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ORIGINAL ARTICLE *Andrology*

Adherence to diet quality indices in relation to semen quality and reproductive hormones in young men

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STUDY QUESTION: Is adherence to an a priori defined diet quality indices [Alternate Healthy Index 2010 (AHEI-2010), relative Mediterranean diet score (rMED) or dietary approaches to stop hypertension (DASH)] associated with semen quality and reproductive hormone levels in young men?

SUMMARY ANSWER: Greater adherence to the DASH diet is related to higher sperm counts.

WHAT IS KNOWN ALREADY: Studies assessing the relationship between dietary intake and male reproductive function have mainly been focused on specific nutrients, food groups or data-driven dietary patterns, but the evidence on a priori defined dietary indices is still scarce.

STUDY DESIGN, SIZE, DURATION: Cross-sectional study of 209 male university students recruited from October 2010 to November 2011 in Murcia Region (Southern Spain).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Healthy young men aged 18–23 years were included in this study. Diet was assessed using a validated food frequency questionnaire and three a priori-defined dietary indices (AHEI-2010, rMED and DASH) were calculated. Linear regression was used to analyze the relation between the three dietary indices and semen quality parameters and reproductive hormone levels accounting for potential confounders and covariates.

MAIN RESULTS AND THE ROLE OF CHANCE: We found statistically significant positive associations between the DASH index and sperm concentration (P, trend = 0.04), total sperm count (P, trend = 0.04) and total motile sperm count (P, trend = 0.02). No associations were observed for other semen parameters or male reproductive hormones.

LIMITATIONS, REASONS FOR CAUTION: Even though we adjusted for several known and suspected confounders we cannot exclude the possibility of residual or unmeasured confounding or chance findings. Subjects were blinded to the study outcomes thus reducing the potential influence on their report of diet. Our sample size may be too small to rule out associations with other semen parameters or reproductive hormones. Causal inference is limited, as usual with all observational studies.

WIDER IMPLICATIONS OF THE FINDINGS: The results suggest that greater adherence to the DASH may help improve sperm counts. This study was carried out on young men from the general population. However, results may differ among other populations (e.g. infertile men). Therefore, further research is needed to confirm these findings and extend these results to other populations.

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Key words: AHEI-2010 / DASH / dietary indices / reproductive hormones / rMED / semen quality

Introduction

Declining semen quality is an ongoing controversy in male reproductive health (Swan *et al*., 2000; Jørgensen *et al*., 2002; Carlsen *et al*., 2005; Axelsson *et al*., 2011; Rolland *et al*., 2013; Levine *et al.* 2017). Several potential culprits have been suggested as explanations for reported trends in semen quality or altered reproductive hormone levels, including concurrent trends in decreased physical activity and increase in sedentary behavior (Gaskins *et al*., 2012; Priskorn *et al*., 2016), obesity (Sermondade et al., 2013), exposure to endocrine-disrupting chemicals (Adoamnei et al., 2018a,b) or change in diet quality (Attaman *et al*., 2012; Salas-Huetos *et al*., 2017). The impact of diet on semen quality is, however, still poorly resolved (Mínguez-Alarcón *et al*., 2018).

Several studies have reported associations between intake of isolated micro or macronutrients, food groups or a posteriori dietary patterns and semen quality and reproductive hormone levels (Mendiola et al., 2009; Vujkovic *et al*., 2009; Mendiola et al., 2010; Gaskins *et al*., 2012; Mínguez-Alarcón et al., 2012; Afeiche et al., 2013; Zareba *et al*., 2013; Afeiche et al., 2014; Chavarro *et al*., 2014; Chiu *et al*., 2015; Cutillas-Tolín *et al*., 2015; Liu *et al*., 2015; Abbasihormozi *et al*., 2017; Mínguez-Alarcón et al., 2017; Oostingh *et al*., 2017; Tiseo *et al*., 2017; Jurewicz *et al*., 2018; Adoamnei *et al*., 2019). A posteriori dietary pattern approach processes the gathered dietary information through multivariate statistical methods, such as principal component analysis (PCA) (Gaskins *et al*., 2012; Cutillas-Tolín *et al*., 2015; Liu *et al*., 2015; Jurewicz *et al*., 2018; Oostingh *et al*., 2017).

