



Open Access

ORIGINAL ARTICLE

Reproduction

Fatty acid intake in relation to reproductive hormones and testicular volume among young healthy men

Lidia Mínguez-Alarcón^{1,2,*}, Jorge E Chavarro^{3,4,5,*}, Jaime Mendiola², Manuela Roca⁶, Cigdem Tanrikut^{7,8}, Jesús Vioque^{9,10}, Niels Jørgensen¹¹, Alberto M Torres-Cantero^{2,12}

Emerging evidence suggests that dietary fats may influence testicular function. However, most of the published literature on this field has used semen quality parameters as the only proxy for testicular function. We examined the association of fat intake with circulating reproductive hormone levels and testicular volume among healthy young Spanish men. This is a cross-sectional study among 209 healthy male volunteers conducted between October 2010 and November 2011 in Murcia Region of Spain. Participants completed questionnaires on lifestyle, diet, and smoking, and each underwent a physical examination, and provided a blood sample. Linear regression was used to examine the association between each fatty acid type and reproductive hormone levels and testicular volumes. Monounsaturated fatty acids intake was inversely associated with serum blood levels of calculated free testosterone, total testosterone, and inhibin B. A positive association was observed between the intake of polyunsaturated fatty acids, particularly of omega-6 polyunsaturated fatty acids, and luteinizing hormone concentrations. In addition, the intake of *trans* fatty acids was associated with lower total testosterone and calculated free testosterone concentrations ($P_{\text{trend}} = 0.01$ and 0.02 , respectively). The intake of omega-3 polyunsaturated fatty acids was positively related to testicular volume while the intake of omega-6 polyunsaturated fatty acids and *trans* fatty acids was inversely related to testicular volume. These data suggest that fat intake, and particularly intake of omega 3, omega 6, and *trans* fatty acids, may influence testicular function.

Asian Journal of Andrology (2017) 19, 184–190; doi: 10.4103/1008-682X.190323; published online: 28 October 2016

Keywords: fat intake; reproductive health; reproductive hormones; *trans* fatty acids; young healthy men

INTRODUCTION

Two meta-analyses^{1,2} and several single-center studies^{3–7} have documented a downward trend in semen quality in Western countries. Some have also reported a concomitant downward trend in circulating testosterone levels among men.^{8–11} These downward trends have coincided with numerous changes in environmental and lifestyle factors that may directly contribute to diminished testicular function. Most notably, increased exposure to endocrine-disrupting chemicals,^{12–15} increased worldwide prevalence of obesity,^{16,17} and worsening diet quality in Western countries^{18–22} have been previously related to semen quality, suggesting that these may explain some of the downward trends in semen quality and testosterone levels.

Most of the published work about fat intake and markers of male testicular function have been focused on semen quality parameters,^{23–26} and only a few on reproductive hormones. In addition, studies examining the relation between fat consumption and male sex

hormones have focused on middle-aged and older men,^{27–30} and to our knowledge, there are no data on the association between dietary fats and reproductive hormones in young men. Moreover, the potential effect of dietary fat intake on testicular volume has not been explored so far. To address these gaps, we examined the relations between dietary fats with serum reproductive hormones and testicular volumes among young men in Spain.

MATERIALS AND METHODS

Study population

The Murcia Young Men's Study (MYMS) was a cross-sectional study conducted between October 2010 and November 2011 in the Murcia Region of Spain. Healthy young university students born in Spain, aged 18 to 23 years, were eligible to join the study. Recruitment flyers were posted at university campuses to invite students to participate in this study. A total of 240 students contacted the study staff, 17 of whom

¹Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA; ²Department of Health and Social Sciences, Division of Preventive Medicine and Public Health, University of Murcia School of Medicine, IMIB-Arrixaca, Espinardo (Murcia), Spain; ³Department of Nutrition, Harvard School of Public Health, Boston, Massachusetts, USA; ⁴Department of Epidemiology, Harvard School of Public Health, Boston, Massachusetts, USA; ⁵Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, Massachusetts, USA; ⁶Roca Fertility, Advanced Clinic Management SLU, Murcia, Spain; ⁷Department of Urology, Massachusetts General Hospital, Boston, Massachusetts, USA; ⁸Department of Surgery, Harvard Medical School, Boston, Massachusetts, USA; ⁹CIBER Epidemiology and Public Health (CIBERESP), Institute of Health III (ISCIII), Madrid, Spain; ¹⁰Department of Public Health, Miguel Hernández University, Elche-Alicante, Spain; ¹¹Department of Growth and Reproduction, University of Copenhagen, Rigshospitalet, Copenhagen, Denmark; ¹²Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Murcia, Spain.

*These authors contributed equally to this work.

Correspondence: Dr. L Mínguez-Alarcón (lminguez@hsph.harvard.edu)

Received: 26 February 2016; Revised: 27 May 2016; Accepted: 28 July 2016

were ineligible. Of the remaining 223 men, 215 completed a study visit and agreed to participate in the study. We further excluded 6 men who reported implausible total caloric intake leaving 209 men (97.2%) for the current analysis. With this sample size, we had 80% statistical power to detect differences of 35% standard deviations of the outcome measures between men in extreme quartiles of dietary factors. Men received a €50 gift card for their participation. During the study visit, men completed questionnaires on lifestyle, diet, and smoking, underwent a physical examination, and provided blood and semen samples. Written informed consent was obtained from all cases. The Research Ethics Committee of the University of Murcia approved this study.

Physical examination and testicular volume assessment

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. The presence of varicocele or other scrotal abnormalities was also evaluated, and classified as grade I (only detected during Valsalva maneuver), grade II (palpable), or grade III (visible). Testicular volume was measured using a Prader orchidometer (Andrology Australia, Clayton, Victoria, Australia). Mean testicular volume was calculated as the mean of right and left testicular volumes. All physical examinations were performed by the same investigator (Jaime Mendiola) to minimize variability in study procedures.

