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Association between adherence to the Mediterranean diet and semen quality parameters in male partners of couples attempting fertility

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STUDY QUESTION: Is adherence to the Mediterranean diet (MedDiet) associated with better semen quality in men of subfertile couples attempting fertility?

SUMMARY ANSWER: Greater adherence to the MedDiet, as assessed through the validated Mediterranean diet score (MedDietScore), was significantly associated with higher sperm concentration, total sperm count and sperm motility.

WHAT IS KNOWN ALREADY: *A-posteriori* dietary pattern approaches have revealed that dietary patterns characterized by high intakes of fruits, vegetables, whole grains, fish and low intake of meat are associated with better semen quality. Yet, whether adherence to the MedDiet is associated with better semen profile remains largely unexplored.

STUDY DESIGN, SIZE, AND DURATION: This was a cross-sectional study of 225 men from couples attending a fertility clinic in Athens, Greece, recruited between November 2013 and May 2016. The study was designed to evaluate the influence of habitual dietary intake and lifestyle on fertility outcomes.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Men aged 26–55 years, 51.1% overweight or obese, 20.9% smokers, with complete dietary data were analyzed. Diet was assessed via a food-frequency questionnaire and adherence to the MedDiet was assessed through the MedDietScore (range: 0–55; higher scores indicating greater adherence to MedDiet). Semen quality was evaluated according to World Health Organization 2010 guidelines. Multiple logistic regression analysis was used to evaluate associations between tertiles of the MedDietScore and the likelihood of having abnormal semen parameters, after adjusting for potential confounders.

MAIN RESULTS AND THE ROLE OF CHANCE: Compared to men in the highest tertile of the MedDietScore ($\geq$37, $N = 66$), a higher percentage of men in the lowest tertile of the score ($\leq$30, $N = 76$) exhibited below the WHO reference values for sperm concentration (47.4% vs 16.7%, $P < 0.001$), total sperm count (55.3% vs 22.7%, $P < 0.001$), total motility (65.8% vs 31.8%, $P < 0.001$), progressive motility (84.2 vs 62.1%, $P = 0.011$) and sperm morphology (50.0 vs 28.8%, $P = 0.023$). In the multivariable adjusted models, men in the lowest tertile of the MedDietScore had ~2.6 times higher likelihood of having abnormal sperm concentration, total sperm count and motility, compared to men in the highest tertile of the score.

LIMITATIONS, REASONS FOR CAUTION: The main limitation of the study stems from its cross-sectional nature, limiting our ability to determine causality.

WIDER IMPLICATIONS OF THE FINDINGS: The results suggest that greater compliance to the MedDiet may help improve semen quality. Whether this translates into differences in male fertility remains to be elucidated. Our findings are consistent with previous studies showing that dietary patterns with some of the characteristics of the MedDiet, i.e. rich in fruit, vegetables, legumes and whole grains, are associated with better measures of semen quality.

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Introduction

In recent years there has been an increase in research on the factors affecting male fertility given the fact that, in almost 40% of infertile couples, the man is the sole or contributing cause in the inability to conceive. Semen analysis is the cornerstone of the laboratory examination for male infertility and a recent comprehensive review has pointed to a tremendous decrease in semen quality parameters among Western populations during the last four decades (Sengupta *et al.*, 2016). Apart from the traditional risk factors (genetic, endocrine, etc.), lifestyle factors, especially diet, have been suggested to play an important role in male fertility (Gabrielsen and Tanrikut, 2016). Unlike other subfertility risk factors that cannot be reversed, diet presents a potential opportunity for intervention, which means that it is an important consideration in the counseling of subfertile men.

Accumulating literature supports the hypothesis that specific nutrients can affect semen quality parameters (Giahi *et al.*, 2016). However, most studies focus on isolated micronutrients or food compounds, while studies on the effects of dietary patterns are scarce. In a study among healthy young men (Gaskins *et al.*, 2012), a 'prudent' dietary pattern characterized by a high intake of fruits, vegetables, chicken, fish and whole grains was associated with higher sperm progressive motility, while the consumption of a 'Western' dietary pattern was unrelated to conventional semen quality parameters. Similarly, in a study among male university students, a 'Mediterranean' dietary pattern derived by principal component analysis (PCA), which was characterized by high intakes of vegetables, fruits and seafood, was positively associated with total sperm count (Cutillas-Tolin *et al.*, 2015). Studies among men of subfertile couples have also revealed that a 'Mediterranean' dietary pattern, characterized by high intakes of fruits, vegetables, fish and whole grains, is related to lower DNA fragmentation, higher sperm motility (Vujkovic *et al.*, 2009), higher sperm concentration and higher levels of testosterone (Jurewicz *et al.*, 2016).

The Mediterranean diet (MedDiet) is a dietary pattern with plenty of well-established health benefits, presenting strong anti-inflammatory and anti-oxidative effects (Trichopoulou *et al.*, 2003), yet the reproductive benefits of this dietary pattern are less clear. Previous studies evaluating the role of dietary patterns on semen quality have relied on *a-posteriori* dietary pattern approaches, which process the collected dietary information through multivariate statistical methods, such as PCA. However, the interpretation of the components derived by PCA is often difficult, subjective and not easily generalized to the reference population. On the other hand, *a-priori* dietary pattern approaches are based on the use of dietary indexes, such as the Mediterranean diet score (MedDietScore) (Panagiotakos *et al.*, 2007), that aim to capture pre-defined healthy patterns, and have the advantage of relying on the current scientific data concerning nutrition, health and disease.

