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Antioxidant intake is associated with semen quality in healthy men

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BACKGROUND: We seek to determine whether dietary and supplement intake of specific micronutrients (zinc and folate) and antioxidants (vitamins C, E and β-carotene) is associated with semen quality. METHODS: Ninety-seven healthy, non-smoking men provided semen and were interviewed. Average daily nutrient intake from food and supplements was derived from a self-administered food frequency questionnaire. Intake levels were summarized as low, moderate and high. Semen volume, sperm concentration, total sperm count, motility, progressive motility and total progressively motile sperm count (TPMS) were measured. RESULTS: After controlling for covariates, a high intake of antioxidants was associated with better semen quality but, in almost all cases, there was no clear dose relationship in that moderate intake groups had the poorest semen quality. For example, positive associations were observed between vitamin C intake and sperm number as reflected in the higher mean count ($P = 0.04$), concentration ($P = 0.05$) and TPMS ($P = 0.09$); between vitamin E intake and progressive motility ($P = 0.04$) and TPMS ($P = 0.05$); and between β-carotene intake and sperm concentration ($P = 0.06$) and progressive motility ($P = 0.06$). Folate and zinc intake were not associated with improved semen quality. CONCLUSIONS: In a convenience sample of healthy non-smoking men from a non-clinical setting, higher antioxidant intake was associated with higher sperm numbers and motility.

Key words: age/antioxidants/diet/semen quality/sperm

Introduction

Approximately 25% of infertility among couples can be attributed to diminished semen quality and other male factors (Templeton, 1995). The aetiology of diminished semen quality is generally poorly understood, although environmental, occupational and lifestyle characteristics, such as age and diet, have been implicated (Vine, 1996; Auger *et al.*, 2001; Kenkel *et al.*, 2001; Eskenazi *et al.*, 2003). In particular, dietary intake of antioxidants, such as vitamins C and E, and β-carotene, and micronutrients, such as folate and zinc, have been demonstrated to be critically important for normal semen quality and reproductive function in a number of studies in both animals and humans (Apgar, 1985; Hunt *et al.*, 1992; Dabrowski and Ciereszko, 1996; Ziegler and Filer, 1996).

In clinical trials, vitamin E supplementation has been found to increase fertilization rates (Geva *et al.*, 1996) possibly by reducing oxidative damage (Comhaire *et al.*, 2000) and lipid peroxidation potential (Geva *et al.*, 1996). Ascorbic acid concentration in the seminal plasma has also been found to be negatively associated with reactive oxygen species activity in sperm of infertile men, and the depletion of ascorbic acid intake has been associated with an increase in oxidative damage in the sperm of healthy men (Fraga *et al.*, 1991). There is only limited evidence, however, that supplementation will improve clinical measures of semen quality. In one small trial of nine infertile men receiving 400 mg/day of α-tocopherol in combination with selenium (Vezina *et al.*, 1996), there were significant increases in sperm motility, percentage of live sperm and percentage of normal sperm, whereas in other studies, there was no effect of even higher levels of vitamin E supplementation (300–1200 mg/day) (Moilanen *et al.*, 1993; Kessopoulou *et al.*, 1995; Moilanen and Hovatta, 1995) alone or in combination with vitamin C (Rolf *et al.*, 1999).

The micronutrients folate and zinc have also been associated with semen quality. Folate levels in blood plasma (Wallock *et al.*, 1997) and in seminal plasma (Wallock *et al.*, 2001) have been positively associated with sperm concentration and count. Zinc levels in seminal plasma have been positively associated with sperm concentration and motility in some studies (Fuse *et al.*, 1999; Chia *et al.*, 2000), but not others (Lewis-Jones *et al.*, 1996; Lin *et al.*, 2000). In clinical trials of men with round cell idiopathic syndrome, folinic acid supplementation (15 mg/day) improved both sperm count and motility (Bentivoglio *et al.*, 1993). Sperm count

also increased after combined zinc sulfate (66 mg) and folic acid (5 mg) treatment in a randomized clinical trial of subfertile men, but not with either alone (Wong *et al.*, 2002). However, in a trial where infertile men were given a much higher dose of 500 mg/day of zinc sulfate alone, there was a significant improvement of count and progressive motility (Omu *et al.*, 1998).

