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Walnuts Improve Semen Quality in Men Consuming a Western-Style Diet: Randomized Control Dietary Intervention Trial[1]

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ABSTRACT

We tested the hypothesis that 75 g of whole-shelled walnuts/day added to the Western-style diet of healthy young men would beneficially affect semen quality. A randomized, parallel two-group dietary intervention trial with single-blind masking of outcome assessors was conducted with 117 healthy men, age 21–35 yr old, who routinely consumed a Western-style diet. The primary outcome was improvement in conventional semen parameters and sperm aneuploidy from baseline to 12 wk. Secondary endpoints included blood serum and sperm fatty acid (FA) profiles, sex hormones, and serum folate. The group consuming walnuts (n = 59) experienced improvement in sperm vitality, motility, and morphology, but no change was seen in the group continuing their usual diet but avoiding tree nuts (n = 58). Comparing differences between the groups from baseline, significance was found for vitality ($P = 0.003$), motility ($P = 0.009$), and morphology (normal forms; $P = 0.04$). Serum FA profiles improved in the walnut group with increases in omega-6 ($P = 0.0004$) and omega-3 ($P = 0.0007$) but not in the control group. The plant source of omega-3, alpha-linolenic acid (ALA) increased ($P = 0.0001$). Sperm aneuploidy was inversely correlated with sperm ALA, particularly sex chromosome nullisomy (Spearman correlation, −0.41, $P = 0.002$). Findings demonstrated that walnuts added to a Western-style diet improved sperm vitality, motility, and morphology.

randomized dietary intervention, semen quality, sperm aneuploidy

INTRODUCTION

Food has been linked to human reproductive success throughout history [1]. Dietary habits and essential nutrients to promote successful reproductive outcomes have been identified for the maternal periconceptional and perinatal period [2–7], but healthy dietary habits and essential nutrients for paternal reproductive fitness are less clear. Selenium in the form of selenoproteins protects sperm from oxidative damage and defines sperm morphology in the epididymis [8–10]; zinc and vitamins C and E have antioxidant properties believed to be important to male fertility [9, 11–15]; low folate intake has been associated with sperm aneuploidy [13], and polyunsaturated fatty acids (PUFAs) have been shown to play critical roles in sperm maturation and membrane function in animal and human laboratory studies [16–18] as well as some but not all clinical investigations of male infertility [19–28]. Although interest in establishing dietary recommendations to optimize male fertility is growing, evidence on which to base recommendations is still being defined, and further research is needed.

Evidence is particularly limited for men who routinely consume Western-style diets that may lack optimal nutrients and PUFA profiles needed for healthy sperm and fertility. Best known sources of dietary PUFAs for persons consuming a Western-style diet include fish and fish oil supplements, flax seed, and tree nuts [29]. Nuts provide a rich source of α-linolenic acid (ALA), a natural plant source of omega-3. Of popular tree nuts, walnuts are particularly rich in ALA, omega-6 FAs, antioxidants, and micronutrients including folic acid.

In the current study, we tested the hypothesis that 75 g of whole-shelled walnuts/day added to a Western-style diet would beneficially affect semen quality. The primary endpoint evaluated with respect to efficacy of the walnuts was improvement in conventional semen parameters (sperm concentration, vitality, motility, morphology) and sperm aneuploidy from baseline to 12 wk. These semen quality parameters provide clinical markers for male subfertility [30]. Secondary endpoints included blood serum and sperm fatty acid (FA) profiles and sex hormones as potential underlying factors associated with conventional semen parameters, and serum folate as a potential underlying factor associated with sperm aneuploidy.

MATERIALS AND METHODS

This was a parallel two-group, randomized dietary intervention trial with single-blind masking of outcome assessors conducted at a major university. Research was approved by the Medical institutional review board, Human Research Protection Program, University of California, Los Angeles, and all research participants gave written, informed consent.