Therefore, in order to expand the current knowledge, in this study we investigated associations between adherence to the Mediterranean dietary pattern and semen quality parameters in male partners of couples attempting fertility, by employing the *a-priori* dietary pattern approach and using the validated MedDietScore.

Materials and Methods

Study population

Couples with primary infertility, seeking evaluation and treatment at the Embryogenesis Assisted Conception Unit in Athens, Greece, were invited to participate in an ongoing prospective cohort study focusing on investigating how background diet and lifestyle patterns impact fertility. Male partners aged 20–55 years and without a medical history of systemic diseases, cryptorchidism or varicocele, microorchidism, vasectomy or hormonal treatment in the last six-months were eligible for the present analysis. There were 243 men evaluated from November 2013 until May 2016, all of whom completed a dietary assessment questionnaire. There were 18 men excluded from the analysis due to the presence of azoospermia, leaving 225 men for this analysis. At enrollment, participants completed a detailed questionnaire requesting information on demographic, socioeconomic and lifestyle factors and medical history.

All procedures were in accord with the Helsinki Declaration and all participants provided written informed consent. The study protocol was approved by the Ethical Review Board of Harokopio University, Athens, Greece.

Physical examination and clinical and lifestyle variables

A physical examination of each participant was performed on the day of semen sampling and the presence of varicocele or other abnormalities was assessed. Body weight and height were measured to the nearest 0.5 kg and 0.5 cm, respectively, and BMI (kg/m^2) was calculated. In addition, waist and hip circumferences and arterial blood pressure measurements were taken. Blood samples were collected from fasting participants and a biochemical evaluation was carried out in the Assisted Conception Unit Laboratory.

Assessment of physical activity was performed through a validated version of the 'International Physical Activity Questionnaire' (iPAQ) (Papathanasiou *et al.*, 2010). The short version of iPAQ provided information on weekly time spent on walking, on vigorous, moderate-intensity, and on sedentary activity. Participants were instructed to refer to all domains of physical activity during a usual week of the past year. Both continuous [sum of weekly Metabolic Equivalent of Task (MET)-minutes/week of walking and moderate- and vigorous-intensity exercise] and categorical indicators of physical activity (inactive/minimally active/highly active) were assessed.

Anxiety was assessed using a validated version of the Spielberger State-Trait Anxiety Inventory (STAI) (Fountoulakis *et al.*, 2006), which is a

40-item self-reported questionnaire, divided into two parts: the first part evaluates state anxiety by inquiring about the current emotional state, and the second part assesses trait anxiety, asking the subjects to describe how they usually feel. The 40 items are rated from 1 to 4 according to frequency of their feelings (i.e. almost never, sometimes, often, and almost always). The range of the score of each part is 20–80. Values of 20–39 suggest low anxiety, values of 40–59 suggest moderate anxiety, while values >59 suggest severe anxiety.

Dietary assessment and evaluation of adherence to the Mediterranean diet

To estimate habitual food and alcohol intake all participants filled out a (validated for the Greek population) food-frequency questionnaire (FFQ), which included 75 items (foods and beverages commonly consumed in Greece and dietary habits) (Bountziouka *et al.*, 2012).

To evaluate the level of adherence to the MedDiet, the MedDietScore was calculated for each participant (Panagiotakos *et al.*, 2007), by taking into account the consumption of food items from nine food groups, as well as olive oil and alcoholic beverages. The components and the scoring system for calculating MedDietScore are presented in Table I. The range of the MedDietScore is 0–55, with higher values indicating greater adherence to the MedDiet.

Semen analysis

Participants were asked to abstain from ejaculation for at least 48 h before sample collection. The semen sample was immediately delivered to the laboratory and incubated in a 37°C incubator. The duration of complete liquefaction (<1 h) was documented, until 1 h was reached. One semen sample was assessed for each subject, particularly the one used during the *in-vitro* fertilization protocol.

Semen analysis was performed with standardized methods. Semen volume was measured by weighing, assuming a semen density of 1.0 g/ml; sperm concentration was evaluated by hemocytometer (Bürker-Türk; Paul Marienfeld GmbH&Co. KG, Lauda-Königshofen, Germany); sperm morphology was identified from semen smears prepared with 10 µl of well-mixed semen, stained with Papanicolaou and assessed using the Tygerberg strict criteria (Menkveld and Kruger, 1995). Sperm motility was graded into total (progressive + non-progressive motility) and progressive motility. Total sperm count (volume × sperm concentration) was also calculated. Reference values from the newest World Health Organization semen analysis manual were used to assess sperm concentration and motility (WHO, 2010).