Although high dose supplementation may impact semen quality, no study has examined whether variation in intake over the normal dietary and supplement intake range is also associated with semen quality. Also, there remains a question as to whether diet can reverse the decrements in semen quality that occur with age (Eskenazi *et al.*, 2003). The purpose of the present analysis is to determine whether normal dietary and supplement intake of specific micronutrients (zinc and folate) and antioxidants (vitamins C, E and β-carotene) is associated with semen quality in a population of healthy nonsmoking men over a wide age range and without a previous history of reproductive problems. In addition, we aim to determine whether nutrient intake can modify the age-related decrements in semen quality we have observed previously in this population.

Materials and methods

Participants

The study population consisted of 97 healthy male volunteers employed by or retired from the Lawrence Livermore National Laboratory (LLNL) in Livermore, California, who participated in the Age and Genetic Effects in Sperm (AGES) Study. The AGES Study was approved by the Internal Review Boards of the participating institutions, and all participants gave written informed consent. Male volunteers were recruited from advertisements, E-mail listserves, posters and newsletters. We enrolled at least 15 men from each age decade from 20 to 60 years of age, and, additionally, 25 men from 60 to 80 years of age. All participants were screened over the telephone to exclude men who had smoked cigarettes in the last 6 months, had current fertility or reproductive problems, had a vasectomy, had a history of prostate cancer or undescended testicles, had received chemotherapy or radiation treatment for cancer or had a previous semen analysis with zero sperm count (Eskenazi *et al.*, 2003). Twenty men were excluded after the preliminary screening due to current smoking ($n = 11$), one testicle ($n = 2$), undescended testicles ($n = 1$), varicocele ($n = 3$), valium use ($n = 1$), chemotherapy ($n = 1$) and hepatitis B infection ($n = 1$). If men had a fever $>101°$ F in the prior 3 months, then they were recruited for participation no earlier than 3 months from the date of the fever.

Dietary assessment

The men were mailed a questionnaire and a semen collection container with instructions. The questionnaire asked about medical and reproductive history, socio-demographic characteristics, occupation, and lifestyle habits and characteristics. Participants also completed a 100-item self-administered Modified Block Food Frequency Questionnaire (Huang *et al.*, 2002). This food frequency questionnaire (FFQ) estimates average daily intake of nutrients based on usual eating habits over the past year with questions about frequency and portion size of each listed food item. Average daily nutrient intake is derived from this questionnaire for: total calories, protein, fat, carbohydrate and micronutrients; average daily intake of vitamins and minerals from supplements; and frequency per day of food groups. The FFQ took ~30 min to complete and was received within 1 week of producing the semen sample. Participants received the results of their semen analysis only after the FFQ was received. We excluded one participant whose nutrient analysis indicated intake of too few foods and calories per day (659 kcal/day), and therefore lacked credibility.

Semen analysis

Semen specimens were produced by masturbation into sterile containers and analysed within 2 h of collection (mean = 45 min). Donors were instructed to abstain for 2–5 days but recorded the actual duration at collection. Replicate specimens were requested when there was indication of loss, low motility or presence of red or white blood cells in the semen. Coded specimens were analysed by established protocols (Shrader *et al.*, 1992) with enhanced quality control (Eskenazi *et al.*, 2003). Semen volume was measured to the nearest 0.1 ml. Sperm concentration was determined in triplicate. Total sperm count was calculated by multiplying the semen volume by the sperm concentration. Motility was assessed visually under 400× phase contrast magnification with a 5 × 5 ocular grid for 150 sperm per sample. Progressively motile sperm were the number of forward-moving sperm that exceeded 25 μm/s (approximately five times the length of the sperm head/s). Total progressively motile sperm (TPMS) was defined as the product of total sperm count and percentage progressive motility.

Statistical analysis

Sperm concentration, total sperm count, progressive sperm motility and TPMS were transformed to achieve normality by taking the square root. Unadjusted and adjusted means and 95% confidence intervals (CIs) for square-root-transformed semen parameters were back-transformed for presentation in the text and tables. Motility-related analyses were performed excluding four participants with azoospermia (men aged 63, 77, 77 and 78 years). For analyses on both semen volume and TPMS, a single outlier (>3 SDs from the mean) was excluded from the population.