Study Subjects and Dietary Intervention

Eligible participants were males, age 21 through 35 yr old, who reported routinely eating a Western-style diet. Men were ineligible for inclusion if they

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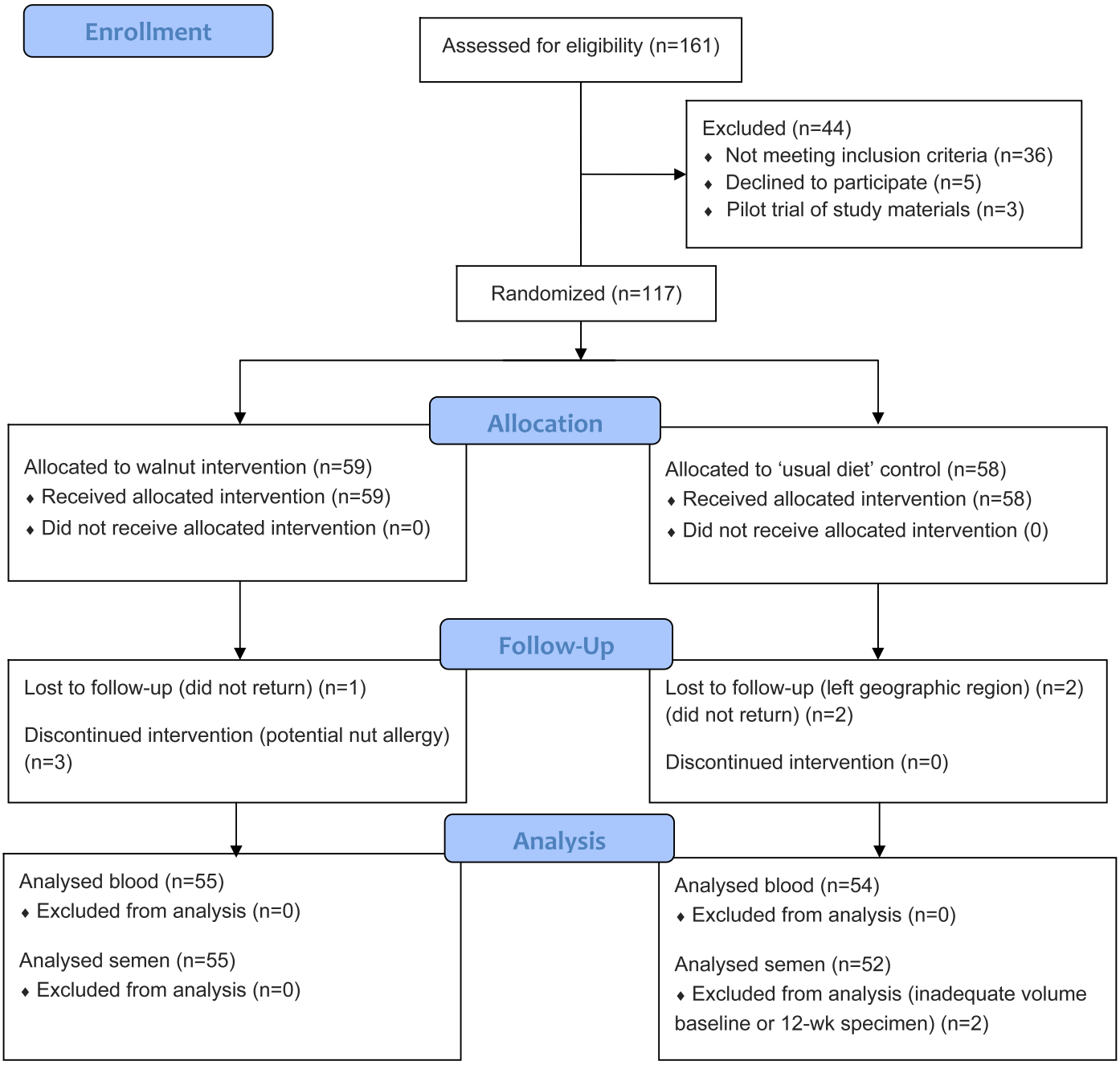

FIG. 1. Flow diagram of walnut randomized control dietary intervention.

had a known food allergy, history of reproductive disorders or vasectomy, were current smokers, were currently taking antioxidant supplements or medications for chronic illness, or were using illicit drugs. Subjects were recruited through flyers posted on campus. A total of 120 men were enrolled from October 2009 to September 2010. Three men completed a pilot trial of the walnut intervention. Subsequently, the remaining 117 participants were randomly assigned to one of two parallel groups: group one continued to consume their usual diet but added 75 g of whole-shelled English walnuts per day (n = 59); and group two continued to consume their usual diet, avoiding consumption of tree nuts (n = 58). Walnuts were supplied in preweighed 1-ounce snack packs provided by the California Walnut Commission, Folsom, California (http://www.walnuts.org).

