations between vitamin C intake and semen volume or sperm morphology were found. Likewise, vitamin A and E intakes were not related to any of the semen quality parameters.

The associations described above were not significantly modified by either smoking or BMI (analyses not shown).

DISCUSSION

In this cross-sectional study of 189 young healthy men, higher intakes of the carotenoids α -carotene and lutein were associated with better sperm motility, while higher lycopene intake was associated with better morphology. Conversely, moderate intake of α -carotene and vitamin C was associated with the highest concentration, count, and motile count.

Dietary antioxidants prevent oxidative damage to spermatozoa by acting as scavengers of reactive oxygen species in seminal plasma (3). A recent systematic review of 17 randomized

trials of antioxidant supplementation showed that a majority reported an improvement in sperm motility in men assigned to antioxidant supplementation compared to placebo (28). However, the majority of these studies were small, of poor methodological quality, and used supraphysiologic doses despite the fact that the dose-response relationships between antioxidant intake and sperm production and function are unknown.

Carotenoids have been the least studied of all dietary antioxidants. In a study in which 30 men with idiopathic oligoasthenoteratospermia were given lycopene for three months, two-thirds showed an improvement in sperm concentration, 43% in motility, and 46% in morphology (29). In a case-control study by Mendiola et al. (2010) comparing 30 men with moderate to severe oligospermia or teratospermia to 31 normospermic but subfertile men, the risk of poor semen quality was shown to decrease with increasing dietary intake of lycopene. The larger cross-sectional study by Eskenazi et al. (2005) of 97 non-smoking healthy men reported a significant trend toward higher sperm concentration and motility with increasing β -carotene intake. In the largest and most recent cross-sectional study of healthy university-aged males from Spain, higher β -carotene and lycopene intakes were shown to correlate with higher total motile sperm counts (20). This is also the only other study to date to have assessed lutein intake, and it found no association between lutein intake and any of the semen quality parameters, including motility (20).

When interpreting these results, it is important to consider that dietary carotenoids are derived almost exclusively from fruits and vegetables. Five foods (tomato soup, tomato juice, salsa, ketchup and fresh tomatoes) explained 98% of lycopene intake; three foods (carrots, lettuce and spinach) explained 59% of β -carotene intake; and two foods (spinach and lettuce) explained 56% of lutein intake. It is possible that another component in these healthful foods is responsible for the observed improvement in sperm motility or, alternately, that the relationship between carotenoid intake and semen quality may be confounded by other healthy behaviors (30). Well-designed randomized trials are needed to confirm if a causal relationship between carotenoid intake and motility exists.

In contrast to other studies, we did not identify any positive linear associations between intakes of antioxidant vitamins and semen quality. Vitamin C intake was found to be related to higher sperm concentration among healthy, non-smoking men in the previously cited cross-sectional study by Eskenazi et al. (2005). Likewise, in the case-control study by Mendiola et al. (2010), vitamin C intake was associated with greater odds of having semen quality above WHO (1999 ed.) reference values. In two randomized trials, supplementation with 1,000 mg/day of vitamin C improved sperm morphology among smokers with proven fertility (31) and sperm motility among subfertile men with high sperm agglutination (32). However, a third trial of combined vitamin C (1,000 mg/day) and vitamin E (800 mg/day) supplementation among subfertile, asthenozoospermic men showed no improvement in sperm concentration, motility or morphology (11). The inconsistent results across studies may be attributable to varying study populations (healthy vs. subfertile men, smokers vs. non-smokers), sample sizes, and intake levels. For example, the mean vitamin C intake in the Mendiola et al. study (57.9 mg among controls) and the median vitamin C intake in the Eskenazi et al. study (165 mg) were lower than the mean vitamin C intake in our study (245 mg), which in turn is only about 25% of the dose of vitamin C used in previous randomized trials. The suggestion that vitamin C may have varying effects on semen quality at different levels of intake is supported by the fact that men in our study who fell in the second quartile of vitamin C intake (corresponding to a median intake of 148 mg/day) had a higher mean sperm concentration, count, motility, and motile count than men in either the lowest or highest quartiles of intake. Clearly, further study on the dose-response relationships between antioxidants and semen quality parameters is needed.