Intakes of micronutrients and antioxidants based on the FFQ were each divided into three categories (based on the intake of the entire sample of 96 men): the lowest quartile of intake (low intake), the middle two quartiles of intake (moderate intake) and the highest quartile of intake (high intake). An antioxidant composite variable was created *a priori* based on the intake of vitamins C, E and β-carotene. Low antioxidant composite intake was defined as intake in the lowest quartile of two or more antioxidants; high antioxidant composite intake was defined as intake in the highest quartile of two or more antioxidants; and moderate intake was defined as all other combinations of intake (there were no participants in this category with a low intake ranking in two antioxidants and a high intake ranking in another antioxidant, and vice versa).

Analysis of covariance (ANCOVA) was used to examine the relationship between micronutrients and semen parameters. Multivariate ANCOVA models were created with continuous semen parameters as dependent variables, and micronutrient and antioxidant categories and potential confounders as independent covariates. Age (years) and duration of abstinence (≤5 versus ≥6 days) were included as covariates in all final models, and time (in minutes) from sample collection to sample analysis was also included in all final models for the motility-related parameters (i.e. for sperm motility, progressive sperm motility and TPMS). Based on information from the literature which suggested associations with semen parameters, the following additional covariates were considered: body mass index (BMI; kg/m^2); history of cigarette smoking; season of

sample collection (autumn, winter, spring–summer); alcohol and coffee use in the 3 months prior to sample collection; hot tub use in the 3 months prior to sample collection; history of urinary tract infection and fertility-related problems; and exposure to work hazards and occupational exposure to ionizing radiation (measured from LLNL badge dosimetry records). A covariate was included in the multivariate model if it was related ($P \leq 0.20$) to both the nutrient categories in univariate analyses and to the measure of semen quality controlling for age and abstinence, and if when placed in a regression model (with age and abstinence, as well as time to processing for motility measures), the coefficient for the nutrient changed by $\geq 10\%$. Least squares means were computed to obtain the predicted value of each intake category with covariates set at their mean value. The final regression models were also re-specified with the three intake groups as ordinal variables (i.e. 1 = low intake, 2 = moderate, 3 = high) to test for trend.

To assess whether higher micronutrient or antioxidant intake reduced the adverse relationships previously observed with age, models were run with interaction terms for age and nutrient levels. In addition, each final adjusted model was stratified by intake level (low, moderate and high), and the coefficients for age in the three strata were compared by calculating differences and 95% CIs from the combined standard errors. If the 95% CI for any two-way comparison did not include zero, we concluded that there was a significant difference in the association of age with the outcome in the two nutrient intake levels (Altman and Bland, 2003).

Final models were re-specified with daily kilocalorie intake in order to examine relative versus absolute intake. Models were also rerun excluding men reporting very low (<1000 kcal; $n = 2$) or very high (>3000 kcal; $n = 2$) daily intake. Because recent vitamin or supplement use may be a response to an illness, the analyses were reanalysed without those men who began taking multivitamins or antioxidant supplements in the past 2 years ($n = 12$). Similarly, although no men currently had fertility or genitourinary problems, the analyses were analysed again excluding men who reported any history of an abnormal semen analysis ($n = 5$). Because the results

Table I. Unadjusted mean (95% CI) of semen quality parameters by selected characteristics of the participants: AGES Study, California, 1997–1998

