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Dietary intake of antioxidant nutrients is associated with semen quality in young university students

Lidia Mínguez-Alarcón[1], Jaime Mendiola[1,*], José J. López-Espín[2], Laura Sarabia-Cos[1,3], Guillermo Vivero-Salmerón[1], Jesús Vioque[4,5], Eva M. Navarrete-Muñoz[4,5], and Alberto M. Torres-Cantero[1,5]

[1]Division of Preventive Medicine and Public Health, Department of Health and Social Sciences, University of Murcia School of Medicine, 30100 Espinardo (Murcia), Spain [2]Center of Operations Research, Miguel Hernández University, 03202 Elche, Spain [3]Reproductive Medicine Department, USP Dexeus Murcia, 30008 Murcia, Spain [4]Departamento de Salud Pública, Universidad Miguel Hernández, 03202 Elche-Alicante, Spain [5]Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP), 28029 Madrid, Spain

*Correspondence address. Tel: +34-868-88-7149; Fax: +34-868-88-3947; E-mail: jaime.mendiola@um.es

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STUDY QUESTION: What are the associations between the dietary intake of antioxidant nutrients and semen parameters in young men?

SUMMARY ANSWER: Our study suggests that some sperm parameters are sensitive to dietary intake of antioxidant nutrients.

WHAT IS KNOWN ALREADY: A few reports have suggested that some dietary factors might be related to semen quality. However, the relationship between the intake of antioxidant nutrients and semen quality in young men remains unexplored.

STUDY DESIGN, SIZE, DURATION: In this cross-sectional study, 215 young men were included between October 2010 and November 2011.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Healthy university students with complete dietary and semen quality data were analyzed. Dietary intake was recorded using a validated food frequency questionnaire. The associations between the energy-adjusted nutrient intake of antioxidants in quartiles and the semen volume, sperm concentration, sperm motility, sperm morphology, total sperm count and total motile sperm count were assessed using multivariate linear regression.

MAIN RESULTS AND THE ROLE OF CHANCE: Out of 240 students who contacted us, 223 (92.9%) were eligible to participate in this study, and 215 attended the clinical appointment. In the multivariate adjusted linear regression models, there was a positive association between dietary intakes of cryptoxanthin ($P_{trend} = 0.03$), vitamin C ($P_{trend} = 0.04$), lycopene ($P_{trend} = 0.03$) and β-carotene ($P_{trend} = 0.04$) and total motile sperm count. The semen volume increased with higher intakes of vitamin C ($P_{trend} = 0.04$).

LIMITATIONS, REASONS FOR CAUTION: Only one sample of semen was taken for each subject. However, there are indications that one semen sample may be sufficient to characterize the semen quality of the individuals in epidemiological studies. Bias due to measurement errors may also occur since there is no perfect method to assess diet. However, any bias due to measurement error would be non-differential and would reduce, not increase, the strength of the associations. Although selection bias in cross-sectional studies might not always be ruled out, our subjects were university student volunteers who were rewarded for their participation and the study was not advertised as a fertility study.

WIDER IMPLICATIONS OF THE FINDINGS: Previous articles in this area have focused mainly on men attending fertility clinics, thus our study brings generalizability to young men of the general population with unknown or untested fertility. Some of our results are in agreement with the previously reported papers.

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Key words: antioxidants / food frequency / sperm parameters / vitamins / semen quality

Introduction

Several reports have suggested a decline in semen quality in recent decades (Carlsen et al., 1992; Auger et al., 1995; Swan et al., 2000; Skakkebaek et al., 2006). A special concern has been raised about the low sperm concentration found in young men in some European countries (Jørgensen et al., 2002). Semen quality may be impaired by environmental exposures (Benoff et al., 2000; Rozati et al., 2002; Duty et al., 2003; Spanò et al., 2005; Swan, 2005; Carreño et al., 2007), lifestyle (Homan et al., 2007; Braga et al., 2012) or dietary factors. Regarding the latter, higher intakes of caffeine (Jensen et al., 2010), meat or milk products (Mendiola et al., 2009), saturated fats (Attaman et al., 2012), soy foods and soy isoflavones (Chavarro et al., 2008) have been associated with a decreased sperm quality.