We found no significant linear associations between intakes of vitamins A and E and any of the semen parameters studied, raising the question as to why certain antioxidants would have an effect on semen quality while others not. Unfortunately, there is little data on the biological mechanisms by which these antioxidants affect semen quality, and how dietary intake relates to their levels in the seminal plasma. It is possible that for some of these micronutrients, increasing intake across the range documented in our study does not result in an appreciable increase in their concentrations in the semen. It is also not known whether different antioxidants are concentrated in different parts of the genital tract. This would explain why certain micronutrients would be associated with motility (if they were concentrated in the epididymis) and others with concentration (seminiferous tubules). Although our study does not address these questions, they provide interesting avenues for future exploration.

Ours is one of the largest studies relating dietary antioxidant intake to semen quality parameters, and only the second such study in young healthy males. As these men were unaware of their fertility status, our results are less likely to be attributable to reverse causation than similar studies of men presenting for infertility evaluation. We used a validated food frequency questionnaire, and assessed intake from food sources as well as supplements. The major limitation of our study was that it was cross-sectional in design, making it difficult to ascribe causal interpretations to the reported associations. Intakes of individual antioxidants were also correlated, making it difficult to determine with certainty whether the reported associations are independent of the intakes of the other antioxidants. Furthermore, the probability of finding chance associations was increased by the inclusion of multiple antioxidants and semen quality parameters in our analysis. Lastly, we collected a single semen sample from each subject. While this may have introduced some measurement error into our results, there is evidence that semen quality parameters do not differ significantly between samples in healthy men (33).

In summary, we found a linear dose-response relationship between higher intakes of the carotenoids α -carotene and lutein and sperm motility and between higher intake of lycopene and sperm morphology in a large group of physically active, university-aged men. Whether these results represent a true causal effect of carotenoids on semen quality and whether they apply to subfertile men is uncertain and deserves further study.

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Figure 1A

Figure 1B

Fig. 1. Adjusted differences in sperm motility by quartiles of (A) -carotene and (B) lutein intake

Table 1
Subject characteristics by quartile of vitamin A, C, E, and carotenoid intake in the Rochester Young Men’s Study