Semen quality assessment

Men were asked to abstain from ejaculation for at least 48 h before sample collection but were not excluded if they failed to follow this instruction ($n = 30$). 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; no lubricants were used. Ejaculate volume was estimated by specimen weight, assuming a semen density of 1.0 g ml^{-1} . Sperm concentration was evaluated by hemocytometer (Improved Neubauer; Hauser Scientific, Inc., Horsham, PA, USA). Spermatozoa were classified as either motile or immotile according to the World Health Organization (WHO) criteria,³¹ and the percentage of motile spermatozoa (progressive + nonprogressive) was calculated. Smears for morphology were prepared, air-dried, fixed, Papanicolaou stained, and assessed using strict criteria.³² Total sperm count (volume \times sperm concentration) was also calculated. The same specialist biologist carried out all the semen analyses. 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. To assess interlaboratory variation in sperm concentration analysis, five sets of duplicate semen samples (600 μl each) were sent by mail during the study period from the University of Copenhagen's Department of Growth and Reproduction to the Murcia Andrology Laboratory. The specimens were coded, undiluted fresh sperm samples from regular semen donors that were preserved by adding 10 μl of a 3 mol l^{-1} sodium azide solution per 1 μl of the ejaculate after liquefaction. No systematic differences in the results were identified. The mean interexaminer coefficient of variation was 4.0%, ranging between 1.7% and 7.1%.

Dietary assessment

We used a validated^{33,34} 101-food item semi-quantitative food frequency questionnaire (FFQ) to assess the usual intake of foods and nutrients (available at: <http://bibliodieta.umh.es/files/2011/07/CFA101.pdf>). Men were asked to report 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 offered nine options for frequency of consumption for each food, ranging from never or less than once a month to 6 or more times per day. Nutrient values for each food in the questionnaire were obtained from food composition tables of the US Department of Agriculture and supplemented with Spanish sources.^{35,36} *Trans* fatty acid intake was derived using published sources for Spanish foods.^{37,38} We calculated nutrient intakes (saturated fatty acids [SFA], monounsaturated fatty acids [MFA], polyunsaturated fatty acids [PUFA], omega-3 [n3], and omega-6 [n6]) by multiplying the frequency of use for each food by the nutrient composition of the portion size specified on the FFQ and by addition across all foods to obtain a total nutrient intake for each individual. Intake of energy-bearing nutrients was adjusted for total energy intake using the multivariate nutrient density method while non-energy-bearing nutrients were adjusted using the nutrient residual method.³⁹

The reproducibility and validity of this FFQ is comparable to other widely used FFQs.^{40–43} The average of correlation coefficients between nutrient intakes estimated using four one-week prospectively collected diet records and those estimated with the FFQ were 0.44 for validity and 0.44 for reproducibility.⁴⁴ This FFQ also showed satisfactory biochemical validity when compared to plasma levels of carotenoids and vitamin C.³⁴

Reproductive hormones measurement

Blood samples were drawn from participants' cubital veins in the afternoon and centrifuged; the serum was separated, stored, and frozen at -80°C . Serum samples were then shipped to Copenhagen, Denmark, on dry ice and stored at -20°C until hormone analysis was performed at Rigshospitalet. The methods have been described previously.⁴⁵ Briefly, hormone assessments were performed simultaneously to reduce intralaboratory variations. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone-binding globulin (SHBG) were determined using time-resolved immunofluorometric assays (DELFI; PerkinElmer, Skovlunde, Denmark). Intra- and inter-assay variations were $<5\%$ in each of the three assays. Serum total testosterone (TT) levels were determined using a time-resolved fluoroimmunoassay (DELFI; PerkinElmer) with intra- and inter-assay variation of $<8\%$. Estradiol (E2) was measured by radioimmunoassay (Pantex, Santa Monica, CA, USA) with an intraassay variation of $<8\%$ and an interassay variation of $<13\%$. Inhibin B levels were determined by a specific two-sided enzyme immunometric assay (Oxford Bio-Innovation Ltd., Bicester, UK) with intra- and inter-assay variation of 13% and 18%, respectively. When inhibin B was above approximately 100 pg ml^{-1} , the intraassay variation was $<7\%$ and the interassay variation was $<6\%$. The majority of the men had levels above 100 pg ml^{-1} . Calculated free testosterone (cFT) was determined using the equation of Vermeulen and colleagues assuming a fixed albumin of 43.8 g l^{-1} .⁴⁶

Statistical analyses

Because the reproductive hormones, fat intakes, and the men's characteristics had a skewed distribution, the median and 5th and 95th percentiles were used to describe those variables. FSH and E2 concentrations were transformed using the natural log (ln) before analysis because they showed nonnormal distributions. Left and right testicular volumes were averaged, and the mean volume was used for analysis. Men were divided into quartiles of intake for total and major subtypes of dietary fat. Men with the lowest intake of each fat category

were considered as the reference group. To test for associations across quartiles of intake, Kruskal-Wallis tests were used for continuous variables, and χ^2 -tests for categorical variables. Linear regression was used to examine the association of each fatty acid category with testicular volume and reproductive hormone levels. Tests for linear trend were performed using the median values of fat categories in each quartile as a continuous variable and reproductive hormones as the response variable. We used analysis of covariance (ANCOVA) to calculate adjusted testicular volume and reproductive hormone levels for each quartile by relevant covariates. Regression coefficients for outcomes that were log-transformed for analysis (FSH and E2 serum levels) were exponentiated ("back-transformed") to allow the presentation of adjusted means in the scale variables that were originally measured. In addition, the presence of nonlinear associations was tested modeling fat intake as a continuous variable and modeling them as a linear and quadratic term. We considered that an association was present when we found a linear trend across quartiles <0.05 .

Confounding was assessed using a hybrid method that combines previous knowledge using directed acyclic graphs (DAGs)⁴⁷ and a statistical method on change in point estimate. The variables considered as potential confounders included factors previously related to semen quality or reproductive hormones in this or other studies, and factors associated with fat intake and reproductive outcomes in this study, regardless of whether they had been previously described as predictors of male reproductive health. Using this method, final models included terms for BMI (kg m^{-2}), smoking (current smoker versus not current smoker), time to blood sampling (min), mean daily alcohol intake (g day^{-1}), and mean daily caffeine intake (mg day^{-1}). Mean daily intakes of micronutrients previously related to semen quality in this population (vitamin C, β -cryptoxanthin, lycopene, and β -carotene)²⁰ were forced into the model. In order to adjust for total energy intake, terms for total calorie intake, intake of protein, and the remaining types of fat were included in the model,³⁹ regardless of statistical significance, to allow the interpretation of the regression parameter of interest as the isocaloric substitution of a specific type of fat for the same amount of energy from carbohydrates. Statistical analyses were performed with the IBM Statistical Package for the Social Sciences 19.0 (IBM Corporation, Armonk, NY, USA).