However, diet may also have a positive contribution as antioxidant intake may have a positive effect on semen quality (Mendiola et al., 2010). It is known that spermatozoa are susceptible to oxidative damage because their plasma membranes are rich in polyunsaturated fatty acids and have low concentrations of scavenging enzymes (De Lamirande and Gagnon, 1995). Reactive oxygen species (ROS) levels are higher and levels of seminal plasma antioxidants are significantly lower in subfertile patients than in normal fertile control subjects (Kao et al., 2008; Abd-Elmoaty et al., 2010).

Although diet might be an important and modifiable source of antioxidant intake, most of the relevant information has come from clinical trials with large doses of antioxidant supplements (Ross et al., 2010). So far, only two observational studies have analyzed the dietary intake of specific antioxidant nutrients and semen quality: in 2005, a study on 97 non-smoking healthy men between 20 and 80 years old from a non-clinical setting (Eskenazi et al., 2005) and, in 2010, a study of men attending infertility clinics (Mendiola et al., 2010). Both studies support the hypothesis of a positive association between the dietary intake of antioxidant nutrients and semen quality.

In spite of the large interest and concern about the semen quality in young men, the relationship between the intake of antioxidant nutrients and semen quality in the young population remains unexplored. The objective of this study is to describe the relationship between the dietary intake of antioxidant nutrients and semen quality in healthy young university students.

Materials and methods

Participants

The Murcia Young Men's Study (MYMS) is a cross-sectional study of healthy young university students (18–23 years old) in the Murcia region (Spain). The MYMS was carried out between October 2010 and November 2011. Written informed consent was obtained from all subjects. The Research Ethics Committee of the University of Murcia approved this study.

Flyers stating, 'Young healthy male university students wanted for research project' were posted at university campuses to invite students to participate in this study. To be included in the MYMS, subjects had to be university students, born in Spain after 31 December 1987 and able to contact their mother and ask her to complete a questionnaire. There were 240 students who contacted us, 17 subjects had some exclusion criteria (had not been born in Spain: 5; had not been born after 31 December 1987: 9 and had not able to contact their mother: 3). Therefore, 223 students (92.9%) met eligibility criteria and were given an appointment to attend the study at the clinic. Finally, 215 (89.6%) agreed to participate in the study, but five men reporting an implausible calorie intake >5000 kilocalories (kcals) were excluded from further analysis. On the day of attendance, men underwent an andrological examination, provided a semen sample and completed questionnaires on lifestyle, food frequency, smoking exposure, psychological status and quality of life. Participants were rewarded for their participation (€50 gift card).

Physical examination

Body weight and height were measured using a digital scale (Tanita SC 330-S, London, UK). Body mass index (BMI) was calculated as weight in kilograms divided by squared height in meters. Testes sizes were measured using a Prader orchidometer. The presence of varicocele or other scrotal abnormalities was also evaluated. The presence of varicocele was classified as no varicocele, only detected during the Valsalva procedure, palpable or visible.

Dietary assessment

We used a semi-quantitative food frequency questionnaire (FFQ) to assess the usual daily intake of foods and nutrients (available at: http://bibliodieta.umh.es/files/2011/07/CFA101.pdf). The FFQ included 101 food items to capture the major sources of the most relevant nutrients, including specific carotenoids. This questionnaire was a modified version from a previous FFQ based on the Harvard questionnaire (Willet et al., 1985), which we adapted and validated for a general adult Spanish population. The validity and reproducibility of the FFQ was satisfactory when comparing the FFQ with four 1-week dietary records. The mean correlation coefficients for 1-year validity and reproducibility of nutrient intakes were 0.47 and 0.40, respectively (Vioque, 1995); this is a similar range to other established diet questionnaires (Willet, 1998). This FFQ also showed satisfactory biochemical validity when compared with plasma levels in an elderly population with high obesity prevalence (Vioque et al., 2007). Pearson correlations between energy-adjusted dietary intakes and plasma concentrations were 0.20 and 0.36 for carotenoids and vitamin C, respectively. Among those with a BMI $<25\ kg/m^2$, correlations were even greater for α- and β-carotene, lycopene, β-cryptoxanthin and vitamin C (0.41, 0.35, 0.23, 0.26 and 0.41, respectively).

Participants in the study were asked how often, on average, they had consumed each food item over the past year. Serving sizes were specified for each food item in the FFQ. The questionnaire had nine possible responses, ranging from 'never or less than once per month' to 'six or more per day'. Nutrient values were primarily obtained from the food composition tables of the US Department of Agriculture publications as well as other published sources for Spanish foods and portion sizes (Palma et al., 2008; U.S. Department of Agriculture, 2010). In order to obtain average daily nutrient intakes from diet for each individual, we multiplied the frequency of use for each food by the nutrient composition of the portion/serving size specified on the FFQ and added the results across all foods.