Statistical analysis

Continuous variables are presented as median (25th, 75th percentiles), and categorical variables as absolute and relative frequencies. The normality of the data was assessed graphically using histograms and with the Shapiro–Wilk test. All seminal parameters showed non-normal distributions. Correlations between dietary, anthropometric and semen analysis parameters were assessed using Spearman's or Pearson's method whenever appropriate. Associations between categorical variables were tested by Fisher's exact test, while differences between categorical and several clinical and nutritional variables were tested by the use of Student's *t*-test and Mann–Whitney test (for the normally distributed and skewed variables, respectively). Comparisons between various variables and tertiles of

Table I The Mediterranean diet score.

How often do you consume	Frequency of consumption (servings/week or otherwise stated)					
Non-refined cereals (whole grain bread, pasta, rice, etc.)	Never	1–6	7–12	13–18	19–31	>32
	0	1	2	3	4	5
Potatoes	Never	1–4	5–8	9–12	13–18	>18
	0	1	2	3	4	5
Fruits	Never	1–4	5–8	9–15	16–21	>22
	0	1	2	3	4	5
Vegetables	Never	1–6	7–12	13–20	21–32	>33
	0	1	2	3	4	5
Legumes	Never	<1	1–2	3–4	5–6	>6
	0	1	2	3	4	5
Fish	Never	<1	1–2	3–4	5–6	>6
	0	1	2	3	4	5
Red meat and products	≤1	2–3	4–5	6–7	8–10	>10
	5	4	3	2	1	0
Poultry	≤3	4–5	5–6	7–8	9–10	>10
	5	4	3	2	1	0
Full fat dairy products (cheese, yoghurt, milk)	≤10	11–15	16–20	21–28	29–30	>30
	5	4	3	2	1	0
Use of olive oil in cooking (times/week)	Never	Rare	<1	1–3	3–5	Daily
	0	1	2	3	4	5
Alcoholic beverages (ml/day, 100 ml = 12 g ethanol)	<300	300	400	500	600	>700 or 0
	5	4	3	2	1	0

The range of the Mediterranean diet score (MedDietScore) is from 0 to 55, with higher values indicating greater adherence to the Mediterranean diet (Panagiotakos *et al.*, 2007).

the MedDietScore were performed using Kruskal–Wallis test, and the Bonferroni correction was used to account for the increase in Type-I error. Analysis of covariance was also used to calculate multivariable adjusted semen quality parameters for each tertile by relevant covariates.

Multiple logistic regression was applied to assess associations between adherence to the MedDiet and the likelihood of having abnormal semen parameters, after adjusting for the following confounders: age (years), BMI (kg/m^2), smoking (current/never/ex-smokers), physical activity (MET-min/week), state/trait-anxiety (score value), total energy intake (kcal/day), educational level (primary–secondary school/Bachelor's degree/Master's–Doctoral degree), income level (low/moderate/high), and family subfertility history (yes/no). Collinearity between independent variables was evaluated through the condition index, while the model's goodness-of-fit was graphically evaluated (standardized residuals against fitted values). Standardized residuals were used to test the model's goodness-of-fit. Tests of linear trend across categories were conducted by modeling the median values of each category as a single continuous variable and assessing significance using Wald test. Statistical Package for Social Sciences software (SPSS, version 21.0, Chicago, Illinois, USA) was used for all the statistical calculations. All reported *P*-values are based on two-sided tests and compared to a significance level of 5%.

Results

The median age of the 225 study participants was 38 (range: 26–55) years. Most men (51.1%) were overweight or obese (BMI ≥ 25 kg/m^2), 20.9% were current smokers, and 93.8% were inactive or minimally active. The median (25th, 75th percentiles) values for semen analysis parameters were: 30 × 10^6/ml (10, 55) for sperm concentration, 60 × 10^6/ml (20, 111) for total sperm count, 20% (10, 35) for percent progressively motile sperm, and 4% (3, 5) for percent morphologically normal sperm. The median MedDietScore was 34.0 (range: 23–46).

Overall, 50 men (22.2%) had a normal semen analysis, while most (77.8%) had at least one semen analysis parameter below the lower benchmarks according to the WHO 2010 criteria. Compared to men with normal semen analysis, those with abnormal semen analysis had higher median BMI (25.6 vs 24.0 kg/m^2, $P = 0.021$) and exhibited lower median values in the MedDietScore (33.0 vs 35.0, $P = 0.002$). As was expected, BMI and waist circumference were both negatively correlated with semen quality measures, except for semen volume, and a negative correlation was also observed between semen parameters and anxiety levels (all $P < 0.05$, data not shown). In contrast, MedDietScore was positively correlated with sperm concentration (Spearman's rho = 0.29, $P < 0.001$), total sperm count (rho = 0.26, $P < 0.001$), total and progressive motility (rho = 0.31 and rho = 0.30, $P < 0.001$, respectively), and with percent morphologically normal sperm (rho = 0.15, $P = 0.025$).