RESULTS

Almost all the participants were Caucasian (98%) with a median (interquartile range [IQR]) age of 20.4 (19.6, 21.4) years. Sixty-eight (33%) of the men were smokers and the median (IQR) BMI was 23.7 (21.8, 25.5). Four (1.9%) men had a history of cryptorchidism and 32 (15.3%) men had a left varicocele diagnosed during the examination. Men in the highest quartile of total fat intake had lower intake of carbohydrates, vitamin C, cryptoxanthin, and alcohol, compared with men in the lowest quartile (Table 1).

Table 2 shows the multivariate-adjusted associations between fatty acid intake and reproductive hormones. In these models, MFA intake was inversely related with blood levels of cFT, TT, and inhibin B ($P_{\text{trend}} < 0.05$). There was a positive relation between intakes of PUFAs, particularly of omega-6, and LH concentrations. In addition, intake of *trans* fatty acids was associated with lower cFT and TT concentrations ($P_{\text{trends}} = 0.01$ and 0.02 , respectively). Relative to men in the lowest quartile of *trans* fatty acid intake, the adjusted difference (95% Confidence Interval [CI]) of TT blood levels for men in the 2nd, 3rd, and 4th quartiles were -0.3 (-3.0 – 2.5), -0.4 (-3.2 – 2.4), and -3.4 (-6.4 – -0.4) nmol l^{-1} , respectively.

We then evaluated the relation between fatty acid intake and testicular volume. Intake of omega-3 PUFAs was positively related to testicular volume while intakes of omega-6 PUFAs and *trans* fatty acids were inversely related to testicular volume (Figure 1). The adjusted median (95% CI) total testicular volumes (ml) for men in increasing quartiles of fatty acid intake were 20.7 (19.7, 21.7), 21.0 (20.1, 22.0), 20.9 (20.0, 21.9), and 22.9 (21.9, 23.9) for omega-3 fatty acids; 22.2 (21.1, 23.3), 21.9 (21.0, 22.8), 21.5 (20.6, 22.4), and 20.0 (18.9, 21.0) for omega-6 fatty acids; and 21.3 (20.3, 22.3), 22.0 (21.1, 22.9), 21.9 (20.9, 22.8), and 20.4 (19.5, 21.4) for *trans* fatty acids. Intake of other fatty acids was not related to testicular volume (data not shown). Further adjustment by physical activity did not modify the associations (data not shown).

DISCUSSION

We examined the cross-sectional associations of fat intake with reproductive hormone levels and testicular volume in a population of young healthy Spanish men. We found inverse associations of monounsaturated fat intake with cFT and TT and inhibin B concentrations. Intake of omega-6 PUFAs was positively associated with LH concentrations and inversely related to testicular volume. Similarly, intake of *trans* fatty acids was inversely related to cFT and TT levels and testicular volume. In addition, we found a positive association between intake of omega-3 PUFAs and testicular volume. The findings for *trans* fatty acids are of particular interest given our previous report of an association between *trans* fat intake and lower sperm count in these men²⁶ and previous experimental work in rodents showing a deleterious effect of *trans* fats on testicular function^{48–51} supporting a causal interpretation of this association.

We found inverse associations of MFA intake with three reproductive hormones (inhibin B, cFT, and TT concentrations). In this population, monounsaturated fats are primarily consumed from animal products (27%: from red and processed meats and dairy products) and from olive oil (20%). Jensen and colleagues found a borderline and inverse association between monounsaturated fatty acid intake and morphologically normal sperm among Danish men from general population.²⁵ Jensen's finding could be interpreted as consistent with our results, suggesting that monounsaturated fat intake may impair male reproductive health. However, we have previously reported no association between monounsaturated fat intake and semen quality in this population²⁶ and others have found no relation between monounsaturated fats and reproductive hormones in older men.^{27,28}

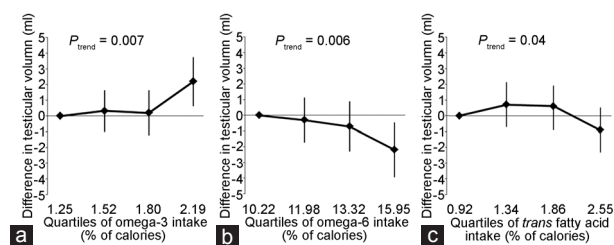


Figure 1: Difference in testicular volume by increasing quartiles of some nutrient intakes. (a) Omega-3. (b) Omega-6. (c) *Trans* fatty acids. Values represent the difference and 95% confidence interval in the mean (right and left) of testicular volume for men in the 2nd, 3rd, and 4th quartile of intakes when compared to men in the first quartile of intake. Models are adjusted for BMI (kg m^{-2}), smoking (current smoker vs not current smoker), time of blood sampling (min), and intakes of caffeine (mg day^{-1}), alcohol (g day^{-1}), calorie (kcal day^{-1}), other fat sources (% of calories), protein (% of calories), β -carotene (mg day^{-1}), vitamin C (mg day^{-1}), cryptoxanthin (mg day^{-1}), and lycopene (mg day^{-1}). BMI: body mass index.

Table 1: Demographic characteristics of men in the Murcia Young Men's Study among total fat