	Vitamin A			Vitamin C			Vitamin E			Total Carotenoids		
	Lowest [IU] (n=47)	Highest [IU] (n=47)	<i>p</i> -value (trend)	Lowest [mg] (n=48)	Highest [mg] (n=48)	<i>p</i> -value (trend)	Lowest [mg] (n=46)	Highest [mg] (n=47)	<i>p</i> -value (trend)	Lowest [IU] (n=48)	Highest [12230 IU] (n=47)	<i>p</i> -value (trend)
Demographics												
Age (years)	19.8 (1.1)	19.9 (0.9)	0.25	19.9 (1.0)	19.6 (0.9)	0.12	19.7 (1.1)	19.8 (1.0)	0.49	19.8 (1.0)	19.6 (0.9)	0.41
Caucasian, <i>n</i> (%)	39 (83)	41 (87)	0.35	44 (92)	37 (77)	0.10	38 (83)	40 (85)	0.74	37 (77)	40 (85)	0.37
Current smoker, <i>n</i> (%)	15 (32)	10 (21)	0.19	15 (31)	11 (23)	0.16	14 (30)	9 (19)	0.28	17 (35)	8 (17)	0.03
Exercise ^a (hours/week)	8.4 (7.2)	14.6 (10.9)	< 0.001	8.5 (6.5)	12.6 (9.9)	0.01	8.8 (6.0)	14.4 (10.6)	< 0.001	8.7 (7.3)	13.5 (10.9)	0.01
Body mass index (kg/m ²)	25.1 (4.9)	25.9 (3.9)	0.20	25.4 (4.4)	25.7 (4.0)	0.59	25.3 (4.1)	25.6 (4.0)	0.60	25.4 (4.4)	25.3 (4.0)	0.90
Dietary Intake												
Total energy intake (kcal/day)	2900 (1109)	2889 (1170)	0.56	2995 (1021)	3086 (1340)	0.52	2682 (977)	2815 (941)	0.37	3010 (1194)	3155 (1447)	0.54
Carbohydrate intake (% calories)	49.8 (7.6)	49.1 (6.4)	0.52	46.4 (6.8)	53.1 (6.2)	< 0.001	47.8 (7.3)	51.4 (6.8)	0.08	48.2 (6.9)	52.2 (5.9)	0.01
Protein intake (% calories)	15.3 (2.6)	17.9 (3.4)	< 0.001	16.2 (3.1)	16.3 (2.8)	0.84	15.7 (2.6)	17.0 (3.5)	0.03	15.6 (3.1)	17.5 (3.1)	0.001
Fat intake (% calories)	31.0 (5.0)	30.7 (5.6)	0.84	32.3 (4.9)	29.8 (5.3)	0.01	30.6 (5.7)	29.8 (5.3)	0.39	31.7 (4.9)	28.9 (4.3)	0.007
Alcohol intake (g/day)	21.5 (22.2)	15.8 (16.0)	0.14	26.1 (23.7)	11.0 (11.2)	0.001	27.1 (27.4)	13.8 (13.8)	0.01	22.4 (22.3)	14.7 (19.4)	0.09
Caffeine intake (mg/day)	89 (101)	96 (255)	0.79	145 (262)	71 (72)	0.03	88 (79)	75 (81)	0.34	121 (251)	82 (102)	0.20
Multivitamin use, <i>n</i> (%)	2 (4)	33 (70)	< 0.001	6 (13)	26 (55)	< 0.001	0 (0)	33 (70)	< 0.001	9 (19)	20 (43)	0.005
Reproductive History												
Abstinence time (hours)	106 (108)	91 (63)	0.61	75 (42)	92 (61)	0.63	83 (47)	99 (63)	0.47	91 (60)	91 (66)	0.90
Cryptorchidism, <i>n</i> (%)	2 (4)	1 (2)	0.75	2 (4)	0 (0)	0.09	2 (4)	0 (0)	0.31	0 (0)	3 (6)	0.08
Variocoele, <i>n</i> (%)	0 (0)	2 (4)	0.15	0 (0)	3 (6)	0.15	1 (2)	2 (4)	0.30	1 (2)	2 (4)	0.30

Percentages may not add up to 100% because of rounding;

^a moderate or strenuous physical activity; *p*-values derived using the Cochrane-Armitage test for trend for categorical variables and general linear models for continuous variables

Table 2

Adjusted^a least-squares mean (95% CI) semen analysis parameters by quartile of carotenoid intake from all sources