In Table II, the distribution of various characteristics of the participants according to tertiles of the MedDietScore is presented. Compared to men in the lowest tertile of the MedDietScore (≤30, $N = 76$), men in the highest tertile (≥37, $N = 66$) had statistically significant higher median values for sperm concentration (40.0 vs 16.0 × 10^6/ml, $P < 0.001$), total sperm count (73.5 vs 31.0 × 10^6/ml, $P < 0.001$), percent total and progressive motility (45.0% vs 25.0%, $P < 0.001$ and 25.0% vs 15.0%, $P = 0.016$, respectively) and percent morphologically normal sperm (5.0% vs 3.5%, $P = 0.018$), and less frequently exhibited abnormal values for all semen analysis parameters, except for volume (Table II). Participants with higher adherence to the MedDiet also had lower BMI values ($P < 0.001$), were less likely to be smokers ($P < 0.001$) and had higher levels of physical activity ($P < 0.001$). In addition, they exhibited lower levels of current and long-term anxiety ($P \leq 0.001$), and had a more favorable blood lipid profile. Adjusted means (95% CI) for the semen quality parameters for each tertile of the MedDietScore by several potential confounders are presented in Table II.

Table III shows the results of the multiadjusted logistic regression analysis exploring the association between tertiles of the MedDietScore and the likelihood of having abnormal semen parameters, after adjusting for the relevant confounders in model 1 (age, smoking status, physical activity, state and trait anxiety, total energy intake, educational level, individual income level and family subfertility history). Men with lower MedDietScores had a higher likelihood of having abnormal sperm concentration [Odds Ratio (OR) for tertile 1 vs tertile 3: 2.65; 95% CI: 1.05–6.71], total sperm count (OR: 2.55; 95% CI: 1.08–6.02), and total motility (OR: 2.88; 95% CI: 1.26–6.62) and progressive motility (OR: 2.74; 95% CI: 1.08–6.92). Further adjustment for BMI (model 2) did not modify the observed associations.

Discussion

In this cross-sectional study among male partners of subfertile couples attempting fertility, we implemented an *a-priori* dietary pattern approach to investigate the relationship between adherence to the MedDiet and semen quality parameters. We found that greater compliance to the MedDiet, as assessed through the validated MedDietScore, was associated with better semen quality measures (i.e. sperm concentration, total sperm count, total and progressive motility). This association was independent of a large number of potential confounders. Moreover, we found that men in the lowest tertile of the MedDietScore had ~2.6 times higher likelihood of having abnormal sperm concentration, total sperm count and motility.

To date, many studies in humans have investigated the relationship between diet and semen quality (Giahi *et al.*, 2016). Most of these studies have focussed on the role of specific nutrients or food compounds, while a few examine overall dietary patterns extracted from multivariate statistical methods, such as PCA (an *a-posteriori* dietary pattern approach). For example, in a cross-sectional study among 161 men of subfertile couples undergoing IVF in the Netherlands (Vujkovic *et al.*, 2009), two dietary patterns were identified using PCA: a 'health conscious' pattern (characterized by a high intake of fruits, vegetables, fish and seafood, whole grains and legumes, and a low intake of mayonnaise, meat products, refined grains and desserts) and a 'traditional Dutch' pattern. The 'health conscious' dietary pattern showed an association with lower sperm DNA fragmentation while the 'traditional Dutch' dietary pattern showed an association with higher sperm concentration. In the study of Jurewicz *et al.* (2016), among 336 men who were attending a fertility clinic for diagnostic purposes, a similar *a-posteriori* approach was employed to identify dietary patterns and relate them to semen quality parameters. In this study, a 'prudent' dietary pattern, (characterized by a high intake of fruits, vegetables, legumes and whole grains) showed a positive association with increased sperm concentration, higher levels of testosterone and decreased DNA fragmentation index. Although the dietary patterns identified in these two studies are clearly different, there is some consistency in the relation of

Table II Characteristics of men attending a fertility clinic by tertiles of the MedDietScore.