Nonetheless, the interpretation of the components derived by PCA can be challenging and subjective (Jolliffe and Cadima, 2016). Conversely, an a priori defined dietary indices approach is based on pre-defined healthy patterns and has advantages including relying on current scientific data regarding nutrition, health and disease (Chiuve *et al*., 2012; Harmon *et al*., 2015). Several a priori-defined indices have been related to well-known health benefits, showing anti-oxidative and anti-inflammatory actions (Trichopoulou *et al*., 2003; Fung *et al*., 2005) and protective factors for chronic diseases or mortality (Fung *et al*., 2010; Mursu *et al*., 2013; Harmon *et al*., 2015; Jacobs *et al*., 2015, 2016; Mattei *et al*., 2017), but the reproductive benefits of these dietary indices are still largely unknown. In general, the overwhelming majority of the literature on diet and male reproductive outcomes has been focused on individual nutrients whereas individual and public health recommendations about diet and health are generally made in terms of diet patterns, particularly in more recent years—food or pattern-based as opposed to nutrient-based—(Willett & Stampfer, 2013; American Heart Association, 2015; U.S. Department of Health and Human Services and U.S. Department of Agriculture, 2015; American Institute for Cancer Research, 2019). Moreover, most diet recommendations for health promotion are based on their impact on the prevention of cardiovascular disease and other major chronic diseases but it is unclear to what extent they may also provide male reproductive benefits. To date, only two studies have examined the relationship between the adherence to dietary indices and semen quality parameters in male partners of couples seeking for fertility treatment (Karayiannis *et al*., 2017; Efrat *et al.,* 2018). Karayiannis et al. (2017) observed a positive relationship between sperm concentration, motility and morphology and the Mediterranean diet score (MDS). Efrat et al. (2018) also reported a positive association between the MDS, healthy eating index (HEI), alternate healthy index (AHEI), alternate MDS (aMED) and dietary approaches to stop hypertension (DASH) and several semen parameters in a comparable population.

However, to our knowledge, no study has examined the relationships between dietary indices and male reproductive parameters in unselected men unaware of their fertility status or testis function. Therefore, we sought to evaluate the associations between these three a priori-defined dietary indices and semen quality and reproductive hormone levels in young men.

Materials and Methods

Study population

The Murcia Young Men's Study is a cross-sectional study of university students 18–23 years old in the Murcia Region (Southern Spain). Study details are described elsewhere (Mendiola *et al*., 2013). Briefly, a total of 215 students agreed to participate and completed the study visit between October 2010–November 2011. Six men who reported implausible total caloric intake (>5000 kcal/day) were excluded, leaving 209 men for the current analysis. At the study visit men underwent an andrological examination, provided semen and blood samples and completed questionnaires on lifestyle and food frequency. The Research Ethics Committee of the University of Murcia approved this study and written informed consent was obtained from all subjects.

Dietary assessment and a priori-defined dietary indices

We used a validated 101-food item semi-quantitative food frequency questionnaire (FFQ) to assess the usual diet (available at: http://bibliodieta.umh.es/files/2011/07/CFA101.pdf) (Vioque *et al*., 2007, 2013). Subjects were asked to report how often, on average, they had consumed each food item over the past year. The questionnaire offered nine options for frequency of consumption for each food, ranging from never or less than once a month to six or more times a day. Nutrient values for each food were obtained from the US Department of Agriculture and supplemented with Spanish sources (Palma *et al*., 2008; U.S. Department of Health and Human Services, 2018). The reproducibility and validity of this FFQ are comparable with other widely used FFQs (Willett, 2012). The mean correlation coefficients between nutrient intakes estimated using prospectively collected diet records and those estimated with the FFQ were 0.44

for validity and 0.44 for reproducibility (Vioque, 1995). This FFQ also showed satisfactory biochemical validity when compared with the plasma levels of carotenoids and vitamin C in further validation studies with other adult populations (Vioque *et al.*, 2007, 2013). The FFQ dietary information was used to calculate the following three a priori-defined dietary indices AHEI-2010, relative MDS (rMED) and DASH.