	Mean (IQR)			P ^a
	Total cohort (n=209)	Q1 (n=54)	Q4 (n=49)	
Age (year)	20.4 (19.6–21.4)	20.8 (19.6–21.4)	20.4 (19.5–21.9)	0.35
Caucasian, n (%)	204 (97.6)	53 (98.1)	48 (98.0)	0.82
BMI (kg m ⁻²)	23.7 (21.8–25.5)	24.1 (22.2–25.8)	23.4 (21.8–25.5)	0.25
Current smoker, n (%)	68 (32.5)	24 (44.4)	14 (28.6)	0.13
Abstinence time (h)	71.0 (59.5–92.0)	78.5 (65.0–92.3)	71.0 (56.0–93.5)	0.22
Varicocele, n (%)	32 (15.3)	5 (9.3)	10 (20.4)	0.93
History of cryptorchidism, n (%)	4 (1.9)	0	1 (2.0)	0.36
Semen volume (ml)	3.0 (2.0–4.0)	3.2 (2.5–4.1)	3.2 (2.3–4.4)	0.40
Sperm concentration (mill ml ⁻¹)	42.9 (21.9–72.2)	50.4 (18.9–71.5)	37.0 (19.3–73.0)	0.50
Total sperm count (mill)	121.5 (65.4–212.7)	134.3 (73.0–210.6)	130.3 (75.2–242.3)	0.80
Sperm motility (PR + NPR) (%)	57.2 (50.7–63.8)	54.5 (48.7–60.8)	58.0 (50.5–67.5)	0.17
Morphologically normal sperm (%)	8.9 (6.0–13.9)	10.0 (6.0–14.0)	8.9 (5.5–13.9)	0.81
Mean testicular volume (ml)	21.0 (19.5–24.0)	22.0 (20.0–24.5)	21.0 (19.0–23.0)	0.08
LH (IU l ⁻¹)	4.0 (2.9–5.2)	4.0 (2.6–5.1)	4.4 (3.2–5.9)	<0.01
FSH (IU l ⁻¹)	2.2 (1.6–3.4)	2.2 (1.5–3.0)	2.3 (1.5–3.7)	0.92
E2 (pmol l ⁻¹)	75.9 (62.8–90.9)	75.2 (60.9–89.1)	81.5 (82.3–100)	0.29
Calculated free testosterone (ng dl ⁻¹)	13.5 (10.8–17.0)	14.2 (11.6–18.7)	12.7 (9.8–16.6)	0.21
Total testosterone (nmol l ⁻¹)	21.2 (17.1–26.6)	22.4 (17.0–27.4)	20.1 (16.0–26.2)	0.67
Inhibin B (pg ml ⁻¹)	193 (147–246)	203 (146–260)	172 (137–223)	0.23
SHBG (nmol l ⁻¹)	30.0 (22.5–38.5)	30.0 (21.0–38.3)	32.0 (22.0–41.1)	0.52
Calories intake (kcal day ⁻¹)	2278.0 (1896.1–2828.6)	2443.6 (2107.3–2946.3)	2263.5 (1899.4–2904.4)	0.27
Carbohydrates intake (g day ⁻¹)	230.2 (202.0–252.9)	267.7 (250.7–297.3)	191.2 (170.4–203.2)	<0.01
Protein intake (g day ⁻¹)	104.9 (94.1–115.4)	99.0 (87.6–113.6)	108.3 (98.8–115.1)	0.17
Vitamin C intake (mg day ⁻¹)	113.1 (77.1–142.3)	129.1 (85.2–161.3)	82.2 (67.1–114.1)	<0.01
Lycopene intake (µg day ⁻¹)	3938.7 (2425.9–5893.4)	3466.9 (2359.7–5528.5)	3483.8 (2176.8–5133.9)	0.39
B-carotene intake (µg day ⁻¹)	2465.3 (1488.1–4213.3)	228.4 (1357.5–3992.2)	2108.9 (1520.5–3174.6)	0.36
Cryptoxantin intake (µg day ⁻¹)	276.8 (160.1–414.9)	342.8 (187.0–519.1)	163.9 (110.9–267.0)	<0.01
Alcohol intake (g day ⁻¹)	6.8 (3.3–13.7)	9.5 (3.2–20.4)	4.7 (1.9–10.6)	0.09
Caffeine intake (mg day ⁻¹)	77.0 (24.6–159.6)	83.4 (37.0–173.1)	44.0 (24.6–163.7)	0.17
Physical activity (h week ⁻¹)	9.0 (6.0–13.0)	9.0 (6.0–14.0)	7.0 (4.0–13.5)	0.32

^aKruskal–Wallis test for continuous variables and the Chi-square test for categorical variables. IQR: interquartile range; BMI: body mass index; PR: progressive receptor; NPR: nonprogressive receptor; LH: luteinizing hormone; FSH: follicle-stimulating hormone; E2: estradiol; SHBG: sex hormone-binding globulin; Q1: 1st quartile; Q4: 4th quartile

Nevertheless, intake of red and processed meats and of dairy foods has also been previously related to poor semen quality,^{18,52,53} suggesting that the observed relation in this study could reflect the relation with these foods rather than with monounsaturated fats *per se*. Given the paucity of data on the relation between fat intake with reproductive hormone concentrations and testicular volume in men, additional work is necessary to evaluate these relations further.

We observed a positive association between intake of omega-6 PUFAs and LH levels coupled with an inverse relation between intake of these fats and testicular volume. Elevated LH levels are characteristic of primary Leydig cell failure. While none of the men in the study had abnormally high LH levels and all had a testicular volume within the normal range, the co-occurrence of higher LH levels and lower testicular volume among men with the highest intake of omega-6 PUFAs may suggest that these fats could negatively influence testicular function acting directly on the testis. As we did not observe a relation to testosterone, it might indicate a situation with a compensated state (i.e., unchanged testosterone on a background of increased stimulation with LH). We did not observe a relation between intake of omega-6 PUFAs with FSH (expected in the case of spermatogenic dysfunction) raising the possibility that the observed relation to testis volume could be due to chance. The literature on the relation between these fats and markers of testicular function is sparse. PUFA intake was

unrelated to sex hormones among 696 British men aged 70 years and older⁵⁴ and also in middle-aged US men.²⁷ We also found that intake of omega-3 PUFAs was not associated with reproductive hormones, but it was positively related with testicular volume. Although there is little consistency in the relation between omega-3 PUFA intake and reproductive hormones among men,^{27–30} it seems that omega-3 consumption may have a positive effect on semen quality parameters, in agreement with our finding regarding testicular volume. Afeiche and colleagues have recently found that fish intake, an important source of omega-3 fatty acids, was positively associated with sperm counts and morphologically normal sperm among men attending a fertility center, and that relation appeared to be mediated through intake of long-chain omega-3 fats.²¹ In agreement with these results, omega-3 fatty acid supplementation increased the total percentage of sperm with normal morphology among oligoasthenoteratospermic Iranian men,²³ and Jensen *et al.*²⁵ found a positive and borderline association between omega-3 fat intake and semen volume among the general population ($P_{\text{trend}} = 0.05$). Moreover, experimental models also showed a positive effect of omega-3 PUFAs on male reproductive health.^{55,56}