	MedDietScore tertile			*P*-value
	1st (≤30)	2nd (31–36)	3rd (≥37)	
N	76	83	66	
Age, y	37 (34–42)	38 (36–42)	38 (35–41)	0.314
Educational level, *n* (%)				0.124
Primary/secondary school	22 (29.2)	21 (25.3)	12 (18.2)	
Bachelor's degree	32 (41.6)	27 (32.5)	21 (31.8)	
Master's/Doctoral degree	22 (29.2)	35 (42.2)	33 (50.0)	
Individual income level, *n* (%)				0.208
Low (<10.000 euros/annually)	2 (2.6)	1 (1.2)	1 (1.5)	
Moderate (10–30.000 euros)	53 (69.7)	52 (62.7)	35 (53.0)	
High (>30.000 euros)	21 (27.7)	30 (36.2)	30 (45.6)	
Total energy intake, kcal/day	2590.4 (2173.8–3054.8)[a]	2092.7 (1982.7–2414.8)[b]	2176.7 (1979.3–2642.7)[b]	<0.001
BMI, kg/m^2	26.7 (24.3–28.8)[a]	24.8 (23.4–26.5)[b]	23.8 (22.8–26.2)[b]	<0.001
BMI category, *n* (%)				<0.001
Normal weight (<25 kg/m^2)	23 (30.3)	43 (51.8)	44 (66.7)	
Overweight (≥25– <30 kg/m^2)	41 (53.9)	36 (43.4)	20 (30.3)	
Obese (≥30 kg/m^2)	12 (15.8)	4 (4.8)	2 (3.0)	
Smoking status, *n* (%)				<0.001
Current smokers	25 (32.9)	18 (21.7)	4 (6.1)	
Never smokers	43 (56.6)	60 (72.3)	56 (84.8)	
Ex-smokers	8 (10.5)	5 (6.0)	6 (9.1)	
Physical activity, MET-min/week	646.5 (330.0–1345.0)[a]	891.0 (447.5–1596.0)[b]	1380.7 (761.7–1887.3)[c]	<0.001
Physical activity level, *n* (%)				0.002
Inactive	36 (47.4)	28 (33.7)	11 (16.7)	
Minimally active	37 (48.7)	51 (61.4)	48 (72.7)	
Highly active	3 (3.9)	4 (4.8)	7 (10.6)	
S-Anxiety (score range 20–80)[a]	43.0 (38.0–47.0)[a]	40.0 (33.0–43.0)[b]	40.5 (33.0–44.0)[b]	0.001
T-Anxiety (score range 20–80)[a]	41.5 (39.0–44.7)[a]	36.0 (31.0–42.0)[b]	36.0 (31.0–41.2)[b]	<0.001
Fasting glucose (mg/dl)[b]	90.0 (83.5–95.5)	83.5 (79.0–95.0)	86.0 (81.0–92.0)	0.089
Triglycerides (mg/dl)[b]	110.0 (81.0–133.0)[a]	103.5 (79.5–125.7)[a]	82.5 (67.2–103.0)[b]	0.008
Total cholesterol (mg/dl)[b]	195.0 (180.2–201.7)[a]	176.0 (159.7–192.0)[b]	175.0 (155.2–187.0)[b]	<0.001
LDL cholesterol (mg/dl)[b]	121.0 (98.0–132.0)[a]	101.5 (92.2–124.7)[b]	99.0 (84.0–121.0)[b]	0.012
HDL cholesterol (mg/dl)[b]	49.0 (42.0–57.0)	47.0 (41.2–53.0)	49.0 (44.0–58.0)	0.282
Family subfertility history, *n* (%)	14 (18.4)	17 (20.5)	16 (24.2)	0.702
Semen analysis				
Volume (ml)[c]	2.0 (1.7, 2.2)	2.2 (2.0–2.3)	2.1 (1.9–2.3)	
Volume <1.5 ml, *n* (%)	15 (19.7)	11 (13.3)	8 (12.1)	0.403
Concentration (×10^6/ml)[c]	30.5 (24.3–36.7)	37.1 (31.5–42.6)	37.9 (31.4–44.4)	
Concentration <15 × 10^6/ml, n (%)	36 (47.4)[a]	18 (21.7)[b]	11 (16.7)[b]	<0.001
Total sperm count (×10^6/ml)[c]	63.8 (47.2–80.4)	86.6 (71.8–101.4)	82.1 (64.7–99.5)	
Total sperm count <39 × 10^6/ml, *n* (%)	42 (55.3)[a]	27 (32.5)[b]	15 (22.7)[b]	<0.001
Motility (%)[c]	32.1 (27.4–36.8)	40.7 (36.5–44.9)	42.6 (37.6–47.5)	
Motility <40% motile, *n* (%)	50 (65.8)[a]	30 (36.1)[b]	21 (31.8)[b]	<0.001
Progressive motility (%)[c]	20.0 (16.2–23.8)	24.1 (20.7–27.5)	26.0 (22.0–30.0)	
Progressive motility <32% motile, *n* (%)	64 (84.2)[a]	62 (74.7)[a]	41 (62.1)[b]	0.011
Morphology (%)[c]	4.3 (3.7–4.9)	4.3 (3.8–4.8)	4.6 (4.0–5.1)	

Continued

Table II *Continued*

	MedDietScore tertile			*P*-value
	1st (≤30)	2nd (31–36)	3rd (≥37)	
Morphology <4% normal, *n* (%)	38 (50.0)[a]	28 (33.7)[b]	19 (28.8)[b]	0.023
Abnormal semen analysis, *n* (%)[d]	66 (86.8)	62 (74.7)	47 (71.2)	0.051

Values represent median (25th, 75th percentiles) or number of subjects (%). For semen analysis parameters, adjusted means (95% CI) are presented. Differences in variables across tertiles of the MedDietScore were tested using Kruskal–Wallis test for continuous variables and Fisher's exact test for categorical variables. Values with different superscript letter are statistically significant different (Bonferroni correction, $P < 0.015$).
[a]Evaluated by the State-Trait Anxiety Inventory (STAI) Y, with higher values suggesting higher levels of anxiety (Fountoulakis *et al.*, 2006).
[b]Values available for 177 study participants.
[c]Means adjusted for age, BMI, smoking status, physical activity, state and trait anxiety, total energy intake, educational level, individual income level and family subfertility history.
[d]Below World Heath Organization reference values for volume, concentration, total sperm count, total motility, progressive motility or morphology (WHO, 2010).
MET = metabolic equivalent of task; S-anxiety = state anxiety, T-anxiety = trait anxiety; LDL = low density lipoprotein, HDL = high density lipoprotein.