Briefly, AHEI-2010 was the updated version of AHEI designed in 2012 by Chiuve and colleagues based on a comprehensive review of the relevant literature and discussions with other nutrition researchers to identify foods and nutrients that have been associated consistently with lower risk of chronic disease in clinical and epidemiologic investigations. The rMED is a variation of the original MDS (Trichopoulou *et al.*, 2003) created by Buckland *et al.* in 2009 to evaluate the adherence to Mediterranean diet and risk of coronary heart disease in Spain. The DASH index was constructed based on the DASH clinical trial in which dietary pattern rich in fruit, vegetables and low-fat dairy products can substantially lower blood pressure (U.S. Department of Health and Human Services, 2018). This a priori defined index was described by Fung *et al.* in 2008. Definition of the scoring system, number of components, total score and components of AHEI-2010, rMED, and DASH scores can be seen on Supplementary Table SI.

Semen analysis and physical examination

Semen analyses were carried out as described in detail elsewhere (Mendiola *et al.*, 2013). Briefly, men were asked to abstain from ejaculation for at least 48 hours before sample collection. Abstinence time was recorded as the time between current and previous ejaculation as reported by the study subject. Ejaculate volumes were estimated by specimen weight, assuming a semen density of 1.0 g/ml. Sperm concentration was evaluated by hemocytometer (Improved Neubauer; Hauser Scientific, Inc., Horsham, PA, USA). The spermatozoa were classified as either motile or immotile (World Health Organization, 2010) to report the percentage of motile spermatozoa [progressive (PR) and non-progressive (NP)]. Total sperm count (TSC; volume × sperm concentration) and total motile sperm count [TMSC; volume × sperm concentration × % motile sperm (PR + NP)] were also calculated. Smears for morphology were made, air-dried, fixed, Papanicolaou stained and assessed using strict criteria (Menkveld *et al.*, 1990). The same specialized biologist carried out all the semen analyses. 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. Body weight and height were measured using a digital scale (Tanita SC 330-S, London, UK). All participants were measured standing barefoot and wearing underwear at the study visit. BMI was calculated as weight in kilograms divided by squared height in meters. The presence of varicocele or other scrotal abnormalities was evaluated and recorded.

Reproductive hormones measurement

Hormone analysis methods have been described previously (Asklund *et al.*, 2007; Cutillas-Tolín *et al.*, 2015). Briefly, blood samples were drawn from participants' cubital veins on the same time of the day of semen sample collection and were stored and frozen. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH) and sex hormone-binding globulin (SHBG) were determined using time-resolved immunofluorometric assays (DELFIA; PerkinElmer, Skovlund, Denmark). Intra- and interassay variations were <5% in each of the three assays. Serum testosterone (T) levels were determined using a time-resolved fluoroimmunoassay (DELFIA; PerkinElmer) with intra- and interassay variation of <8%. Estradiol (E2) was measured by radioimmunoassay (Pantex, Santa Monica, CA) 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 interassay variation of 13% and 18%, respectively. Free testosterone (cFT) was calculated using the equation of Vermeulen et al. (1999) assuming a fixed albumin of 43.8 g/L.