We are not aware of previous reports between the relation of *trans* fat intake and testosterone levels and testicular volume. Nevertheless, our findings are in agreement with our previous report on the relation between intake of these fats and total sperm count

Table 2: Multivariate adjusted^a associations of fat intake with reproductive hormones (n=209)

Median for each quartile of intake	LH (IU l ⁻¹)		FSH (IU l ⁻¹)		E2 (pmol l ⁻¹)		Calculated free testosterone (ng dl ⁻¹)		Total testosterone (nmol l ⁻¹)		Inhibin B (pg ml ⁻¹)		SHBG (nmol l ⁻¹)	
	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI	Mean	95% CI
Saturated														
Q1 (9.44% of calories)	3.8	3.3, 4.3	2.4	2.0, 2.8	70.4	64.8, 76.5	13.4	12.0, 14.8	20.7	18.8, 22.6	199	176, 221	31.5	28.3, 34.6
Q2 (11.31% of calories)	4.5	4.0, 5.0	2.3	1.9, 2.6	78.8	73.0, 85.1	14.9	13.6, 16.2	22.4	20.6, 24.2	206	185, 226	31.3	28.4, 34.2
Q3 (12.63% of calories)	4.3	3.8, 4.7	2.6	2.2, 3.0	77.7	72.0, 83.9	14.0	12.7, 15.3	22.2	20.4, 24.0	189	168, 210	33.2	30.3, 36.1
Q4 (15.53% of calories)	4.4	3.8, 4.9	2.0	1.7, 2.4	76.2	69.6, 83.5	14.8	13.2, 16.3	21.8	19.6, 23.9	219	194, 244	29.3	25.8, 32.7
<i>P</i> _{trend}	0.30		0.34		0.33		0.35		0.58		0.42		0.53	
Monounsaturated														
Q1 (13.86% of calories)	4.4	3.9, 5.0	2.26	1.9, 2.7	77.8	70.8, 85.5	15.4	13.8, 16.9	23.6	21.4, 25.8	228	203, 254	32.9	29.4, 36.4
Q2 (15.74% of calories)	4.1	3.7, 4.6	2.21	1.9, 2.6	77.5	71.5, 83.9	14.8	13.5, 16.1	22.5	20.6, 24.3	211	189, 232	31.1	28.1, 34.0
Q3 (17.65% of calories)	3.8	3.3, 4.3	2.27	1.9, 2.7	77.1	70.7, 83.7	15.0	13.6, 16.3	21.5	19.5, 23.4	182	160, 205 ^b	27.7	24.6, 30.7 ^b
Q4 (20.43% of calories)	4.6	4.0, 5.1	2.50	2.1, 3.0	70.4	64.2, 77.2	11.7	10.1, 13.2 ^b	19.3	17.1, 21.5 ^b	188	163, 213	34.0	30.5, 37.4
<i>P</i> _{trend}	0.80		0.46		0.17		0.003		0.01		0.04		0.76	
Polyunsaturated														
Q1 (4.84% of calories)	3.8	3.2, 4.3	2.31	1.9, 2.8	72.2	65.6, 78.9	13.2	11.6, 14.7	20.7	18.5, 22.9	202	177, 228	32.9	29.4, 36.4
Q2 (5.64% of calories)	4.1	3.7, 4.6	2.18	1.9, 2.5	75.2	69.6, 91.2	13.9	12.6, 15.2	21.0	19.2, 22.8	205	184, 226	30.0	27.0, 32.9
Q3 (6.31% of calories)	4.3	3.8, 4.8	2.26	1.9, 2.6	76.9	70.9, 83.4	15.4	14.0, 16.7	23.3	21.4, 25.2	204	182, 226	31.2	28.1, 34.3
Q4 (7.62% of calories)	4.7	4.2, 5.3 ^b	2.52	2.1, 3.0	79.2	72.3, 86.8	14.5	13.0, 16.0	22.2	20.0, 24.3	199	175, 224	31.5	28.0, 34.9
<i>P</i> _{trend}	0.03		0.42		0.19		0.26		0.32		0.84		0.83	
Omega-3														
Q1 (0.51% of calories)	4.5	4.0, 5.0	2.43	2.1, 2.9	73.2	67.2, 79.7	14.2	12.8, 15.6	22.1	20.1, 24.1	205	183, 228	32.3	29.1, 35.5
Q2 (0.62% of calories)	4.4	3.9, 4.9	2.20	1.9, 2.6	77.6	71.5, 84.1	14.1	12.7, 15.4	21.0	19.1, 22.9	176	155, 198	28.8	25.8, 31.8
Q3 (0.74% of calories)	4.2	3.7, 4.6	2.53	2.2, 3.0	76.4	70.4, 82.9	14.1	12.8, 15.5	22.1	20.2, 24.0	196	175, 218	32.5	29.4, 35.6
Q4 (0.91% of calories)	3.8	3.3, 4.3	2.08	1.7, 2.5	75.9	69.4, 83.0	14.5	13.0, 16.0	21.9	19.8, 24.0	233	210, 257	31.8	28.4, 35.2
<i>P</i> _{trend}	0.07		0.37		0.68		0.76		0.97		0.06		0.84	
Omega-6														
Q1 (4.08% of calories)	3.6	3.1, 4.2	2.29	1.9, 2.8	71.9	65.3, 79.2	13.2	11.6, 14.8	20.6	18.3, 22.8	217	191, 243	31.8	28.2, 35.5
Q2 (4.87% of calories)	4.2	3.7, 4.6	2.23	1.9, 2.6	75.5	69.9, 81.6	13.9	12.6, 15.2	21.1	19.3, 22.9	194	174, 215	30.8	27.9, 33.8
Q3 (5.42% of calories)	4.3	3.8, 4.8	2.21	1.9, 2.6	76.5	70.5, 83.0	15.2	13.8, 16.6	23.0	21.1, 24.9	208	186, 230	31.1	28.0, 34.2
Q4 (6.73% of calories)	4.9	4.3, 5.4 ^b	2.50	2.1, 3.0	79.2	72.3, 86.8	14.6	13.1, 16.1	22.4	20.3, 24.6	191	167, 216	31.8	28.3, 35.3
<i>P</i> _{trend}	0.006		0.45		0.22		0.29		0.26		0.32		0.90	
Trans														
Q1 (0.37% of calories)	4.1	3.6, 4.6	2.27	1.9, 2.7	76.4	70.2, 83.3	15.5	14.0, 16.9	22.8	20.8, 24.8	206	183, 229	30.0	26.8, 33.2
Q2 (0.54% of calories)	4.4	3.9, 4.9	2.35	2.0, 2.8	80.3	74.1, 87.2	14.6	13.2, 16.0	22.6	20.7, 24.4	201	179, 223	32.9	29.8, 36.0
Q3 (0.74% of calories)	4.5	4.1, 5.0	2.28	2.0, 2.7	78.2	72.3, 84.5	14.1	12.8, 15.4	22.4	20.6, 24.2	216	195, 237	33.3	30.3, 36.2
Q4 (1.03% of calories)	4.9	3.5, 4.4	2.32	2.0, 2.7	68.7	63.2, 74.6	12.8	11.4, 14.2 ^b	19.4	17.4, 21.3 ^b	187	164, 209	29.4	26.3, 32.6
<i>P</i> _{trend}	0.56		0.94		0.05		0.01		0.02		0.34		0.61	