Table III Multiple logistic regression models exploring the association between tertiles of the MedDietScore and the likelihood of having abnormal semen parameters.[a]

Semen parameter	MedDietScore tertile			*P* for trend
	1st	2nd	3rd	
Concentration ($<15 \times 10^6$/ml vs $\geq 15 \times 10^6$/ml)				
Model 1	2.65 (1.05–6.71)*	1.19 (0.48–2.91)	1(ref)	0.04
Model 2	2.69 (1.05–6.90)*	1.03 (0.41–2.58)	1	0.04
Total sperm count ($<39 \times 10^6$/ml vs $\geq 39 \times 10^6$/ml)				
Model 1	2.55 (1.08–6.02)*	1.36 (0.62–2.99)	1(ref)	0.03
Model 2	2.61 (1.09–6.27)*	1.20 (0.53–2.70)	1	0.04
Total motility (<40% vs ≥ 40%)				
Model 1	2.88 (1.26–6.62)*	1.14 (0.54–2.39)	1(ref)	0.02
Model 2	2.88 (1.23–6.78)*	0.98 (0.46–2.12)	1	0.03
Progressive motility (<32% vs ≥32%)				
Model 1: adj. for age	2.74 (1.08–6.92)*	1.77 (0.84–3.73)	1(ref)	0.02
Model 2	2.67 (1.03–6.90)*	1.57 (0.73–3.37)	1	0.04
Morphology (<4% normal spermatozoa vs ≥ 4%)				
Model 1	1.53 (0.66–3.53)	1.22 (0.57–2.62)	1(ref)	0.32
Model 2	1.56 (0.64–3.50)	1.07 (0.49–2.34)	1	0.34

Data represent odds ratios (95% CI). Model 1: Odds ratios adjusted for age, smoking status, physical activity, state & trait anxiety, total energy intake, educational level, individual income level and family subfertility history. Model 2: Model 1 + adjustment for BMI.
*$P < 0.05$.
[a]Based on World Health Organization reference values for semen characteristics (WHO, 2010).

the dietary patterns high in fruits, vegetables, legumes and whole grains with semen quality.

The so-called MedDiet is also characterized by a high intake of fruits, vegetables, legumes and whole grains, and a low intake of meat and saturated fatty acids. Adherence to the MedDiet has been shown to confer multiple health benefits (Sofi *et al.*, 2010), and a recent study among 215 healthy male university students showed that higher adherence to a 'Mediterranean' pattern identified by PCA was positively associated with total sperm count (Cutillas-Tolin *et al.*, 2015), a result in accordance with our findings. Comparable studies from infertile populations have also shown that higher adherence to a 'Prudent' dietary pattern, bearing close resemblance to the Mediterranean pattern, is associated with higher total sperm count and motility (Vujkovic *et al.*, 2009; Jurewicz *et al.*, 2016).

The favorable effect(s) of the MedDiet on semen parameters may be mediated by various mechanisms. The MedDiet is naturally high in nutrients with favorable anti-inflammatory properties and low in pro-inflammatory nutrients. Inflammation may affect reproduction through anatomical or functional alteration of the male accessory gland, and/or direct negative effects on the spermatozoa (La Vignera *et al.*, 2013). The association between adherence to the MedDiet and semen quality could also be mediated through an increased intake of omega-3 fatty acids found in fish and seafood. Compared with other cells or tissues, sperm and testicular cellshave a higher concentration of long-chain

(LC-) polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA). The increase in DHA levels in the sperm membrane during sperm maturation suggests that testes have high levels ofactive fatty acid metabolism, resulting in the preferential accumulation of LC-PUFAs due to efficient metabolization of PUFAs into the long-chain metabolites. Furthermore, seafood is characterized by a high proportion of fat-soluble vitamins which play a crucial role in fertilization (Blomberg Jensen, 2014). Finally, the MedDiet is a dietary patern low in saturated and trans-fatty acids, which have been shown to adversely affect semen quality (Chavarro *et al.*, 2014).

The MedDiet is also characterized by high intakes of fruits and vegetables rich in antioxidants, such as beta-carotene and vitamins E and C, which have been suggested to improve semen quality. Carotenoid intake, for example, has been associated with higher sperm motility in young healthy males (Zareba *et al.*, 2013), while vitamin A supplementation was recently shown to enhance spermatogenesis progression *in vitro* (Dumont *et al.*, 2016). Numerous health benefits have been ascribed to antioxidants, mainly because of their protective effect against generation of reactive oxygen species which may negatively affect sperm motility and sperm–oocyte fusion (Ko *et al.*, 2014). Furthermore, antioxidants are thought to protect sperm against endogenous oxidative damage by neutralizing hydroxyl, superoxide and hydrogen peroxide radicals, thus preventing sperm agglutination. With an accent placed on plant food consumption rich in antioxidants as opposed to meat and meat products, the MedDiet could therefore exert favorable effects on semen indicators.

In the present analysis, we have considered the confounding effect of several factors which are known to be critical determinants of normal reproductive function and to affect semen quality (such as age, BMI, smoking and physical activity). Recent reports confirm a negative association between semen quality parameters and overall and central adiposity, cigarette smoking and lower physical activity levels (Eisenberg *et al.*, 2014; Gaskins *et al.*, 2014; Pärn *et al.*, 2015; Sharma *et al.*, 2016). Evidence suggests that increased male age and family subfertility history are also associated with a decline in semen quality (Sharma *et al.*, 2015). Moreover, findings from observational studies suggest that state and trait anxiety (Vellani *et al.*, 2013) and low educational levels (Pärn *et al.*, 2015) may also represent significant factors affecting semen quality. Alcohol consumption has also been associated with a deterioration of sperm parameters (Condorelli *et al.*, 2015). In our analysis, we did not adjust for alcohol intake because alcohol consumption was taken into account in the construction of the MedDietScore. Of note, we also performed a sensitivity analysis by calculating the MedDietScore without considering alcohol consumption and found similar results (data not shown). Other strengths of this study are also the use of a previously validated FFQ for use in the Greek population and the evaluation of compliance to the MedDiet by using an *a-priori* dietary pattern approach and calculation of the MedDietScore.