	n (%)[a]	Volume (ml)	Concentration (10^6/ml)[b]	Count (10^6)[b]	Motility (%)	% Progressively motile[b]	TPMS (10^6/ml)[b]
Overall	96	2.8 (2.5–3.1)	107 (85–132)	292 (226–367)	37 (33–42)	17 (14–21)	62 (45–82)
Age							
20s	19 (20)	3.0 (2.4–3.5)	122 (75–182)	350 (209–528)	47 (37–57)	25 (17–34)	95 (48–157)
30s	20 (21)	3.5 (3.0–4.1)	89 (63–119)	312 (218–424)	49 (42–55)	25 (20–31)	74 (49–105)
40s	15 (16)	3.7 (2.7–4.7)	142 (77–227)	511 (231–900)	40 (28–51)	29 (11–30)	108 (37–216)
50s	17 (18)	2.3 (1.7–2.8)	117 (67–181)	252 (132–412)	32 (22–42)	14 (8–23)	44 (16–85)
60+	25 (26)	1.9 (1.4–2.5)[e]	88 (40–153)	167 (68–309)[d]	21 (14–28)[e]	8 (4–13)[e]	23 (9–43)[e]
Abstinence							
2–5 days	72 (75)	2.7 (2.3–3.0)	95 (73–120)	243 (182–311)	40 (35–45)	19 (15–23)	56 (39–76)
>5 days	24 (25)	3.3 (2.6–3.9)[c]	149 (91–221)[c]	469 (265–728)[d]	29 (20–38)[d]	13 (8–21)[c]	84 (39–147)
Time to processing							
≤45 min	51 (53)	2.9 (2.4–3.3)	101 (72–134)	281 (190–389)	41 (35–47)	20 (15–26)	71 (44–104)
>45 min	45 (47)	2.7 (2.3–3.2)	115 (82–155)	305 (211–417)	33 (28–39)[c]	14 (10–19)[c]	53 (34–76)
Ever smoked							
No	75 (78)	3.1 (2.7–3.4)	105 (84–128)	325 (249–410)	40 (35–44)	19 (16–24)	75 (54–99)
Yes	21 (22)	1.8 (1.3–2.3)[e]	118 (53–209)	190 (75–359)[c]	29 (20–38)[d]	11 (6–16)[d]	25 (8–51)[d]
Alcohol use past 3 months							
No	35 (36)	3.4 (2.9–3.9)	99 (68–136)	339 (224–478)	33 (26–41)	16 (10–22)	63 (35–98)
Yes	61 (64)	2.5 (2.1–2.8)[e]	112 (83–147)	267 (188–360)	40 (34–45)	19 (15–23)	62 (41–87)
Body mass index							
≤25	49 (51)	3.0 (2.5–3.4)	117 (85–153)	331 (235–443)	39 (33–44)	19 (15–24)	78 (50–111)
>25	47 (49)	2.6 (2.2–3.0)	98 (68–133)	255 (166–361)	36 (29–43)	16 (11–21)	48 (29–72)[c]
History of urinary tract infection							
No	84 (88)	2.9 (2.5–3.2)	112 (88–138)	315 (241–399)	39 (35–44)	19 (15–23)	71 (51–93)
Yes	12 (13)	2.3 (1.5–3.2)	81 (25–169)	157 (51–321)[c]	24 (11–36)[d]	9 (3–18)[d]	21 (4–51)[d]
Season of sample collection							
Autumn	34 (35)	2.8 (2.2–3.3)	111 (70–162)	303 (176–464)	40 (33–48)	17 (11–24)	78 (40–129)
Winter	38 (40)	2.9 (2.4–3.4)	115 (81–154)	326 (216–458)	35 (29–42)	17 (13–22)	60 (37–87)
Spring/Summer	24 (25)	2.7 (2.1–3.3)	92 (55–137)	228 (144–332)	37 (27–46)	18 (11–26)	48 (25–78)
Any vitamin supplement use							
No	40 (42)	3.0 (2.5–3.5)	95 (61–135)	286 (177–422)	39 (32–47)	17 (12–23)	63 (36–98)
Yes	56 (58)	2.6 (2.3–3.0)	117 (89–149)	296 (216–389)	36 (31–41)	18 (13–22)	62 (41–87)
Kilocalories/day							
500–999	2 (2)	2.3 (0.0–11.8)	130 (49–888)	313 (139–558)	50 (0–208)	38 (0–445)	105 (0–1660)
1000–1499	32 (33)	3.3 (2.8–3.8)	113 (75–158)	360 (229–522)	38 (29–46)	17 (11–24)	74 (41–116)
1500–1999	38 (40)	2.5 (2.0–3.0)	102 (67–145)	253 (159–370)	36 (30–43)	17 (12–23)	55 (33–84)
2000–2499	16 (17)	3.2 (2.5–3.9)	102 (54–164)	332 (162–563)	41 (32–51)	21 (13–30)	82 (29–162)
2500–2999	6 (6)	1.2 (0.6–1.9)	114 (5–366)	108 (2–381)	31 (5–57)	9 (1–25)	14 (0–56)
3000+	2 (2)	3.2 (0.0–17.8)	127 (54–893)	386 (0–8714)	30 (0–233)	15 (0–453)	47 (5–132)