Participants were randomized in a 1:1 manner to either the walnut intervention or control group by using a computerized random proportion model in permuted blocks of 10 [31]. Randomization was performed at the Center for Human Nutrition, with each allocation assignment placed in a sealed envelope and transported to the Robbins' laboratory where subjects were enrolled. Participants opened the envelope after informed consent at the baseline visit. Subsequent to the baseline visit, allocation was concealed from the researchers. The dietary trial lasted 12 wk, during which participants attended two research clinic visits, one at baseline and one at the 12-wk interval. Prior to completing the study, 3 men were withdrawn by the researchers due to possible nut allergy, 2 left the geographic area, and 3 were otherwise lost to follow-up (Fig. 1) [32]. There were no significant differences in baseline demographics, hormone, and FA profiles, and semen parameters between the group of 8 men who did not complete the study and those of the 109 men who did ($P > 0.05$).

Dietary Measures

Usual diet at baseline was determined using a self-administered 2007 National Cancer Institute diet history questionnaire [33], and 3-day food record [31]. The food frequency questionnaire assessed underlying dietary patterns, while the 3-day food record provided measurement of recent dietary intake. To evaluate compliance, 24-h dietary recalls were conducted over the telephone by trained interviewers twice each month on randomly chosen days throughout the

12-wk study. The Automated Self-Administered 24-h Dietary Recall (ASA24) software template was used for data collection and entry [34]. Researchers were blinded as to intervention or control group status when collecting and entering ASA24 data.

Height and body weight were measured, and body mass index (BMI) was calculated as kg/m^2. Men reported usual exercise level, which was recorded as to specific activity, intensity, and duration. Total physical activity level was calculated and recorded in units of metabolic equivalent of task (MET)-min per week (MET-min/wk) according to the International Physical Activity Questionnaire scoring protocol [35]. One MET equals the energy expended during rest (3.5 ml $O_2 \cdot kg^{-1} \cdot min^{-1}$).

Semen Analysis

All men were instructed to abstain for 2 to 3 days prior to providing the study specimen, with abstinence period verified by self-report on day of sample collection. Semen samples were collected in a private clinical examination room located adjacent to the research laboratory, allowing analysis within 60 min of sample production. A single researcher, who was blinded as to intervention versus control group status, performed the conventional semen analyses. Each sample was allowed to liquefy at room temperature, and routine semen analysis was performed according to standard procedures described in the World Health Organization laboratory manual for examination and processing of human semen [30]. An IVOS sperm analyzer (Hamilton-Thorne Biosciences; http://www.hamiltonthorne.com) was used for count and motility parameters according to version 12.1 protocols. Samples with high concentrations were diluted using autologous sperm-free seminal plasma to achieve concentrations below $\sim 50 \times 10^6$ cells/ml for IVOS motility assessments. The value for motile cells was set at path velocity (VAP) of >5.0 μ/sec. Minimum values for progressively motile cells were set at VAP of 25 μ/sec and straightness (STR) of 80%. Standards were purchased from Fertility Solutions (http://fertilitysolutions.com). Vitality was determined by membrane exclusion of eosin vital dye (Fisher Scientific; http://www.fishersci.com). For sperm morphology, slides were scored by a single technician who was blinded as to intervention or control group status and who had completed American Society of Andrology Sperm Morphology laboratory training. Test slides for quality control validation were from Fertility Solutions. Strict criteria [36] were used at a magnification of ×100. For sperm aneuploidy, 10 μl of semen was smeared onto slides, air dried, and evaluated for aneuploidy of chromosomes X, Y, and 18 using sperm fluorescence in situ hybridization (FISH) adapted from Robbins et al. [37]. A single technician, blinded as to walnut intervention versus control group, systematically analyzed 5000 sperms per sample according to strict scoring criteria [38].