Our study has several limitations. First, due to its cross-sectional design causal inference is limited. It is not possible to predict whether the beneficial effect of the MedDiet on semen quality measures translates into a higher probability of successful conception. However, previous work showing that higher adherence to the MedDiet is associated with a lower risk of difficulty conceiving (Toledo *et al.*, 2011), and higher biochemical pregnancy rates among women undergoing IVF (Vujkovic *et al.*, 2010), suggest a greater reproductive success where the study participants have a higher adherence to this dietary pattern. Another limitation is that only one semen sample from each study participant was analyzed. Nonetheless, some studies have shown that one sample is enough to assess semen quality in epidemiological studies (Stokes-Riner *et al.*, 2007). A third limitation is that the study participants were selected from an IVF clinic, were generally unhealthy or overweight and did not exercise, so our finding may not be extrapolated to a healthy population. Finally, men adhering more closely to the MedDiet were generally healthier and although we have adjusted for a large number of known and suspected confounders, it is difficult to know whether diet, or other health related factors, could have an impact on semen quality.

In summary, in this study among Greek men of infertile couples, we employed an *a-priori* dietary pattern approach to evaluate adherence to the MedDiet and its relation to semen quality. We found that greater adherence to this traditional diet was associated with a lower likelihood of having an abnormal semen profile, which suggests that diet modificatons and compliance to the MedDiet may help improve at least one measure of semen quality. Whether the beneficial effect of the MedDiet on semen parameters translates into a higher probability of successful conception remains to be elucidated. Furthermore, due to the cross-sectional nature of the study, it is not possible to assess whether advice to adhere more closely to the MedDiet would improve semen quality; this needs to be addressed in future intervention studies.

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Authors' roles

D.K., M.K. and N.Y. were involved in study concept and design. D.K., C.M., L.D. and M.M. contributed to the acquisition of data. D.K. analyzed data, wrote the manuscript and had a primary responsibility for final content; M.K. and N.Y. supervised the analysis and critically revised the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

None declared.

References

Blomberg Jensen M. Vitamin D and male reproduction. *Nat Rev Endocrinol* 2014;**10**:175–86.

Bountziouka V, Bathrellou E, Giotopoulou A, Katsagoni C, Bonou M, Vallianou N, Barbetseas J, Avgerinos PC, Panagiotakos DB. Development repeatability and validity regarding energy and macronutrient intake of a semi-quantitative food frequency questionnaire: methodological considerations. *Nutr Metab Cardiovasc Dis* 2012;**22**:659–667.

Chavarro JE, Minguez-Alarcon L, Mendiola J, Cutillas-Tolin A, Lopez-Espin JJ, Torres-Cantero AM. Trans fatty acid intake is inversely related to total sperm count in young healthy men. *Hum Reprod* 2014;**29**: 429–440.

Condorelli RA, Calogero AE, Vicari E, La Vignera S. Chronic consumption of alcohol and sperm parameters: our experience and the main evidences. *Andrologia* 2015;**47**:368–379.

Cutillas-Tolin A, Minguez-Alarcon L, Mendiola J, Lopez-Espin JJ, Jorgensen N, Navarrete-Munoz EM, Torres-Cantero AM, Chavarro JE. Mediterranean and western dietary patterns are related to markers of testicular function among healthy men. *Hum Reprod* 2015;**30**: 2945–2955.

Dumont L, Oblette A, Rondanino C, Jumeau F, Bironneau A, Liot D, Duchesne V, Wils J, Rives N. Vitamin A prevents round spermatid nuclear damage and promotes the production of motile sperm during in vitro maturation of vitrified pre-pubertal mouse testicular tissue. *Mol Hum Reprod* 2016. doi:10.1093/molehr/gaw063.

Eisenberg ML, Kim S, Chen Z, Sundaram R, Schisterman EF, Buck Louis GM. The relationship between male BMI and waist circumference on semen quality: data from the LIFE study. *Hum Reprod* 2014;**29**:193–200.

Fountoulakis KN, Papadopoulou M, Kleanthous S, Papadopoulou A, Bizeli V, Nimatoudis I, Iacovides A, Kaprinis GS. Reliability and psychometric properties of the Greek translation of the State-Trait Anxiety Inventory form Y: preliminary data. *Ann Gen Psychiatry* 2006;**5**:2.

Gabrielsen JS, Tanrikut C. Chronic exposures and male fertility: the impacts of environment diet and drug use on spermatogenesis. *Andrology* 2016. doi:10.1111/andr.12198.