^aAdjusted for BMI (kg m⁻²), smoking (current smoker vs not current smoker), time of blood sampling (min), and intakes of caffeine (mg day⁻¹), alcohol (g day⁻¹), calorie (kcal day⁻¹), other fat sources (percentage of calories), protein (percentage of calories), β-carotene (mg day⁻¹), Vitamin C (mg day⁻¹), cryptoxanthine (mg day⁻¹), and lycopene (mg day⁻¹); ^bDifferent to mean in the lowest quartile of intake at *P*<0.05. LH: luteinizing hormone; FSH: follicle-stimulating hormone; E2: estradiol; SHBG: sex hormone-binding globulin; CI: confidence interval; BMI: body mass index; Q1: 1st quartile; Q2: 2nd quartile; 3rd quartile; Q4: 4th quartile

in this study population.²⁶ Specifically, we had previously reported that men in the highest quartile of *trans* fat intake had 37% lower total sperm count;²⁶ these same men were also found to have 15% lower T levels and 4% lower testicular volume than men in the lowest quartile of intake. These findings are also in agreement with a study among fertility patients where sperm *trans* fat levels, a biomarker of intake, were inversely related to sperm concentration.¹⁹ Moreover, rodent models of *trans* fat supplementation provide strong evidence supporting a causal interpretation of these relations. Supplementing the diet of male mice with *trans* fatty acids results in reproductive impairment including the accumulation of *trans* fats in the testis,^{48,49} decreased serum testosterone concentrations and sperm counts, and at the highest levels of supplementation, arrest of spermatogenesis and testicular degeneration.^{48,50,51} Findings from these rodent models closely parallel our findings in humans. However, given the scarcity of data

in humans, it is important that these relations are further evaluated in different populations.

We cannot discount the limitations of our study. Reverse causation is possible due to the cross-sectional study. However, we selected young men with untested fertility and we did not communicate the results of the semen analyses or reproductive hormones to them, reducing the possibility of this bias. Second, as is the case in all observational epidemiologic studies, we cannot exclude the possibility of unmeasured confounding. Nevertheless, we adjusted for a large number of known and suspected confounders. Adjustment for these factors had a minimal impact on the results, suggesting that any unmeasured confounding is unlikely to have a major impact on the results. Based on the number of tested associations, we cannot completely rule out the possibility that observed associations may result from chance. Third, misclassification of dietary intakes is possible as well. However, we used a previously



validated FFQ.^{33,34,57} Fourth, associations with testicular volume are more difficult to evaluate since little is known about how much this outcome could be influenced by environmental factors, such as diet, among adult men. Whether and to what extent testicular volume might change in response to modifiable environmental factors remains an open question. Interpretation of these findings since we used an orchidometer to assess testicular volume which limited the precision of the measurements. Fifth, only one serum sample was obtained for each man opening the possibility of error due to within-person variability in reproductive hormones. Nevertheless, previous work has shown that a single sample can be used to classify men's reproductive hormone function.⁵⁸ Sixth, the intra- and inter-assay variation for assessing inhibin B levels could be considered as high (13%–18%); however, the majority of the men had inhibin B levels above 100 pg ml⁻¹, which is translated to a lower intra- and inter-assay variation (<7%).

In summary, we found that omega-3 fatty acids may be positively associated with testicular function as indicated by testicular volume whereas intake of omega-6 fatty acids and *trans* fatty acids appear to be negatively related to it. Some of these findings, most notably those for omega-3 and *trans* fats, are in agreement with animal experimental data as well as preclinical and clinical data. However, because data in humans on this topic are scarce, further work is necessary to clarify the nature of these relations as well as their clinical relevance.

AUTHOR CONTRIBUTIONS

LMA and JEC carried out the nutrition studies and drafted the manuscript. NJ and JM carried out the hormone analysis. JEC, LMA, CT, JV, and NJ participated in the design of the study and performed the statistical analysis. AMTC, NJ, JM, and MR conceived of the study, and participated in its design and coordination and helped draft the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declared no competing interests.

ACKNOWLEDGMENTS

The authors gratefully acknowledge C. Ruiz, E. Belmonte, F. Mas, and all the Quirón Dexeus Murcia clinic staff for their assistance in data collection; and the young men of the study for their participation. We would also like to thank L. Sarabia and G. Vivero for semen analyses, K. Ruiz and E. Estrella for database management, and E.M. Navarrete-Muñoz for dietary assessment. This work was supported by The Seneca Foundation, Regional Agency of Science and Technology, grant n° 08808/PI/08, Ministerio de Ciencia e Innovación, Instituto de Salud Carlos III (FIS), grant n° PI10/00985, and grant P30 DK46200 from the National Institutes of Health.