Nutrient intakes were adjusted for total energy intake by calculating the residuals from a linear regression with the log e of the nutrient modeled as the dependent variable and the log e of total energy intake as the independent variable (Willet, 1998).

Semen analysis

Men were asked to abstain from ejaculation for at least 48 h before sample collection. Nonetheless, subjects were not excluded if they had not abstained for that period of time ($n = 30$). Abstinence time was recorded as the time between current and previous ejaculation as reported by the study subject. Men collected semen samples by masturbation at the clinic.

Ejaculate volumes were estimated by specimen weight, assuming a semen density of 1.0 g/ml. Sperm concentration was evaluated by hemocytometer (Improved Neubauer; Hauser Scientific, Inc., Horsham, PA, USA). For the assessment of sperm concentration, samples were diluted in a solution of 0.6 m NaHCO3 and 0.4% (v/v) formaldehyde in distilled water. The hemocytometer chamber was loading with the dilution and the spermatozoa were allowed to settle in a humid chamber. From the same dilution, two chambers of the hemocytometer were assessed and at least 200 spermatozoa per replicate were counted. The two replicate counts were compared to see if they were acceptably close. If so, their averages were calculated and used in the analyses; if not, new dilutions were prepared. The spermatozoa were classified as either motile or immotile (World Health Organization, 2010) to report the percentage of motile spermatozoa (progressive and not progressive). Briefly, a 10 μl of well-mixed semen was placed on a clean glass slide that had been kept at 37°C and covered with a 22 × 22 mm coverslip. The preparation was placed on the heating stage of a microscope at 37°C and immediately examined at ×400 magnification. Total sperm count (volume × sperm concentration) and total motile sperm count (volume × sperm concentration × % motile sperm) were also calculated. Smears for morphology were made, air-dried, fixed, Papanicolaou stained and assessed using strict criteria (Menkveld *et al.*, 1990). The same specialized biologist carried out all the semen analyses (L.S.C.). An external quality control on semen samples throughout the study period was carried out in collaboration with the University of Copenhagen's Department of Growth and Reproduction.

Statistical analyses

Semen volume, sperm concentration, total sperm count, total motile sperm count and percentage of morphologically normal sperm showed non-normal distributions and were transformed using the natural log (ln) before analysis. Nutrient intakes were adjusted for total energy intake using the nutrient residual method (Willet, 1998) and further categorized in quartiles. Men with the lowest intake of each micronutrient were considered as the reference group. Linear regression was used to examine the association of each antioxidant with semen quality parameters. Tests for linear trend were performed using the median values of micronutrient intake in each category as a continuous variable and semen parameters as the response variable. The potential effect of BMI (kg/m^2), ejaculation abstinence time (hours), total calorie intake (kcal/day), alcohol intake (g/day), caffeine intake (mg/day), light-to-extreme exercise (hours/week), presence of varicocele (yes versus no), smoking (current smoker versus not current smoker), time to start of semen analysis (minutes) and season (winter versus spring, summer or fall) were assessed using lineal regression models. When inclusion of a potential covariate resulted in a change in the β-coefficient of <10%, the variable was not retained in final models. We used analysis of covariance (ANCOVA) to calculate adjusted semen parameters for each nutrient quartile by relevant covariates. Multivariate ANCOVA models were created with continuous semen parameters as dependent variables, and antioxidant categories and covariates as independent variables. We considered that an association was present when we found a statistically significant linear trend across quartiles, or a statistically significant difference in semen parameters between any of the quartiles. All tests were two-tailed and the level of statistical significance was set at 0.05. Statistical analyses were performed with the statistical package IBM SPSS 19.0 (IBM Corporation, Armonk, New York, USA).