Gaskins AJ, Afeiche MC, Hauser R, Williams PL, Gillman MW, Tanrikut C, Petrozza JC, Chavarro JE. Paternal physical and sedentary activities in relation to semen quality and reproductive outcomes among couples from a fertility center. *Hum Reprod* 2014;**29**:2575–2582.

Gaskins AJ, Colaci DS, Mendiola J, Swan SH, Chavarro JE. Dietary patterns and semen quality in young men. *Hum Reprod* 2012;**27**:2899–2907.

Giahi L, Mohammadmoradi S, Javidan A, Sadeghi MR. Nutritional modifications in male infertility: a systematic review covering 2 decades. *Nutr Rev* 2016;**74**:118–130.

Jurewicz J, Radwan M, Sobala W, Radwan P, Bochenek M, Hanke W. Dietary patterns and their relationship with semen quality. *Am J Mens Health* 2016. doi:10.1177/1557988315627139.

Ko EY, Sabanegh ES Jr, Agarwal A. Male infertility testing: reactive oxygen species and antioxidant capacity. *Fertil Steril* 2014;**102**:1518–1527.

La Vignera S, Condorelli RA, Vicari E, Tumino D, Morgia G, Favilla V, Cimino S, Calogero AE. Markers of semen inflammation: supplementary semen analysis? *J Reprod Immunol* 2013;**100**:2–10.

Menkveld R, Kruger TF. Advantages of strict (Tygerberg) criteria for evaluation of sperm morphology. *Int J Androl* 1995;**18**:36–42.

Panagiotakos DB, Pitsavos C, Arvaniti F, Stefanadis C. Adherence to the Mediterranean food pattern predicts the prevalence of hypertension hypercholesterolemia diabetes and obesity among healthy adults; the accuracy of the MedDietScore. *Prev Med* 2007;**44**:335–340.

Papathanasiou G, Georgoudis G, Georgakopoulos D, Katsouras C, Kalfakakou V, Evangelou A. Criterion-related validity of the short International Physical Activity Questionnaire against exercise capacity in young adults. *Eur J Cardiovasc Prev Rehabil* 2010;**17**:380–386.

Pärn T, Grau Ruiz R, Kunovac Kallak T, Ruiz JR, Davey E, Hreinsson J, Wånggren K, Salumets A, Sjöström M, Stavreus-Evers A *et al*. Physical activity, fatness, educational level and snuff consumption as determinants of semen quality: findings of the ActiART study. *Reprod Biomed Online* 2015;**31**:108–119.

Sengupta P, Dutta S, Krajewska-Kulak E. The disappearing sperms: analysis of reports published between 1980 and 2015. *Am J Mens Health* 2016. doi:10.1177/1557988316643383.

Sharma R, Agarwal A, Rohra VK, Assidi M, Abu-Elmagd M, Turki RF. Effects of increased paternal age on sperm quality reproductive outcome and associated epigenetic risks to offspring. *Reprod Biol Endocrinol* 2015; **13**:35.

Sharma R, Harlev A, Agarwal A, Esteves SC. Cigarette smoking and semen quality: a new meta-analysis examining the effect of the 2010 World Health Organization Laboratory Methods for the Examination of Human Semen. Eur Urol 2016. doi:10.1016/j.eururo.2016.04.010.

Sofi F, Abbate R, Gensini GF, Casini A. Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. *Am J Clin Nutr* 2010;**92**:1189–1196.

Stokes-Riner A, Thurston SW, Brazil C, Guzick D, Liu F, Overstreet JW *et al*. One semen sample or 2? Insights from a study of fertile men. *J Androl* 2007;**28**:638–643.

Toledo E, Lopez-del Burgo C, Ruiz-Zambrana A, Donazar M, Navarro-Blasco I, Martínez-González MA, de Irala J. Dietary patterns and difficulty conceiving: a nested case-control study. *Fertil Steril* 2011;**96**:1149–1153.

Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* 2003;**348**:2599–2608.

Vellani E, Colasante A, Mamazza L, Minasi MG, Greco E, Bevilacqua A. Association of state and trait anxiety to semen quality of in vitro fertilization patients: a controlled study. *Fertil Steril* 2013;**99**:1565–1572.

Vujkovic M, de Vries JH, Dohle GR, Bonsel GJ, Lindemans J, Macklon NS, van der Spek PJ, Steegers EA, Steegers-Theunissen RP. Associations between dietary patterns and semen quality in men undergoing IVF/ICSI treatment. *Hum Reprod* 2009;**24**:1304–1312.

Vujkovic M, de Vries JH, Lindemans J, Macklon NS, van der Spek PJ, Steegers EA, Steegers-Theunissen RP. The preconception Mediterranean dietary pattern in couples undergoing in vitro fertilization/intracytoplasmic sperm injection treatment increases the chance of pregnancy. *Fertil Steril* 2010;**94**:2096–2101.

World Health Organization. *WHO Laboratory Manual for the Examination and Processing of Human Semen*, 5th edn.. Geneva, Switzerland: WHO Press, 2010.

Zareba P, Colaci DS, Afeiche M, Gaskins AJ, Jørgensen N, Mendiola J, Swan SH, Chavarro JE. Semen quality in relation to antioxidant intake in a healthy male population. *Fertil Steril* 2013;**100**:1572–1579.