TPMS = total progessively motile sperm.
[a]$n = 95$ for volume; $n = 96$ for concentration and total count; $n = 92$ for motility and progressive motility; $n = 91$ for TPMS.
[b]Concentration, count, % progressively motile sperm and total progressively motile sperm were transformed to achieve normality by taking the square root. Square-root values were back-transformed for presentation in the table.
[c]$P \leq 0.10$.
[d]$P < 0.05$.
[e]$P < 0.01$.

in these additional analyses were similar to the final models, they are not presented.

All *P*-values presented are two-sided. The SAS statistical software package version 9.0 (SAS Institute Inc., Cary, NC) and Stata version 8.0 (STATA Corporation, College Station, TX) were used in the analyses.

Results

Table I describes the socio-demographic characteristics, habits and medical history of the 96 participants included in the study. The men ranged in age from 22 to 80 years, and were, on average, 46 years of age (SD = 16) with a median age of 44. The population was predominantly Caucasian, college educated and in good health. About a quarter of men had been smokers in the past and 64% had used alcohol in the past 3 months. Men were on average of high normal body mass (mean = 24.9 ± 3.1 kg/m^2) and reported an average intake of 1731 kcal/day (SD = 497). Approximately 60% of the men reported taking dietary supplements at the time of interview.

The men had mean semen volumes of 2.8 ml; sperm concentration of 107 × 10^6/ml; sperm count of 292 × 10^6; and TPMS of 62 × 10^6. As shown in Table I, age and period of abstinence were related to most measures of semen quality. A history of smoking and urinary tract infection were also related to many of the measures, but such history is also more common in older men. Men who reported regularly taking any vitamin supplements did not differ statistically in any of the semen parameters from men who did not. Similarly, men who took single-entity vitamin C or E did not differ significantly in any of the measures of semen quality from those who did not, with one exception: those who took a vitamin C supplement had somewhat higher sperm concentration ($P = 0.10$) (data not shown).

For the most part, the demographic characteristics of the men were not associated with overall diet and supplement intake (data not shown). However, supplement users compared with non-supplement users tended to be older ($P = 0.03$), to consume no alcohol in the previous 3 months ($P = 0.05$) and to consume fewer calories (mean intake 1650 versus 1844 kcal; $P = 0.06$), although their dietary intake of nutrients was similar. Increasing levels of folate intake were associated with lower BMI ($r = -0.26$; $P = 0.01$), but increasing kilocalorie intake ($r = 0.23$; $P = 0.02$). Intake of folate ($r = -0.26$; $P = 0.01$), zinc ($r = -0.33$; $P = 0.001$) and vitamin E ($r = -0.25$; $P = 0.01$) from diet alone was negatively correlated with age, but nutrient intake from diet and supplement use together did not differ by age for any of the nutrients.

As shown in Table II, >65% of men based on food intake alone and >40% based on supplement use and food intake combined reported intakes for vitamin E, zinc and folate below the dietary reference intake (DRI) (Institute of Medicine, 2000a, b, 2002). About 40% of the men had intakes which fell below the DRI for vitamin C intake based on dietary intake alone and 20% based on supplements and diet combined. For the micronutrients in Table II, 92–100% of those in the high intake groups took supplements (multiple or single-entity) versus 48–64% in the moderate intake groups versus 5–38% in the low intake groups. In the respective high intake group, 100% took single-entity vitamin C supplements, 83% took vitamin E and 29% took β-carotene supplements, and in the moderate intake group, 17% took vitamin C, 10% took vitamin E and none took β-carotene supplements; in the low intake group, no one took single-entity supplements.