Results

Our study population was Caucasian (99%), with a mean age of 19.2 years [standard deviation (SD): 5.5] and a BMI of 24.0 (SD: 3.4). The majority of men (98%) considered themselves to have a good or excellent general health. Almost 32% were smokers, and among those, 9% also reported the use of marijuana. Of the men, 55% reported alcohol consumption (liquor) and half of these took at least two drinks per week (one drink: 330 cc of liquor). The mean duration of ejaculation abstinence time was 79.3 h (SD: 37.4) and the mean time from semen collection to the start of semen analysis was 37 min (SD: 15.9). The mean sperm concentration was 52.1 × 10^6/ml (SD: 37.1) and the mean percentage of motile sperm was 56.5% (SD: 10.9). The mean value for morphologically normal sperm was 10.3% (SD: 6.3%). The testicular volume was 20.7 ml (SD: 3.6) for the left testicle and 22.0 ml (SD: 3.4) for the right. Of the young men, 15% presented varicocele in the left testis.

Table I presents the covariate mean values by the first and fourth quartiles of the adjusted dietary intake of antioxidants. These were assessed due to the influence of covariates, for example there was a significant positive relationship between the abstinence time and the total motile sperm count ($P < 0.05$), and a statistically significant negative association between the time to the start of semen analysis and the percentage of motile sperm ($P < 0.05$).

Table II presents the multivariate adjusted model of dietary intake of antioxidant nutrients and semen parameters. The semen volume was associated with vitamin C intake ($P_{trend} = 0.04$), being higher for Q2, Q3 and Q4 than for Q1 of intake. The median intake of vitamin C for the first quartile was 63 mg per day. Differences were also found in the semen volume and lycopene intakes in the Q2 and Q4 compared with the lowest quartile of intake. The semen volume was also higher in the Q3 than in Q1 of β-carotene intake. However, the *P* for trends was not statistically significant for lycopene or β-carotene and semen volume. Cryptoxanthin ($P_{trend} = 0.03$) and β-carotene ($P_{trend} = 0.04$) were associated with total motile sperm count. Lycopene and vitamin C were also associated with higher total motile sperm count ($P_{trend} = 0.03$ and 0.04, respectively), and significant differences were found between the lowest and highest quartiles for both nutrients. Other semen parameters did not show statistically significant differences with the dietary intake of antioxidant nutrients.

Discussion

Our study suggests a positive association between the dietary intake of several antioxidant nutrients (cryptoxanthin, vitamin C, lycopene and β-carotene) and the total motile sperm count in young healthy males. The semen volume also increased with higher intakes of vitamin C, lycopene and β-carotene.

The association between vitamin C and total progressively motile sperm was found by Eskenazi *et al.* in an older population (Eskenazi *et al.*, 2005), although vitamin C was not associated with semen volume. Vitamin C was also associated with being normozoospermic in a case–control study in a clinical setting, though specific semen parameters were not assessed (Mendiola *et al.*, 2010). Vitamin C is a water-soluble antioxidant for ROS found in the seminal plasma at higher concentrations than in the blood plasma (Agarwal and Sekhon, 2011). In an open-label supplementation trial, vitamin C improved sperm count, sperm motility and sperm morphology in oligozoospermic patients (Akmal *et al.*, 2006). The reference daily intake or recommended daily intake (RDI) for vitamin C is 60 mg per day, which is the median value of the first quartile in our study population.

Table I Covariate values by the first and fourth quartiles of adjusted dietary intake of antioxidant nutrients.