REFERENCES

- Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. *BMJ* 1992; 305: 609–13.
- Swan SH, Elkin EP, Fenster L. The question of declining sperm density revisited: an analysis of 101 studies published 1934–1996. *Environ Health Perspect* 2000; 108: 961–6.
- Auger J, Kunstmann JM, Czyglik F, Jouannet P. Decline in semen quality among fertile men in Paris during the past 20 years. *N Engl J Med* 1995; 332: 281–5.
- Swan SH, Brazil C, Drobnis EZ, Liu F, Kruse RL, *et al*. Geographic differences in semen quality of fertile U.S. males. *Environ Health Perspect* 2003; 111: 414–20.
- Mendiola J, Jorgensen N, Mínguez-Alarcón L, Sarabia-Cos L, Lopez-Espin JJ, *et al*. Sperm counts may have declined in young university students in Southern Spain. *Andrology* 2013; 1: 408–13.
- Rolland M, Le Moal J, Wagner V, Royere D, De Mouzon J. Decline in semen concentration and morphology in a sample of 26,609 men close to general population between 1989 and 2005 in France. *Hum Reprod* 2013; 28: 462–70.
- Jorgensen N, Vierula M, Jacobsen R, Pukkala E, Perheentupa A, *et al*. Recent adverse trends in semen quality and testis cancer incidence among Finnish men. *Int J Androl* 2011; 34: e37–48.
- Andersson AM, Jensen TK, Juul A, Petersen JH, Jorgensen T, *et al*. Secular decline in male testosterone and sex hormone binding globulin serum levels in Danish population surveys. *J Clin Endocrinol Metab* 2007; 92: 4696–705.
- Travison TG, Araujo AB, Hall SA, McKinlay JB. Temporal trends in testosterone levels and treatment in older men. *Curr Opin Endocrinol Diabetes Obes* 2009; 16: 211–7.
- Nyante SJ, Graubard BI, Li Y, McQuillan GM, Platz EA, *et al*. Trends in sex hormone concentrations in US males: 1988–1991 to 1999–2004. *Int J Androl* 2012; 35: 456–66.
- Perheentupa A, Makinen J, Laatikainen T, Vierula M, Skakkebaek NE, *et al*. A cohort effect on serum testosterone levels in Finnish men. *Eur J Endocrinol* 2013; 168: 227–33.
- Boisen KA, Kaleva M, Main KM, Virtanen HE, Haavisto AM, *et al*. Difference in prevalence of congenital cryptorchidism in infants between two Nordic countries. *Lancet* 2004; 363: 1264–9.
- Bray F, Richiardi L, Ekblom A, Pukkala E, Cuninkova M, *et al*. Trends in testicular cancer incidence and mortality in 22 European countries: continuing increases in incidence and declines in mortality. *Int J Cancer* 2006; 118: 3099–111.
- Jacobsen R, Moller H, Thoresen SO, Pukkala E, Kjaer SK, *et al*. Trends in testicular cancer incidence in the Nordic countries, focusing on the recent decrease in Denmark. *Int J Androl* 2006; 29: 199–204.
- Nassar N, Bower C, Barker A. Increasing prevalence of hypospadias in Western Australia, 1980–2000. *Arch Dis Child* 2007; 92: 580–4.
- Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. *Int J Obes* 2008; 32: 1431–7.
- Ng M, Fleming T, Robinson M, Thomson B, Graetz N, *et al*. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the global burden of disease study 2013. *Lancet* 2014; 384: 766–81.
- Mendiola J, Torres-Cantero AM, Moreno-Grau JM, Ten J, Roca M, *et al*. Food intake and its relationship with semen quality: a case-control study. *Fertil Steril* 2009; 91: 812–8.
- Chavarro JE, Furtado J, Toth TL, Ford J, Keller M, *et al*. Trans-fatty acid levels in sperm are associated with sperm concentration among men from an infertility clinic. *Fertil Steril* 2011; 95: 1794–7.
- Mínguez-Alarcón L, Mendiola J, Lopez-Espin JJ, Sarabia-Cos L, Vivero-Salmeron G, *et al*. Dietary intake of antioxidant nutrients is associated with semen quality in young university students. *Hum Reprod* 2012; 27: 2807–14.
- Afeiche MC, Gaskins AJ, Williams PL, Toth TL, Wright DL, *et al*. Processed meat intake is unfavorably and fish intake favorably associated with semen quality indicators among men attending a fertility clinic. *J Nutr* 2014; 144: 1091–8.
- Gaskins AJ, Colaci DS, Mendiola J, Swan SH, Chavarro JE. Dietary patterns and semen quality in young men. *Hum Reprod* 2012; 27: 2899–907.
- Safarinejad MR. Effect of omega-3 polyunsaturated fatty acid supplementation on semen profile and enzymatic anti-oxidant capacity of seminal plasma in infertile men with idiopathic oligoasthenoteratospermia: a double-blind, placebo-controlled, randomised study. *Andrologia* 2011; 43: 38–47.
- Attaman JA, Toth TL, Campos H, Hauser R, *et al*. Dietary fat and semen quality among men attending a fertility clinic. *Hum Reprod* 2012; 27: 1466–74.
- Jensen TK, Heitmann BL, Jensen MB, Halldorsdottir T, Andersson AM, *et al*. High dietary intake of saturated fat is associated with reduced semen quality among 701 young Danish men from the general population. *Am J Clin Nutr* 2013; 97: 411–8.
- Chavarro JE, Mínguez-Alarcón L, Mendiola J, Cutillas-Tolin A, Lopez-Espin JJ, *et al*. Trans fatty acid intake is inversely related to total sperm count in young healthy men. *Hum Reprod* 2014; 29: 429–40.
- Field AE, Colditz GA, Willett WC, Longcope C, McKinlay JB. The relation of smoking, age, relative weight, and dietary intake to serum adrenal steroids, sex hormones, and sex hormone-binding globulin in middle-aged men. *J Clin Endocrinol Metab* 1994; 79: 1310–6.
- Nagata C, Takatsuka N, Kawakami N, Shimizu H. Relationships between types of fat consumed and serum estrogen and androgen concentrations in Japanese men. *Nutr Cancer* 2000; 38: 163–7.
- Demark-Wahnefried W, Price DT, Polascik TJ, Robertson CN, Anderson EE, *et al*. Pilot study of dietary fat restriction and flaxseed supplementation in men with prostate cancer before surgery: exploring the effects on hormonal levels, prostate-specific antigen, and histopathologic features. *Urology* 2001; 58: 47–52.
- Giltay EJ, Geleijnse JM, Heijboer AC, de Goede J, Oude Griep LM, *et al*. No effects of n-3 fatty acid supplementation on serum total testosterone levels in older men: the Alpha Omega Trial. *Int J Androl* 2012; 35: 680–7.
- World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva: WHO Press; 2010.
- Menkveld R. The basic semen analysis. In: Oerhinger SC, Kruger TF, editors. Male Infertility. Diagnosis and Treatment. Abingdon: Informa Healthcare; 2007.
- Vioque J, Navarrete-Munoz EM, Gimenez-Monzo D, Garcia-de-la-Hera M, Granada F, *et al*. Reproducibility and validity of a food frequency questionnaire among pregnant women in a Mediterranean area. *Nutr J* 2013; 12: 26.
- Vioque J, Weinbrenner T, Asensio L, Castello A, Young I, *et al*. Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects. *Br J Nutr* 2007; 97: 977–86.
- Palma I, Farran P, Cervera P. [Tables of Food Composition for household measures of