Blood Collection

Blood samples were collected in two 16 × 100 mm × 8.5 ml Vacutainer Plus plastic serum collection tubes (Becton Dickinson Diagnostics; http://paswhitepapers.bd.com). Within 30 min of collection, samples were centrifuged at 3000 rpm for 15 min, serum pipetted into 2-ml polypropylene tubes, and then stored at −80°C until analysis. Frozen serum was allowed to thaw at room temperature prior to respective assays.

Blood Serum and Sperm FA Analysis

Blood serum FAs were converted to methyl esters (FAME) using a methanol-benzene mixture and acetylchloride according to the method by Bagga et al. [39]. After samples underwent heat treatment for 60 min at 100°C, sodium carbonate was added, and samples centrifuged at 913 × *g* for 10 min at 4°C. Benzene supernatant was used to separate and quantify the FAMEs by use of a 5890A series II gas chromatography unit (Agilent Technologies; http://www.home.agilent.com) fitted with a model 7673 automatic split-injection system and flame ionization detector and SP2380 stabilized phase-fused silica capillary column (30 m × 0.32 mm inside diameter, 0.25 μm film thickness; Supelco, Inc.; http://www.manta.com). Quantification was based on recovery of a known quantity of internal standard (heptadecanoic acid; NuChek Preparation Inc.; http://www.nu-chekprep.com) and on the response ratio of FA standards purchased from NuChek Preparation Inc. For quality control a pooled serum sample was used with each batch of serum samples. Using this pooled serum sample, we established the following coefficients of variation: $C_{16:0}$, 1.6%; $C_{16:1}$, 2.7%; $C_{18:0}$, 2.6%; $C_{18:1}$, 3.4%; $C_{18:2}$, 0.7%; $C_{18:3}$, 0.7%; $C_{20:2}$, 8.7%; $C_{20:4}$, 0.7%; $C_{20:5}$, 3.2%; $kC_{22:5}$, 4.2%; and $C_{22:6}$, 2.2%. Semen was centrifuged at 600 × *g* for 6 min at 4°C to separate semen plasma and sperm. The sperm pellet was dispersed in 50 μl of PBS and mixed with methanol-benzene mixture and treated like the serum. Methylated FAs were separated and quantified using the same gas chromatography conditions as described for serum FA analysis.

Blood Hormone Assays

Levels of total testosterone (T), estradiol (E2), follicle stimulating hormone (FSH), luteinizing hormone (LH), and sex hormone binding globulin (SHBG) were determined using chemiluminescent immunometric assays with an Immulite 1000 automatic analyzer (Siemens Medical Solutions Diagnostics; http://www.usa.siemens.com). Hormone controls, standards (Siemens Medical Solutions Diagnostics), and serum pools generated in our laboratory were analyzed for quality control. The coefficient of variation for T was 6.8%–13%, 6.3%–15% for E2, 2.3%–3.7% for FSH, 4.8%–6.5% for LH, and 4.1%–7.7% for SHBG. Duplicate samples with >10% coefficient of variation were reanalyzed. Sensitivity of the assays reported by the manufacturer are 0.5 nmol/L for T, 55 pmol/L for E2, 0.1 mIU/ml for FSH and LH, and 0.2 nmol/L for SHBG.

Serum Folate Assay

Serum folate was determined by a chemiluminescent competitive binding technique using natural folate binding protein (Elecsys Folate III assay; Cobas; Roche Diagnostics; https://www.cobas-roche.co.uk). Controls and standards (Cobas, Roche Diagnostics) and serum pools generated in the laboratory were analyzed for quality control. The intra-assay coefficient of variation ranged from 4.7% to 7.0%. Limit of detection ranged from 3.41 to 45.4 nmol/L.