Range values for Q1 and Q4	BMI	Total caloric intake (kcal)	Smokers (%)	Alcohol intake (g/day)	Caffeine intake (mg/day)	Abstinence time (hours)	Time to perform analysis (minutes)
α-Carotene							
Q1 (3.8–118 μg/day)	24.5 (3.7)	2489 (994)	33	9.6 (11.8)	99.4 (90.4)	75.0 (28.9)	40.1 (12.2)
Q4 (404–1651 μg/day)	23.6 (3.2)	2774 (941)	26	7.6 (6.3)	126 (141)	82.5 (49.6)	39.4 (9.5)
β-Carotene							
Q1 (101–1492 μg/day)	24.2 (3.3)	2464 (787)	28	8.7 (10.8)	95.8 (99.5)	76.4 (22.0)	39.2 (11.2)
Q4 (4228–10 996 μg/day)	24.5 (3.8)	2647 (931)	29	7.4 (6.8)	137 (158)	79.7 (42.2)	38.0 (9.0)
Lutein+zeaxanthin							
Q1 (226–784 μg/day)	24.2 (3.3)	2379 (762)	37	9.8 (10.9)	110 (111)	72.5 (20.4)	37.5 (11.4)
Q4 (2481–10 229 μg/day)	24.7 (4.1)	2557 (1062)	34	7.8 (6.6)	130 (163)	78.3 (36.6)	40.4 (12.4)
Lycopene							
Q1 (1330–2446 μg/day)	24.1 (3.4)	2477 (766)	34	8.3 (7.8)	115 (113)	78.2 (30.5)	39.6 (10.4)
Q4 (5869–14 583 μg/day)	24.2 (3.4)	2383 (732)	32	9.4 (9.3)	124 (167)	75.2 (28.9)	34.3 (9.2)
Vitamin B6							
Q1 (1–1.9 mg/day)	23.5 (3.4)	2582 (778)	26	11.6 (12.7)	108 (123)	76.2 (29.6)	38.6 (12.2)
Q4 (2.9–5.2 mg/day)	24.4 (3.8)	2507 (973)	12	6.7 (6.8)	115 (131)	88.3 (51.3)	38.3 (9.2)
Vitamin B12							
Q1 (4.0–8.7 μg/day)	24.2 (3.4)	2506 (974)	41	11.8 (11.8)	114 (148)	74.4 (27.7)	38.1 (11.0)
Q4 (15.7–55.9 μg/day)	24.7 (3.9)	2560 (1007)	32	8.7 (8.7)	111 (89.6)	77.2 (41.7)	38.5 (10.5)
Vitamin C							
Q1 (7.8–76.6 mg/day)	23.8 (3.2)	2573 (1004)	35	10.9 (12.7)	104 (111)	74.3 (24.3)	37.9 (11.1)
Q4 (143–518 mg/day)	24.2 (4.1)	2512 (809)	37	8.5 (7.1)	139 (161)	75.9 (28.8)	38.3 (10.6)
Vitamin D							
Q1 (0.43–2.4 μg/day)	24.3 (3.7)	2586 (884)	39	11.3 (8.7)	108 (124)	80.6 (28.2)	37.3 (10.6)
Q4 (4.9–14.1 μg/day)	23.6 (2.9)	2615 (1045)	25	7.5 (6.4)	143 (154)	81.4 (44.7)	38.6 (11.2)
Vitamin E							
Q1 (4.4–8.4 mg/day)	24.0 (3.7)	2640 (1081)	34	10.6 (11.5)	120 (119)	78.1 (19.4)	37.5 (10.4)
Q4 (11.7–21.3 mg/day)	24.2 (3.8)	2697 (930)	22	6.4 (6.2)	110 (106)	85.3 (50.8)	38.2 (10.9)
Folate							
Q1 (97.8–241 μg/day)	23.9 (2.9)	2633 (1114)	37	11.4 (11.6)	107 (121)	72.9 (27.9)	38.5 (11.0)
Q4 (336–605 μg/day)	24.6 (3.8)	2573 (835)	26	9.2 (7.8)	140 (165)	87.5 (49.1)	38.7 (9.8)
Cryptoxanthin							
Q1 (2.7–157 μg/day)	23.7 (3.2)	2655 (1023)	30	9.8 (12.3)	102 (111)	73.6 (25.2)	38.8 (10.9)
Q4 (405–856 μg/day)	23.9 (3.5)	2380 (838)	34	9.3 (7.7)	135 (159)	74.5 (30.2)	38.4 (10.4)

Note: Continuous variables are shown as the mean and standard deviation unless otherwise indicated.

Our study raise doubts about whether the current RDI may underestimate vitamin C requirements needed with regard to semen quality.

For β-carotene, Eskenazi *et al*. also found that men with higher intake of β-carotene had better sperm concentrations and progressive sperm motility than men with low intake (Eskenazi *et al*., 2005). In that study lycopene and cryptoxantin were not analyzed. Mendiola *et al*. (2010) found that lycopene but not β-carotene was associated with good semen quality. No previous studies have reported an association between cryptoxanthin and total motile sperm count. However, a study published in 2008 suggested that cryptoxanthin plays a role repairing DNA oxidation damage, in addition to acting as an antioxidant in human cells (Lorenzo *et al*., 2009).

For other nutrients such as α-carotene, lutein + zeaxanthin, vitamin b6, vitamin b12, vitamin D, vitamin E and folate, we did not find an association with sperm parameters. Similarly, folate intake did not improve semen quality in 97 healthy non-smoking men (Eskenazi *et al*., 2005) although in a clinical setting, Mendiola *et al*. found higher intake of folate in normozoospermic controls (Mendiola *et al*., 2010). Conversely, vitamin E was not associated with good sperm quality in that case–control study (Mendiola *et al*., 2010) but

Table II Multivariate adjusted model of dietary intake of antioxidant nutrients and semen parameters.