Table III summarizes the relationship of dietary and supplement intake and semen quality adjusting for age, abstinence and other potential confounders. Neither high folate nor zinc intake was associated with improved measures of semen quality. In fact, zinc intake was somewhat negatively associated with volume ($P = 0.07$ ANCOVA). Overall, after controlling for covariates, a high intake of antioxidants was associated with better semen quality than low or moderate intake but, in almost all cases, there was not a clear dose relationship in that the moderate intake groups had the poorest semen quality. For example, vitamin C intake was positively associated with sperm number as reflected in the higher mean count ($P = 0.04$), concentration ($P = 0.05$) and TPMS ($P = 0.09$); vitamin E intake was positively associated with progressive motility ($P = 0.04$) and TPMS ($P = 0.05$); and β-carotene intake was positively associated with sperm concentration ($P = 0.06$) and progressive motility ($P = 0.06$). When the intakes of the three antioxidants (vitamins C and E and β-carotene) were considered together as a composite score (see Table III), antioxidant intake was positively related to sperm concentration ($P = 0.06$), motility

Table II. Reported micronutrient and antioxidant intake from diet and supplements: AGES Study, California, 1997–1998

Micronutrient	Median intake	Definitions of level of intake[a]			DRI[b]	% Below DRI	
		Low (<25th percentile)	Moderate (25th to 74th percentile)	High (≥75th percentile)		Diet only	Diet and supplements
Folate (μg)	484	115–333	344–682	722–1150	400	65%	40%
Zinc (mg)	12	3.8–8.1	8.3–22	22–74	11	78%	48%
Vitamin C (mg)	165	26–99	107–400	437–3394	90	39%	20%
Vitamin E[c] (mg)	25	2.2–9.6	9.9–118	142–833	15	84%	42%
β-Carotene (μg)	2579	376–1167	1263–3942	3973–33 444	–	–	–

[a]Level of intake including supplements; actual values used in the definition of low ($n = 24$), moderate ($n = 48$) and high ($n = 24$) intake.
[b]Dietary reference intakes (DRIs), Institute of Medicine 2000–2002 (none available for β-carotene).
[c]Vitamin E is presented as α-tocopherol equivalents.

Table III. Adjusted means (95% CIs) for semen quality parameters by level of micronutrient or antioxidant intake: AGES Study, California, 1997–1998

	Folate	Zinc	Vitamin C	Vitamin E	β-Carotene	Antioxidant composite
Semen volume (ml)[a]						
Low	2.8 (2.3–3.3)	2.5 (2.0–3.1)	2.9 (2.4–3.4)	2.7 (2.2–3.2)	2.5 (2.0–3.0)	2.8 (2.3–3.3)
Moderate	2.9 (2.5–3.3)	3.0 (2.6–3.3)	2.6 (2.2–3.1)	2.8 (2.4–3.2)	3.0 (2.6–3.4)	2.7 (2.4–3.1)
High	2.3 (1.7–2.8)	2.2 (1.7–2.8)[g]	2.5 (2.0–3.1)	2.5 (2.0–3.1)	2.5 (2.0–3.0)	2.4 (1.8–3.0)
Sperm concentration (10^6/ml)[b]						
Low	136 (88–194)	113 (69–169)	133 (87–189)	107 (66–158)	102 (61–152)	107 (64–160)
Moderate	116 (81–158)	120 (85–162)	95 (65–131)	116 (82–156)	108 (76–146)	112 (81–148)
High	120 (75–175)	137 (87–197)	171 (117–235)[h]	160 (106–226)	177 (123–242)[g,k]	191 (126–270)[g,k]
Total sperm count (10^6)[c]						
Low	301 (149–506)	242 (120–405)	337 (197–514)	222 (104–384)	240 (119–402)	238 (114–407)
Moderate	203 (103–338)	282 (174–416)	199 (113–310)	224 (118–364)	267 (164–395)	206 (107–338)
High	231 (110–398)	289 (153–470)	385 (231–578)[h]	275 (129–475)	345 (196–535)	330 (159–563)
Sperm motility (%)[d]						
Low	30 (20–39)	34 (26–42)	32 (24–40)	32 (23–40)	31 (23–40)	30 (22–39)
Moderate	33 (25–40)	34 (27–40)	32 (26–38)	27 (19–34)	33 (27–39)	28 (21–35)
High	27 (19–35)	32 (24–40)	36 (29–44)	35 (26–44)	37 (29–45)	38 (28–47)[g]
Progressive sperm motility (%)[e]						
Low	14 (8–21)	15 (9–22)	16 (10–22)	18 (12–24)	14 (9–20)	13 (8–20)
Moderate	14 (9–21)	16 (11–21)	14 (10–20)	14 (10–18)	13 (9–18)	11 (7–15)
High	11 (6–17)	15 (9–23)	17 (11–25)	23 (17–31)[h]	22 (15–30)[g,j]	22 (14–32)[i,j]
Total progressively motile sperm (TPMS) (10^6/ml)[f]						
Low	51 (21–94)	53 (25–93)	50 (24–87)	53 (26–89)	43 (17–80)	49 (23–84)
Moderate	29 (11–56)	38 (19–65)	31 (13–56)	31 (14–56)	41 (21–68)	28 (11–51)
High	32 (10–66)	49 (20–90)	68 (35–110)[g]	74 (38–122)[h]	63 (30–108)	85 (44–139)[i]