Statistical Analyses

Summary statistics are reported as percents for nominal data or means ± SD for continuous data. FAs are expressed as a percentage of total FAs. An unpaired Student *t*-test was used to test for differences between control and intervention group, and a paired Student *t*-test was used to test for differences within group from baseline to 12 wk for variables that followed a normal (Gaussian) distribution, or, if the distribution was non-normal, variables were transformed to approximate normal distribution. Mann-Whitney *U* test and Wilcoxon matched-pairs signed-rank test were used for comparisons of non-normally distributed data. Spearman correlation coefficients and Pearson correlation coefficients were used to calculate pair-wise correlations. Two-tailed significance level was defined as a P value of <0.05. Excluding samples with abstinence intervals outside a range of 1.5–3 days did not change study conclusions, thus these samples were retained in the final analyses. Sample size of 50 men per group for sperm parameters and 40 for sperm aneuploidy were estimated for detecting a modest difference of the difference between the two groups from baseline to 12 wk, 80% power, and α level of 0.05. Stata 12 statistical software (http://stata.com) was used for analyses.

RESULTS

Characteristics of the study population (age, education level, race, BMI, body weight, METs) did not differ between the walnut intervention and control group at baseline, indicating that the randomization procedure had been successful. No significant changes in BMI, body weight, or METs were noted in either group at the 12-wk postdietary intervention visit (Table 1). Days of abstinence prior to collecting the semen study sample did not differ between the walnut intervention and control groups at baseline (3.2 [SD 4.2] and 3.3 [SD 4.2] days, respectively) or at the postdietary intervention visit (3.4 [SD 1.7] and 3.2 [SD 2.1] days, respectively) ($P > 0.05$).

FA profiles in blood serum did not differ between the walnut intervention and control groups at baseline ($P > 0.05$) (Table 2). Grouped omega-6 and omega-3 FAs in serum showed significant increases at the 12-wk follow-up visit within the walnut group ($P = 0.0004$ and $P = 0.0007$, respectively) but not in the control group. When the 12-wk changes in the walnut group were compared to the changes in the control group, the differences were significant for omega-6 ($P = 0.004$) and omega-3 ($P = 0.003$). Among the omega-3 FAs, only ALA showed a significant change, and this was an increase seen only in the walnut intervention group ($P =$

TABLE 1. Selected characteristics of the study population at baseline with change at 12-wk dietary intervention visit.

		Education (%)				Race (%)					BMI mean (SD)		Mean weight (lbs) (SD)		Mean exercise metabolic equivalents (SD)	
Group	Mean age (yr) (SD)	High school	AA or AS	BA or BS	Master's, MD, or PhD	White	Asian	Hispanic	Black	Mixed and other	Baseline	Change[a]	Baseline	Change[a]	Baseline	Change[a]
Walnut (n = 59)	25.6 (4.0)	15.8	22.8	42.1	19.3	35.6	32.2	17.0	3.4	11.8	24.98 (4.0)	−0.66 (0.7)	174.7 (35.1)	0.14 (5.0)	2458 (1935)	352 (2099)
Control (n = 58)	24.8 (3.7)	10.5	43.9	31.6	14.0	41.4	29.3	20.7	1.7	6.9	25.59 (3.5)	0.12 (0.8)	174.1 (20.3)	−0.41 (5.2)	3629 (2056)	320 (2294)
P value[b]	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[a] Change values presented for 12-wk follow-up are calculated based on 109 men who completed the study; METs (n = 103).
[b] NS indicates $P > 0.05$.

TABLE 2. Selected FAs in blood serum and sperm at baseline with change at 12-wk dietary intervention visit.[a]

	α-linolenic acid (ALA)				Docosahexaenoic acid (DHA)				Omega-6[b]				Omega-3[c]			
	Serum[d]		Sperm[e]		Serum[d]		Sperm[e]		Serum[d]		Sperm[e]		Serum[d]		Sperm[e]	
Group	Baseline	Change[f]	Baseline	Change[f]	Baseline	Change[f]	Baseline	Change[f]	Baseline	Change[f]	Baseline	Change[f]	Baseline	Change[f]	Baseline	Change[f]
Walnut, mean (SD)	0.897 (0.37)	0.52 (0.87)	2.1 (0.9)	0.03 (0.6)	2.32 (0.77)	−0.99 (0.51)	28.7 (5.8)	0.89 (4.5)	41.2 (4.5)	2.8 (4.0)	16.3 (3.3)	1.2 (3.9)	3.49 (0.84)	0.42 (0.87)	32.9 (5.3)	0.8 (4.3)
Control, mean (SD)	0.933 (0.37)	−0.02 (0.39)	2.0 (0.7)	0.02 (0.5)	2.05 (0.71)	0.11 (0.66)	22.8 (5.0)	0.64 (4.4)	42.5 (3.8)	0.78 (3.4)	15.8 (2.6)	0.4 (2.8)	3.26 (0.72)	0.09 (0.69)	33.1 (4.6)	0.8 (4.1)
P value[g]	NS	0.0001	NS	NS	NS	NS	NS	NS	NS	0.004	NS	NS	NS	0.003	NS	NS