Median for each quartile	Volume		Motile sperm		Morphologically normal sperm		Sperm concentration		Total sperm count		Total motile sperm count	
	(ml)	95% CI	%	95% CI	%	95% CI	(10^6/ml)	95% CI	(10^6)	95% CI	(10^6)	95% CI
α-Carotene												
Q1 (73.9 μg/day)	2.8	2.4–3.3	56.3	53.4–59.2	9.5	8.0–11.4	41.3	31.7–53.8	118	94.2–148	62.4	48.1–81.0
Q2 (178 μg/day)	3.4	2.1–2.9	57.5	54.7–60.2	7.7	6.5–9.2	35.3	27.3–45.7	90.8	72.5–113	46.2	35.9–59.6
Q3 (280 μg/day)	3.0	2.5–3.5	58.1	55.4–60.8	9.7	8.2–11.5	41.1	31.9–53.0	142	113–176	81.0	63.1–104
Q4 (795μg/day)	3.0	2.5–3.6	55.2	52.4–55.0	7.8	6.6–9.3	35.5	24.9–42.4	108	85.5–135	61.2	46.9–79.9
P_{trend}	0.26		0.33		0.33		0.26		0.94		0.73	
β-Carotene												
Q1 (1158 μg/day)	2.6	2.2–3.0	56.3	53.5–59.1	8.6	7.2–10.3	44.9	34.8–58.0	113	89.1–143	59.6	46.2–76.7
Q2 (1927 μg/day)	2.5	2.1–3.0	56.8	53.9–59.6	8.1	6.7–9.6	29.8	23.0–38.8	84.1	65.7–107	47.6	36.5–61.9
Q3 (3192 μg/day)	3.3	2.8–3.9*	58.0	55.3–60.7	9.6	8.1–11.3	36.8	28.6–47.4	119	94.0–150	66.5	51.7–85.5
Q4 (5286 μg/day)	2.9	2.4–3.4	56.2	53.3–59.0	8.4	7.0–10.0	39.2	30.2–50.8	129	100–164	76.1	58.4–99.1
P_{trend}	0.22		0.97		0.97		0.98		0.13		0.04*	
Lutein+zeaxanthin												
Q1 (618 μg/day)	2.9	2.4–3.4	56.7	53.9–59.5	7.8	6.5–9.3	38.9	30.1–50.1	112	88.1–141	59.2	45.9–76.3
Q2 (115 μg/day)	2.9	2.4–3.4	57.4	54.6–60.2	9.3	7.8–11.1	34.7	26.7–45.1	108	84.6–138	61.9	47.6–80.6
Q3 (1858 μg/day)	2.5	2.1–3.0	56.4	53.6–59.2	9.0	7.6–10.7	33.7	26.1–43.6	91.7	72.2–116	50.7	39.2–65.5
Q4 (3157 μg/day)	3.0	2.6–3.6	56.9	54.0–59.7	8.6	7.2–10.3	43.3	33.2–56.5	135	105–172	79.5	60.8–104
P_{trend}	0.70		0.94		0.94		0.45		0.28		0.15	
Lycopene												
Q1 (1780 μg/day)	2.4	2.0–2.8	57.0	54.2–59.8	8.7	7.3–10.3	39.6	30.6–51.2	111	88.1–138	48.6	37.6–62.6
Q2 (3199 μg/day)	3.2	2.8–3.8*	56.7	53.9–59.5	8.2	6.9–9.7	33.9	26.1–43.9	126	101–156	69.5	53.7–90.0
Q3 (4647 μg/day)	2.7	2.3–3.1	55.2	52.4–58.1	8.1	6.8–9.7	33.4	25.8–43.5	102	81.3–126	53.6	41.3–69.6
Q4 (7053 μg/day)	3.0	2.6–3.6*	58.3	55.5–61.2	9.7	8.2–11.6	43.5	33.5–56.4	143	114–179	79.9	61.7–103*
P_{trend}	0.15		0.57		0.57		0.52		0.10		0.03*	
Vitamin B6												
Q1 (1.8 mg/day)	2.8	2.4–3.4	56.6	53.9–59.4	8.9	7.5–10.6	44.2	34.1–57.2	118	92.5–149	65.9	50.9–85.3
Q2 (2.2 mg/day)	2.8	2.4–3.3	56.7	53.9–59.4	8.7	7.3–10.4	37.9	29.2–49.4	109	85.6–139	63.1	48.4–82.2
Q3 (2.5 mg/day)	2.9	2.5–3.5	59.0	56.3–61.8	8.5	7.1–10.1	33.1	25.6–42.9	108	84.7–137	62.8	48.3–81.5
Q4 (3.1 mg/day)	2.7	2.2–3.2	54.8	52.0–57.7	8.5	7.1–10.1	35.1	27.0–45.6	106	82.1–136	54.8	41.8–72.0
P_{trend}	0.72		0.48		0.48		0.20		0.56		0.34	
Vitamin B12												
Q1 (6.5 μg/day)	2.9	2.5–3.4	57.4	54.6–60.1	8.3	7.0–9.9	37.1	28.8–47.8	109	85.5–137	63.9	49.3–82.8
Q2 (9.9 μg/day)	2.7	2.3–3.2	58.1	55.3–60.9	8.2	6.9–9.8	47.1	36.4–61.0	132	103–167	75.6	58.3–98.0
Q3 (13.0 μg/day)	3.1	2.6–3.7	56.1	53.4–58.9	9.1	7.7–10.8	30.4	23.6–39.3	99.5	77.9–126	55.5	42.7–72.0
Q4 (21.8 μg/day)	2.5	2.1–3.0	55.7	52.8–58.6	9.0	7.5–10.7	36.9	28.4–47.8	103	80.8–132	53.4	41.0–69.5
P_{trend}	0.28		0.30		0.30		0.61		0.48		0.16	