[a]All volume models were adjusted for age, abstinence and smoking.
[b]All concentration models were adjusted for age and abstinence. Concentration was square-root transformed for analysis and back-transformed for presentation in the table.
[c]All models of count were adjusted for age, abstinence, smoking and season. In addition, models for vitamin C and β-carotene were adjusted for body mass index (BMI), and models for folate, vitamin E and antioxidant composite were adjusted for BMI and history of urinary tract infection (UTI). Count was square-root transformed for analysis and back-transformed for presentation in the table.
[d]Models for motility were adjusted for age, abstinence, time to sample analysis and season. In addition, models for folate, vitamin E and antioxidant composite were adjusted for UTI.
[e]Models for progressive sperm motility were adjusted for age, abstinence and time to sample analysis. In addition, models for zinc, vitamin C and β-carotene were adjusted for smoking, and models for folate and antioxidant composite were adjusted for smoking and UTI. Progressive motility was square-root transformed for analysis and back-transformed for presentation in the table.
[f]Models for TPMS were adjusted for age, abstinence and time to sample analysis. In addition, the model for zinc was adjusted for smoking and season; models for vitamin C, vitamin E and β-carotene were adjusted for smoking, season and BMI; the model antioxidant composite was adjusted for smoking, BMI and UTI; and the model for folate was adjusted for smoking, season, BMI and UTI. TPMS was square-root transformed for analysis and back-transformed for presentation in the table.
[g]ANCOVA $P < 0.10$.
[h]ANCOVA $P < 0.05$.
[i]ANCOVA $P < 0.01$.
[j]Adjusted test for trend $P < 0.10$.
[k]Adjusted test for trend $P < 0.05$.

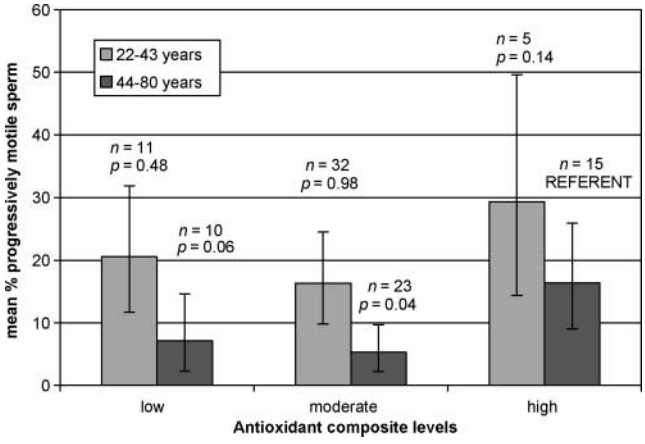

Figure 1. Adjusted mean progressive sperm motility with 95% confidence intervals, by age and antioxidant intake: AGES Study, California, 1997–1998.

($P = 0.09$) and progressive motility ($P = 0.005$), which together contributed to more progressively motile sperm ($P = 0.006$). Men in the high intake levels for the antioxidant composite had adjusted sperm concentration, on average, $> 80 \times 10^6$/ml higher than men with low antioxidant intake, and had 36×10^6/ml more progressively motile sperm than men with low intake levels.