Statistical analyses

Descriptive statistics are shown using crude data. Continuous variables were summarized by median and interquartile range (IQR: 25th–75th) and categorical variables given as number (*n*) and percentage (%). To test for associations of baseline characteristics across quartiles of the three a priori-defined dietary indices, ANOVA and $\chi 2$ tests were used for continuous and categorical variables, respectively. Linear regression was used to examine the associations between the three dietary indices and semen parameters and reproductive hormones. Semen volume, sperm concentration, TSC, TMSC, percentage of morphologically normal sperm, serum FSH and estradiol levels, showed non-normal distributions and were transformed using the natural log (ln) before analysis. Sperm motility and other hormones were used untransformed. The three dietary indices were categorized in quartiles and the lowest quartile of each dietary index was considered as the reference group.

The potential effect of several important or potential covariates or confounders [e.g. age (years), BMI (kg/m^2), presence of varicocele (yes/no), smoking (yes/no), ejaculation abstinence time (hours), moderate-vigorous exercise (hours/week), total caloric intake (kcal/-day)] were assessed. When inclusion of a potential covariate resulted in a change in the β-coefficient of more than 10%, the variable was retained in the final models. These variables included factors previously related to serum reproductive hormones or semen quality in this or other studies, regardless of whether they had been previously described as predictors of male reproductive health. The final covariates included in the models were: total caloric intake, moderate-vigorous exercise, presence of varicocele, ejaculation abstinence time and time to start of analysis (for sperm motility only). For reproductive hormones, similar models were calculated adjusting for time to blood sampling (minutes).

We examined linearity of associations between the three a priori-defined dietary indices and semen parameters and reproductive hormone levels by regressing reproductive parameters on quartiles of dietary indices and testing for linearity of trend using median measure in each quartile as a continuous variable in the linear regression models (*P*, trend across quartiles). We considered that a relationship was present when we found a statistically significant linear trend in semen parameters or reproductive hormones across dietary indices quartiles. All tests were two-tailed and the level of statistical significance was set at 0.05. Statistical analyses were performed with the statistical package IBM SPSS 25.0 (IBM Corporation, Armonk, New York, USA.

Table I **Demographic characteristics of men in the Murcia Young Men's Study according to quartiles of adherence to dietary quality indices (*n* = 209).**

	AHEI-2010		rMED		DASH	
Median value for Q1 and Q4	**Q1 (45.0) (n = 55)**	**Q4 (66.5) (n = 52)**	**Q1 (6.0) (n = 76)**	**Q4 (13.0) (n = 37)**	**Q1 (17.0) (n = 69)**	**Q4 (30.0) (n = 42)**
Age (years)	20.7(18.0–22.7)	20.2 (18.1–22.3)	20.4 (18.1–22.7)	20.4 (18.3–23.0)	20.7 (18.3–22.9)	20.2 (18.1–23.1)
BMI (kg/m^2)	23.1 (19.0–34.6)	24.0 (19.9–29.9)	23.6 (19.4–30.6)	23.8 (20.4–30.9)	23.7 (19.2–30.8)	23.8 (20.5–26.5)
Calories intake (kcal/day)	2470 (1458–3682)	2337 (1230–3992)	2271 (1249–3634)	2496 (1391–3873)	1775 (1127–2411)	3277* (2399–4499)
Current smoker, n (%)	22 (40.7)	14 (26.9)	21 (28.0)	14 (38.9)	20 (29.4)	14 (33.3)
Alcohol intake (g/day)	6.2 (0.0–30.5)	6.7 (0.6–26.5)	5.9 (0.0–39.3)	14.0 (0.47–38.0)	5.4 (0.0–25.7)	10.6* (1.4–32.5)
Physical activity (h/wk)	6.0 (0.0–19.2)	5.0 (0.0–17.0)	6.0 0.0–17.5)	5.7 (0.0–14.5)	5.0 (0.0–13.5)	7.0* (0.3–19.9)
Abstinence time (hours)[a]	83.0 (39.8–144)	76.5* (40.6–145)	71.5 (39.9–71.5)	69.0 (39.6–177)	72.0 (39.0–119)	69.0 (39.6–249)
Presence of Varicocele, n (%)	13 (23.6)	4 (9.6)	16 (21.1)	3* (8.1)	10 (14.5)	6 (14.3)
Taken any medication[b], n (%)	10 (18.2)	12 (23.1)	16 (21.1)	10 (27.0)	16 (23.2)	10 (23.8)
Suffered Prolonged disease[c], n (%)	2 (3.6)	2 (3.8)	7 (9.2)	1 (3.0)	7 (10.1)	5 (7.7)