Continued

Table II *Continued*

Median for each quartile	Volume		Motile sperm		Morphologically normal sperm		Sperm concentration		Total sperm count		Total motile sperm count	
	(ml)	95% CI	%	95% CI	%	95% CI	(10^6/ml)	95% CI	(10^6)	95% CI	(10^6)	95% CI
Vitamin C												
Q1 (62.6 mg/day)	2.3	1.9–2.7	56.9	54.0–59.8	8.4	7.0–10.0	41.9	32.3–54.5	95.1	72.9–118	49.2	37.8–63.8
Q2 (93.4 mg/day)	3.0	2.6–3.6*	56.9	54.1–59.7	8.9	7.5–10.6	40.0	31.0–51.7	120	94.4–152	66.7	51.6–86.1
Q3 (127 mg/day)	3.0	2.5–3.5*	57.5	54.7–60.3	8.7	7.3–10.4	27.2	21.2–35.1	100	78.6–128	56.2	43.3–73.0
Q4 (175 mg/day)	3.0	2.6–3.6*	56.1	53.4–58.9	8.7	7.3–10.3	42.9	33.4–55.3	130	102–165	77.4	59.9–100*
P_{trend}	0.04*		0.74		0.74		0.85		0.13		0.04*	
Vitamin D												
Q1 (1.8 μg/day)	2.6	2.2–3.0	56.3	53.5–59.1	8.8	7.4–10.5	40.8	31.4–52.9	103	81.1–131	59.4	45.6–77.2
Q2 (2.8 μg/day)	3.0	2.5–3.5	57.5	54.6–60.3	7.9	6.6–9.4	33.5	25.9–43.3	111	87.3–141	62.1	47.8–80.6
Q3 (4.2 μg/day)	2.8	2.4–3.4	57.9	55.1–60.7	9.5	8.0–11.3	38.2	29.4–49.7	126	98.2–161	72.6	55.6–94.7
Q4 (5.9 μg/day)	2.8	2.4–3.4	55.6	52.8–58.5	8.5	7.2–10.2	37.6	29.0–48.8	102	80.3–130	54.1	41.7–70.2
P_{trend}	0.59		0.70		0.70		0.93		0.97		0.72	
Vitamin E												
Q1 (7.3 mg/day)	2.7	2.2–3.2	56.4	53.6–59.2	8.6	7.2–10.2	36.4	27.9–47.4	89.6	70.1–114	49.9	38.5–64.9
Q2 (9.2 mg/day)	3.0	2.6–3.6	58.9	56.1–61.6	8.3	7.0–9.8	37.8	29.3–48.8	114	90.0–144	66.8	51.8–86.1
Q3 (10.6 mg/day)	2.8	2.3–3.3	57.6	54.8–60.3	10.0	8.4–11.9	41.7	32.0–54.3	124	97.5–158	71.7	55.2–93.1
Q4 (13.0 mg/day)	2.7	2.3–3.2	54.3	51.4–57.1	7.9	6.7–9.4	34.1	26.2–44.3	116	90.1–149	59.9	45.8–78.3
P_{trend}	0.93		0.20		0.20		0.77		0.15		0.36	
Folate												
Q1 (210 μg/day)	2.7	2.3–3.2	57.3	54.6–60.1	7.8	6.5–9.3	40.1	30.8–52.3	107	83.2–136	60.2	46.1–78.6
Q2 (264 μg/day)	2.6	2.2–3.1	59.1	56.4–61.7	8.9	7.5–10.6	41.1	31.8–53.0	112	88.4–142	65.0	50.2–84.0
Q3 (302 μg/day)	3.0	2.6–3.6	56.4	53.8–59.1	9.2	7.8–10.9	33.4	25.9–43.3	109	85.2–138	60.6	46.6–78.6
Q4 (382 μg/day)	2.8	2.4–3.4	55.0	52.2–57.7	8.6	7.2–10.2	35.5	27.4–46.1	113	88.3–145	60.0	46.2–79.5
P_{trend}	0.54		0.12		0.12		0.40		0.79		0.95	
Cryptoxanthin												
Q1 (105 μg/day)	2.5	2.1–2.9	56.1	53.2–58.9	8.5	7.1–10.1	41.8	32.0–54.4	102	80.4–131	53.8	41.3–70.0
Q2 (209 μg/day)	2.7	2.3–3.2	57.0	54.2–59.9	8.9	7.5–10.6	34.2	26.3–44.4	97.0	75.9–123	54.3	41.8–70.5
Q3 (318 μg/day)	3.0	2.6–3.6	56.0	53.3–58.8	7.8	6.6–9.3	34.5	26.8–44.6	115	90.5–146	63.8	49.4–82.4
Q4 (505 μg/day)	3.1	2.6–3.6	58.1	55.4–60.9	9.5	8.0–11.2	39.9	30.8–51.7	128	100–163	77.0	59.3–100
P_{trend}	0.06		0.34		0.36		0.98		0.12		0.03*	