Daily Intake	N	Sperm Concentration (millions/mL)	Total Sperm Count (millions)	Sperm Motility (% motile)	Sperm Morphology (% normal)
Total Carotenoids (IU/day)					
5065	48	39 (30, 51)	117 (87, 158)	55 (51, 59)	8.7 (7.4, 10.0)
5066 – 8525	47	48 (37, 63)	164 (122, 221)	58 (54, 62)	8.6 (7.3, 9.9)
8526 – 12229	47	54 (41, 71)	159 (118, 215)	60 (56, 64)	9.5 (8.2, 10.8)
12230	47	41 (31, 54)	123 (91, 167)	60 (56, 65)	7.7 (6.4, 9.0)
p-value (trend)		0.87	0.90	0.07	0.34
-Carotene (µg/day)					
353	48	35 (27, 46)	112 (83, 152)	55 (51, 59)	8.4 (7.1, 9.7)
354 – 634	46	52 (40, 69)	169 (125, 230)	58 (54, 62)	7.8 (6.5, 9.2)
635 – 993	48	49 (37, 64)	138 (103, 186)	62 (58, 66)	8.9 (7.7, 10.2)
994	47	46 (35, 61)	144 (107, 195)	59 (55, 63)	9.3 (8.0, 10.6)
p-value (trend)		0.32	0.49	0.13	0.19
-Carotene (µg/day)					
2520	47	38 (29, 50)	115 (85, 155)	54 (50, 58)	8.6 (7.2, 9.9)
2521 – 4274	48	52 (39, 68)	174 (130, 235)	59 (55, 63)	9.0 (7.7, 10.3)
4275 – 6058	47	52 (40, 69)	160 (118, 215)	59 (55, 63)	8.9 (7.6, 10.2)
6059	47	40 (30, 53)	118 (87, 159)	61 (57, 65) ^b	8.1 (6.7, 9.4)
p-value (trend)		0.97	0.72	0.06	0.51
-Cryptoxanthin (µg/day)					
119	49	44 (33, 58)	130 (96, 175)	56 (52, 60)	9.3 (8.0, 10.6)
120 – 190	46	50 (38, 67)	171 (126, 232)	61 (57, 65)	8.7 (7.4, 10.0)
191 – 268	47	42 (32, 56)	130 (96, 175)	59 (55, 63)	8.0 (6.7, 9.3)
269	47	44 (34, 59)	131 (97, 178)	58 (54, 62)	8.4 (7.1, 9.7)
p-value (trend)		0.83	0.72	0.61	0.29
Lutein (µg/day)					
1734	47	40 (30, 52)	130 (96, 176)	57 (53, 61)	8.7 (7.4, 10.0)
1735 – 2807	48	48 (37, 63)	148 (110, 200)	55 (51, 59)	7.9 (6.6, 9.2)
2808 – 4479	47	53 (40, 69)	165 (122, 223)	61 (57, 65)	9.0 (7.7, 10.3)

Daily Intake	N	Sperm Concentration (millions/mL)	Total Sperm Count (millions)	Sperm Motility (% motile)	Sperm Morphology (% normal)
4480	47	41 (31, 54)	117 (87, 159)	61 (57, 65)	8.9 (7.6, 10.2)
<i>p</i> -value (trend)		0.92	0.49	0.06	0.58
Lycopene (µg/day)					
4808	48	40 (30, 53)	115 (85, 156)	56 (51, 60)	8.0 (6.7, 9.3)
4809 – 8610	47	48 (36, 63)	159 (118, 215)	59 (55, 63)	7.7 (6.4, 9.0)
8611 – 13507	46	45 (34, 59)	127 (94, 172)	59 (55, 63)	9.2 (7.9, 10.5)
13508	48	48 (37, 64)	161 (119, 218)	60 (56, 64)	9.7 (8.4, 11.0)
<i>p</i> -value (trend)		0.47	0.28	0.21	0.02

^a Adjusted for age, BMI, smoking, abstinence time, total energy intake, caffeine intake, alcohol intake, and moderate or strenuous physical activity;

^b *p*-value < 0.05 for comparison between highest and lowest quartile; *p*-values derived by linear regression

Table 3

Adjusted^a least-squares mean (95% CI) semen analysis parameters by quartile of vitamin A, C, and E intake