[a] Values for FAs are presented as percentage of total FAs.
[b] Omega-6 FAs = linoleic + arachidonic acid.
[c] Omega-3 FAs = α-linolenic + eicosapentaenoic + docosahexaenoic acid.
[d] Values for blood serum FAs are based on results for 109 men who contributed both baseline and 12-wk blood samples.
[e] Values for sperm FAs are based on results for a subset of 58 men who contributed baseline and 12-wk semen samples with adequate cell counts remaining after semen analysis for FA analysis.
[f] Change values presented for 12-wk follow-up are calculated based on results for 109 men who completed the study; METs (n = 103).
[g] NS indicates $P > 0.05$.

TABLE 3. Selected sperm parameters at baseline and change at 12-wk dietary intervention visit.[a]

	Concentration $\times 10^6$/ml		Vitality %		Motility % (progressive plus nonprogressive)		Morphology % (normal forms)		Sperm X, Y, 18 chromosome aneuploidy %	
Group	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
Walnut group										
Mean (SD)	71.4 (61.1)	2.2 (42.6)	76.4 (13.2)	5.5 (10.0)	51.8 (20.6)	5.7 (13.4)	7.4 (3.5)	1.1 (2.7)	1.2 (0.4)	0.1 (0.4)
Range	2.9–326.3		10–93		2–90		1–18.5		0.5–2.4	
Control group										
Mean (SD)	71.8 (54.5)	−6.3 (49.6)	78.0 (14.4)	0.51 (7.4)	53.5 (19.6)	−0.53 (10.4)	7.1 (2.9)	0.1 (2.3)	1.3 (0.7)	0.04 (0.4)
Range	1.0–316.2		0–95		0–86		1–14		0.7–4.8	
P value[b]	NS	NS	NS	0.003	NS	0.009	NS	0.03	NS	NS

[a] Sperm parameters and change values are based on results for 107 men who provided pre-post semen samples.
[b] NS indicates $P > 0.05$.

0.0001). FA profiles in sperm were not significantly different between the walnut intervention and control groups at baseline or at the 12-wk postintervention visit, except for eicosadienoic acid, for which within-group differences in sperm between baseline and 12 wk were not significant for either group ($P > 0.05$); however, in a comparison of changes in the walnut group (an increase) to changes in the control group (a decrease), the difference became significant ($P = 0.02$).

Sperm concentration, vitality, motility, and morphology (normal forms) were not different between the walnut intervention and control groups at baseline (Table 3). Postdietary intervention measures showed improvement within the walnut group for sperm vitality ($P = 0.0001$), motility ($P = 0.004$), and morphology normal forms ($P = 0.003$) but not within the control group ($P > 0.05$). In a comparison of changes from baseline in the walnut group to changes from baseline in the control group, the following were significant: vitality ($P = 0.003$), motility ($P = 0.009$), and morphology normal forms ($P = 0.03$). Progressive motility increased within the walnut group (mean increase, 3.1% [SD 8.0], $P = 0.001$) but not within the control group. In a comparison of changes from baseline in progressive motility in the walnut group to changes in the control group, the difference was significant ($P = 0.02$). Sex hormones were not different between the groups at baseline or at 12 wk, except for FSH. The walnut group started with a lower FSH level than controls ($P = 0.04$) and remained lower at postintervention. Other than 2 subjects who were outliers, FSH values were within normal limits for healthy men of this age (data not shown) [40].