Semen parameters are presented by the adjusted mean and 95% CI unless otherwise indicated. Tests for linear trend were performed using the median value for each quartile. Multivariate model adjusted for season, BMI, presence of varicocele, total calorie intake, light-to-extreme exercise, alcohol and caffeine intake, smoking, time-to-start analysis and abstinence time.
*Statistically significant.

it was associated with progressive sperm motility and total progressively motile sperm in healthy individuals (Eskenazi *et al.*, 2005). Supplementation with selenium and vitamin E in infertile men improved sperm quality and had protective effects especially on motility (Moslemi and Tavanbakhsh, 2011).

Some possible limitations of our study design should be discussed. Only one sample of semen was taken for each subject. However, there are indications that one semen sample may be sufficient to characterize the semen quality of the individuals in epidemiological studies (Carlsen *et al.*, 2005; Stokes-Riner *et al.*, 2007). Bias due to measurement errors may also occur since there is no perfect method to assess diet. However, the FFQ used in this study was previously validated in an adult population of the same area in Spain and it has been used in other populations (Guxens *et al.*, 2011). Any bias in assessing diet should not be differential which should reinforce our results. And finally, there might be selection bias as the subjects were university student volunteers. However, during the recruitment, the study was not advertised as a fertility study and participation was ensured because subjects were rewarded for participating. The proportion of individuals with andrological anomalies was within the expected range in this population.

In conclusion, our study suggests that some sperm parameters are sensitive to dietary intake of antioxidant nutrients, and that current recommendations of vitamin C intake may be insufficient to reach the optimum benefit in terms of semen quality.

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Authors' roles

A.M.T.C., J.M. and G.V.S. were involved in study conception. A.M.T.C. and J.V. were involved in study design. G.V.S., J.M. and L.S.C were involved in study execution and adquisition of data. L.M.A, J.J.L.E. and E.M.N.M. contributed to data analysis and interpretation. L.M.A, J.M., J.V. and A.M.T.C. drafted the manuscript. All authors provided substantial intellectual contributions and approved the final version of the manuscript.

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Conflict of interest

None declared.

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