Daily Intake	N	Sperm Concentration (millions/mL)	Total Sperm Count (millions)	Sperm Motility (% motile)	Sperm Morphology (% normal)
Vitamin A (IU/day) (All)					
1779	47	43 (32, 56)	125 (92, 170)	57 (53, 61)	9.0 (7.6, 10.3)
1780 – 2532	48	54 (41, 71)	160 (118, 216)	57 (53, 61)	8.6 (7.4, 9.9)
2533 – 3806	47	44 (34, 58)	154 (114, 209)	62 (58, 66)	8.6 (7.3, 9.9)
3807	47	40 (30, 54)	121 (89, 166)	58 (54, 62)	8.3 (6.9, 9.6)
<i>p</i> -value (trend)		0.44	0.54	0.81	0.51
Vitamin A (IU/day) (Food)					
1524	47	43 (32, 57)	123 (90, 168)	58 (54, 62)	9.3 (8.0, 10.7)
1525 – 2022	48	51 (38, 67)	160 (118, 218)	56 (52, 60)	8.2 (6.9, 9.5)
2023 – 2900	47	46 (35, 61)	144 (106, 196)	59 (55, 64)	8.6 (7.3, 9.9)
2901	47	41 (31, 54)	132 (97, 179)	60 (56, 65)	8.4 (7.1, 9.7)
<i>p</i> -value (trend)		0.62	0.97	0.21	0.51
Vitamin C (mg/day) (All)					
125	48	49 (37, 65)	155 (114, 211)	59 (55, 63)	8.9 (7.6, 10.2)
126 – 183	48	52 (39, 68)	162 (119, 219)	60 (56, 64)	8.0 (6.7, 9.3)
184 – 269	45	43 (32, 57)	115 (84, 158)	56 (52, 60)	8.2 (6.9, 9.6)
270	48	38 (29, 50)	128 (94, 174)	59 (55, 63)	9.3 (8.0, 10.7)
<i>p</i> -value (trend)		0.12	0.34	0.84	0.35
Vitamin C (mg/day) (Food)					
121	47	43 (33, 57)	144 (106, 195)	59 (55, 63)	8.7 (7.3, 10.0)
122 – 157	47	65 (50, 85)	199 (148, 268)	60 (56, 64)	9.6 (8.3, 10.9)
158 – 209	47	44 (33, 57)	127 (94, 171)	59 (55, 64)	7.8 (6.5, 9.1)
210	48	34 (26, 44)	104 (77, 140)	55 (51, 59)	8.4 (7.1, 9.8)
<i>p</i> -value (trend)		0.04	0.03	0.19	0.48
Vitamin E (mg/day) (All)					
6.9	46	46 (35, 61)	136 (99, 187)	59 (54, 63)	8.8 (7.5, 10.2)
7.0 – 9.1	52	49 (37, 64)	149 (111, 199)	61 (57, 65)	9.1 (7.9, 10.4)
9.2 – 14.3	44	42 (31, 56)	132 (95, 181)	59 (55, 63)	8.1 (6.7, 9.5)
14.4	47	43 (32, 57)	140 (102, 191)	55 (51, 59)	8.4 (7.0, 9.7)

Daily Intake	N	Sperm Concentration (millions/mL)	Total Sperm Count (millions)	Sperm Motility (% motile)	Sperm Morphology (% normal)
<i>p</i> -value (trend)		0.67	0.99	0.07	0.57
Vitamin E (mg/day) (Food)					
6.8	49	42 (32, 55)	124 (92, 169)	56 (52, 60)	8.6 (7.3, 9.9)
6.9 – 7.9	47	38 (29, 51)	126 (93, 170)	56 (52, 60)	7.7 (6.4, 9.0)
8.0 – 10.1	47	52 (39, 68)	156 (116, 211)	61 (57, 65)	9.3 (8.0, 10.6)
10.2	46	50 (38, 66)	155 (114, 211)	60 (56, 64)	9.0 (7.6, 10.3)
<i>p</i> -value (trend)		0.24	0.27	0.12	0.45

^a Adjusted for age, BMI, smoking, abstinence time, total energy intake, caffeine intake, alcohol intake, and moderate or strenuous physical activity;

^b *p*-value < 0.05 for comparison between highest and lowest quartile; *p*-values derived by linear regression