Sperm aneuploidy for chromosomes X, Y, and 18 (reported as percentages of sperm with numerical abnormality of total sperm scored) was not different between the walnut (n = 54) and control (n = 49) groups at baseline or at the 12-wk postintervention visit (Table 3). However, within the walnut group, sperm aneuploidy was decreased at 12 wk (paired *t*-test, $P = 0.003$). Specifically, decreases were seen in sex chromosome disomy and sperm missing a sex chromosome (paired *t*-test, $P = 0.002$ and $P = 0.01$, respectively). No significant changes from baseline occurred within the control group. At the 12-wk visit, sperm ALA was inversely correlated with the proportion of sperm missing a sex chromosome (Spearman correlation, −0.41, $P = 0.002$) and percentage of sperm with any numerical chromosomal abnormality (Spearman correlation −0.34, $P = 0.01$). No significant correlations were found between sperm aneuploidy measures and other omega-3s.

Serum folate levels were not significantly different between the walnut and control groups at baseline, with mean values for the walnut group of 18.6 (SD 6.3, range, 7.1–44.2 nmol/L; and control group, 19.6 [SD 5.5], range 9.6–40.1 nmol/L). Changes from baseline to 12 wk compared between the groups was not significant ($P > 0.05$). Serum folate was not correlated with sperm aneuploidy measures at baseline or at the 12-wk follow-up visit ($P > 0.05$).

DISCUSSION

We found that 75 g of walnuts per day added to a Western-style diet improved sperm vitality, motility, and morphology (normal forms) in a group of healthy young men compared to those in a control group of men consuming a usual diet but avoiding tree nuts. Improved semen quality was associated with increases in blood serum omega-6 FA and in the plant source of omega-3 (ALA) but not with other omega-3s. These findings are consistent with reports showing a distinct change in FA profiles during sperm maturation and differentiation that are key to cellular functions such as phagocytosis of residual bodies by Sertoli cells affecting sperm morphology and provision of fluidity to sperm membrane for motility [16, 41–44].

In clinical populations, Attaman et al. [19] reported dietary intake of omega-3 FA correlated positively with normal sperm morphology; Chavarro et al. [45] reported higher *trans*-FA levels in sperm were associated with lower sperm concentration; Gulaya et al. [26] reported lower levels of sperm DHA in oligoasthenozoospermic compared with normospermic men; and Aksoy et al. [23] and Safarinejad et al. [21] reported higher omega-6:omega-3 ratios in infertile versus fertile men, respectively. Other researchers have reported no difference in PUFAs across fertile and infertile men [22, 25]. Safarinejad [46] conducted a double-blind control trial of fish oil supplements in an infertility clinic population located in Tehran, Iran. Participants were men with idiopathic oligoasthenoteratospermia randomized to receive 1.12 g of eicosapentaenoic acid (EPA)/day plus 0.72 g of DHA/day or a corn oil placebo. The group taking omega-3 supplements showed statistically significant increases in EPA and DHA in red blood cells, sperm cells, and seminal plasma, with concomitant improvement in sperm concentration, count, motility, and morphology.

Both the Safarinejad [46] randomized controlled trial with EPA and DHA omega-3 supplements and our randomized controlled trial that enriched usual diet with ALA omega-3 through eating walnuts found improved sperm motility and morphology (normal forms). In our study, the walnut dietary intervention resulted in a changed serum FA profile for ALA

but not EPA and DHA, suggesting beneficial omega-3 effects on sperm from walnuts were related to the plant source of omega-3 ALA. Safarinejad [46] reported changes in sperm DHA and EPA associated with DHA/EPA supplements; however, in our study, a significant increase in serum ALA for the walnut group ($P = 0.0001$) was not reflected as a significant change in sperm ALA ($P > 0.05$). Differences in the study findings may reflect the fact that walnuts as a natural food contain multiple nutrients that act synergistically with dietary ALA [47] for cellular level changes of benefit to sperm, or possibly, our 12-wk intervention was not adequate to effect changes in sperm FA (the Safarinejad trial of EPA/DHA supplements lasted 32 wk) [46]. Despite this, in the present study, the percentage of sperm with abnormalities in chromosome number were inversely correlated with sperm ALA, as was the proportion of sperm missing a sex chromosome. No significant correlations were found with other omega-3s.