- habitual consumption in Spain: CESNID). 4th ed. Madrid: McGraw Hill; 2008. [Article in Spanish].
- 36 US Department of Agriculture. Agricultural Research Service 2010 National Nutrient Database for Standard Reference, Release 23. Nutrient Data Laboratory 2010. Available from: <http://www.ars.usda.gov/ba/bhnrc/ndl>. [Last accessed on 2016 Sep 27].
 - 37 Vicario IM, Grigoul V, Leon-Camacho M. Multivariate characterization of the fatty acid profile of spanish cookies and bakery products. *J Agric Food Chem* 2003; 51: 134–9.
 - 38 Larqué E, Garaulet M, Pérez-Llamas F, Zamora S, Tebar J. [Fatty acid composition and nutritional relevance of most widely consumed margarines in Spain]. *Int J Fats Oils* 2003; 54: 65–70. [Article in Spanish].
 - 39 Willett W. *Nutritional Epidemiology*. 3rd ed. Oxford: Oxford University Press; 2013.
 - 40 Willett W, Sampson L, Stampfer M, Rosner B, Bain C, *et al*. Reproducibility and validity of a semiquantitative food frequency questionnaire. *Am J Epidemiol* 1985; 122: 51–65.
 - 41 Subar AF, Thompson FE, Kipnis V, Midthune D, Hurwitz P, *et al*. Comparative validation of the Block, Willett, and National Cancer Institute food frequency questionnaires: the Eating at America's Table Study. *Am J Epidemiol* 2001; 154: 1089–99.
 - 42 Ocke MC, Bueno-de-Mesquita HB, Pols MA, Smit HA, van Staveren WA, *et al*. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. *Int J Epidemiol* 1997; 26 Suppl 1: S49–58.
 - 43 Block G, Woods M, Potosky A, Clifford C. Validation of a self-administered diet history questionnaire using multiple diet records. *J Clin Epidemiol* 1990; 43: 1327–35.
 - 44 Vioque J. [Validity of the evaluation of dietary intake]. In: Serra-Majem L, Aranceta Bartrina J, editors. [Nutrition and Public Health. Methods, Scientific Basis and Applications]. 2nd ed. Barcelona: Masson-Elsevier; 2006. [Article in Spanish].
 - 45 Asklund C, Jorgensen N, Skakkebaek NE, Jensen TK. Increased frequency of reproductive health problems among fathers of boys with hypospadias. *Hum Reprod* 2007; 22: 2639–46.
 - 46 Vermeulen A, Verdonck L, Kaufman JM. A critical evaluation of simple methods for the estimation of free testosterone in serum. *J Clin Endocrinol Metab* 1999; 84: 3666–72.
 - 47 Weng HY, Hsueh YH, Messam LL, Hertz-Picciotto I. Methods of covariate selection: Directed acyclic graphs and the change-in-estimate procedure. *Am J Epidemiol* 2009; 169: 1182–90.
 - 48 Jensen B. Rat testicular lipids and dietary isomeric fatty acids in essential fatty acid deficiency. *Lipids* 1976; 11: 179–88.
 - 49 Privett OS, Phillips F, Shimasaki H, Nozawa T, Nickell EC. Studies of effects of trans fatty acids in the diet on lipid metabolism in essential fatty acid deficient rats. *Am J Clin Nutr* 1977; 30: 1009–17.
 - 50 Veaute C, Andreoli MF, Racca A, Bailat A, Scalerandi MV, *et al*. Effects of isomeric fatty acids on reproductive parameters in mice. *Am J Reprod Immunol* 2007; 58: 487–96.
 - 51 Hanis T, Zidek V, Sachova J, Klir P, Deyl Z. Effects of dietary trans-fatty acids on reproductive performance of Wistar rats. *Br J Nutr* 1989; 61: 519–29.
 - 52 Afeiche MC, Williams PL, Gaskins AJ, Mendiola J, Jorgensen N, *et al*. Meat intake and reproductive parameters among young men. *Epidemiology* 2014; 25: 323–30.
 - 53 Eslamian G, Amirjannati N, Rashidkhani B, Sadeghi MR, Hekmatdoost A. Intake of food groups and idiopathic asthenozoospermia: a case-control study. *Hum Reprod* 2012; 27: 3328–36.
 - 54 Allen NE, Appleby PN, Davey GK, Key TJ. Lifestyle and nutritional determinants of bioavailable androgens and related hormones in British men. *Cancer Causes Control* 2002; 13: 353–63.
 - 55 Li Y, Liu L, Wang B, Xiong J, Li Q, *et al*. Impairment of reproductive function in a male rat model of non-alcoholic fatty liver disease and beneficial effect of N-3 fatty acid supplementation. *Toxicol Lett* 2013; 222: 224–32.
 - 56 Yan L, Bai XL, Fang ZF, Che LQ, Xu SY, *et al*. Effect of different dietary omega-3/omega-6 fatty acid ratios on reproduction in male rats. *Lipids Health Dis* 2013; 12: 33.
 - 57 Vioque J. [Validity of the evaluation of dietary intake]. In: Serra-Majem L, Aranceta J, Mataix J, editors. [Nutrition and Public Health. Methods, Scientific Basis and Applications]. Barcelona: Masson; 1995. [Article in Spanish].
 - 58 Bjornerem A, Straume B, Oian P, Berntsen GK. Seasonal variation of estradiol, follicle stimulating hormone, and dehydroepiandrosterone sulfate in women and men. *J Clin Endocrinol Metab* 2006; 91: 3798–802.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 3.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as the author is credited and the new creations are licensed under the identical terms.