In all the models where nutrient intake was related to semen quality, age also remained significantly related, suggesting that the amount of nutrient intake did not eliminate the age relationships that we have reported previously (Eskenazi *et al.*, 2003). When we examined the interaction of age and nutrient intake, there was some evidence that the slope of age decreased with higher nutrient intake. For example, for progressive sperm motility, the slope of the age relationship was about half as steep for the moderate ($\beta = -0.04$) and the high ($\beta = -0.04$) antioxidant composite intake groups than for the low intake group ($\beta = -0.08$), but these differences were not significant. In Figure 1, we further examine this relationship by dichotomizing the group

by the median age (44 years) and examining the age differences in progressive sperm motility within each level of antioxidant intake. Among older men, those with high intake had better progressive sperm motility than older men with low ($P = 0.06$) and moderate intake ($P = 0.004$). However, progressive sperm motility of older men with high antioxidant intake was non-significantly lower than that in younger men with high intake ($P = 0.14$), but was similar to that of younger men with low ($P = 0.48$) and moderate ($P = 0.98$) intake. A similar pattern was observed for TPMS.

Discussion

Our results suggest that higher antioxidant consumption over the normal range of intake from diet and supplements is associated with greater sperm numbers and motility in our population of healthy men over a wide age range. Although antioxidant intake did not eliminate associations between age and semen quality, there was some evidence that the slope of age decreased with higher intake. Although we had hypothesized that zinc and folate intake would also be associated with improved semen quality, we found no evidence for this association in our population.

We assessed dietary and supplement intake based on self-report on an FFQ. By categorizing intake into low, moderate and high, we were able to guard against effects being driven by unusually high intake by some men who took vitamin supplements. Although the FFQ ascertains eating habits over the last year, participants generally 'telescope' their report so that their dietary report may reflect recent patterns of intake (Willett, 1998). Such a reporting bias would be to the benefit of this study, since the critical period for spermatogenesis was the preceding 3 month period.

Although we did control for a number of important potential confounders, men with high antioxidant intake may differ from men with lower intake in ways which remained unmeasured and/or unknown. For example, although the men in the higher intake group had similar overall caloric intake compared with men with lower antioxidant intake (data not shown), they were of lower body mass, suggesting that men with higher intake may be more physically active. Also, men with higher intake (and those with moderate intake) were less likely to have smoked in the past, although this difference was not significant. Thus, it remains possible that uncontrolled residual confounding reflecting other health behaviours may explain the better semen quality of men with higher antioxidant intake.

The results of our analyses are biologically plausible. Antioxidants may play a critical role in protecting male germ cells against oxidative damage (Fraga *et al.*, 1991). The production of reactive oxygen species has been associated with loss of motility and a decreased capacity for sperm–oocyte fusion (Aitken *et al.*, 1989; Agarwal *et al.*, 2003). Kessopoulou *et al.* (1995) showed that vitamin E levels after treatment improved the *in vitro* function of the human sperm as evidenced by the zona-binding test. Fraga *et al.* (1991) found that seminal plasma levels of vitamin C were directly associated with the level of oxidative damage in human sperm DNA.

Although several clinical intervention trials of antioxidants have found improvement in semen characteristics, the health and habits of the men in our study may also contribute to differences in findings from some of the clinical trials. Rolf *et al.* (1999) hypothesized that the length of vitamin C and E administration in their study (i.e. 8 weeks) may have been too short to improve semen quality in infertile men if the effect is on the testis. In contrast, in our study, the men's intake of antioxidants reflected their normal diets and supplement use behaviour, although the association was seen in only the high intake group where almost all were taking supplements. The effects of small differences in intake may have greater impact in our study, because all the men in this study were non-smokers. Smokers normally need two to three times the intake of vitamin C to maintain blood plasma levels comparable with those of non-smokers (Fraga *et al.*, 1996), and some studies include both smokers and non-smokers (Comhaire *et al.*, 2000).

In summary, we found that higher antioxidant intake over the normal dietary and supplement use range was associated with higher sperm numbers and higher motility in a sample of healthy non-smoking volunteers, and that antioxidant intake, to some extent, may attenuate the impact of age on sperm motility. At present, a large proportion of the US population ingests insufficient amounts of antioxidants, e.g. $>75\%$ of adult American men do not meet the 15 mg/day RDA for vitamin E (Institute of Medicine, 2000a), and only 17.7% of men ate at least the recommended five fruit and vegetable servings per day in 2002 [Centers for Disease Control and Prevention (CDC), 2002]. Our findings support the suggestion that a healthy diet with supplement use may be an inexpensive and safe way to improve semen quality and fertility.

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