Important differences between the Safarinejad [46] EPA/DHA supplement trial and our walnut intervention dietary trial include the study populations. The EPA/DHA supplement trial was conducted in Iran among men (mean age, ~32) attending an infertility clinic and diagnosed with idiopathic oligoasthenoteratospermia, whereas the walnut intervention included healthy young men (mean age, ~25) without known history of infertility, consuming a typical Western-style diet. Both studies generate important findings relevant to the different populations. For men diagnosed with idiopathic oligoasthenoteratospermia, the DHA/EPA intervention improved count, motility, and morphology (normal forms) on a background of abnormal sperm parameters associated with known infertility. In the case of healthy young men eating walnuts, the intervention improved sperm parameters of vitality, motility, morphology (normal forms), and sperm aneuploidy on a heterogeneous background of semen parameters for men of unproven fertility. For example, at baseline, 22% of study participants fell below the World Health Organization lower reference limit (fifth centile, 95% confidence interval, 38–42) for total motile percent [30]. In the walnut intervention group but not the control group the greatest proportion of men experiencing improvement in total motile percent at 12 wk was seen for men who had been in the lowest quintiles of motility (both total motile percent and progressive motility percent) at baseline.

Strengths of the present study include the 12-wk dietary intervention period that spanned cells differentiating throughout one full cycle of spermatogenesis. Additionally, investigating walnuts as a natural food source allowed evaluation of cumulative effects of multiple nutrients as opposed to individual nutrients in commercial dietary supplements. Finally, in the present study, beneficial changes in blood serum FA profiles occurred (increased PUFAs, decreased saturated FAs) that are consistent with a literature showing these same effects of walnuts on blood lipids in studies designed to evaluate cardiovascular risk reduction [48–51]. The choice of 75 g of walnuts for the current study was derived from review of these and other studies showing doses at which blood lipid levels would be expected to change but not result in weight gain in healthy young males, and be high enough to answer the previously untested research question related to male reproductive health.

A limitation of the present design was collection of blood specimens for hormone analysis throughout the day. This was done to facilitate retention of this busy group of young men. To control for diurnal variability, each man was matched as to time of day blood was collected at baseline to same time of day at 12 wk. Greater than 80% of the study population returned at 12-wk within a 2-h window of their baseline time and hormone levels at baseline and 12 wk were correlated ($P < 0.001$). Sex hormone and gonadotropin levels were assessed as a measure of reproductive fitness and, except for 2 subjects who were outliers, fell within mean reference intervals commonly reported for this age group of healthy men [40]. The present study did not find an association between folate and sperm aneuploidy, whereas the study by Young et al. [13] did. This could reflect differences in exposure assessment and study populations. Young et al., [13] averaged dietary folate intake for men age 20–70 yr based on annual consumption patterns assessed by a food frequency questionnaire. The present study of men age 21–35 measured folate in blood serum, which reflects recent dietary intake and can fluctuate. Baseline and 12-wk measures were found to correlate (Pearson, 0.49; $P < 0.0001$) but more folate research is needed.

Approximately 70 million couples worldwide experience subfertility or infertility with 30%–50% of these cases attributed to the male partner [52]. In the United States, prevalence of men seeking help for fertility is estimated at ~3.3–4.7 million [53]. Semen quality is a predictor of male fertility [30], and some studies suggest human semen quality has declined in specific geographic regions of the world, particularly industrialized nations where pollution, lifestyle habits such as smoking, and trends toward a Western-style diet are hypothesized as potential causes [54–56]. Although importance of diet to human reproductive success is clear, existing dietary recommendations primarily focus on women's reproductive health with less attention given to men. Efforts to identify male-specific dietary recommendations that optimize fertility and promote offspring health should be encouraged.

We evaluated walnuts as a whole food contributing not only essential FAs but also micronutrients important for sperm development and function and found that 75 g of walnuts per day improved sperm vitality, motility, and morphology (normal forms) in a group of healthy, young men eating a Western-style diet. Whether adding walnuts to the diet will go beyond the shifts in sperm parameters as seen in this study to improving birth outcomes for men within fertility clinic populations or in the general population is not yet known and will require further research.

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