Continuous variables are shown as median and interquartile range (IQR: 25th–75th) unless otherwise indicated. Kruskal–Wallis test for continuous variables and the $\chi 2$ test for categorical variables.

*P-value < 0.05 among quartiles within each dietary index.

[a] Abstinence time: period calculated as difference between time of current ejaculation and self-reported time of previous ejaculation;

[b] Taken any medication during 3 months prior to participation in study (mostly antibiotics or medication against allergy);

[c] Long-lasting disease (including diabetes/thyroid disease), sexually transmitted diseases (diagnosed with epididymitis, chlamydia or gonorrhea). AHEI-2010, Alternate Healthy Index 2010; rMED, Relative Mediterranean Diet Score; DASH, Dietary Approaches to Stop Hypertension.

Results

Demographic characteristics, semen parameters and reproductive hormone levels among a priori-defined dietary indices quartiles are summarized in Table I. Study subjects were healthy young university students [median (IQR): 20.4 [19.6–21.4] years] with BMI of 23.6 (IQR: 21.8–25.5) kg/m^2. Almost one-third of the subjects smoked (31.9%) and varicocele was detected in 15% of the participants. Median abstinence time was 71.0 hours (IQR: 59.0–92.0 hours), median sperm concentration 43.4 mill/mL (IQR: 22.0–72.3 mill/mL), morphologically normal sperm 9.0% (IQR: 6.0%–14.0%), progressive motility 48.3% (IQR: 41.4%–55.3%) and semen volume 3.0 mL (IQR: 2.0–4.0 mL). In general, all hormones showed serum levels within normal ranges. Median and IQR values for AHEI-2010, rMED and DASH indices were 56 (IQR: 50–62.5), 9 (IQR: 7–11) and 22 (IQR: 19–27), respectively. Diet indices were modestly to highly correlated with each other, having a Spearman's rho correlation coefficients between 0.19 and 0.59 with P-values <0.01 (Supplementary Table SII).

Men with higher adherence to DASH had greater total energy and alcohol intake than men with low adherence ($P < 0.05$). Physical activity was greater in men with high adherence to DASH compared to low adherence men ($P = 0.02$), and abstinence time was significantly lower in men with high adherence to AHEI-2010 compared to low ones ($P = 0.03$). Lastly, men with high adherence to rMED index compared to low ones presented significantly lower number of varicoceles ($P = 0.04$).

Greater adherence to the DASH diet was positively associated to TSC (P, trend = 0.04) and sperm concentration (P, trend = 0.04) in multivariate adjusted models (Table II). These differences were further magnified when examining TMSC (Fig. 1). Relative to men in the lowest quartile (Q1) of adherence to DASH index, the adjusted difference (95%CI) of TMSC (millions) for men in the second, third and fourth quartile were 21.1% (−18.5; 60.6), 42.2% (1.6; 82.7) and 73.8% (19.2; 128), respectively (P, trend = 0.02). Similar results were obtained when further adjustment by AHEI-2010 and rMED was carried out (P, *trends* for sperm counts ≤0.04). Moreover, when examining which specific components of DASH were related to TMSCs, fruits was the most important one ($\beta = 0.11$, $P = 0.059$; Supplementary Table SIII). Adherence to the other diet indices examined was not related to semen quality parameters (Table II). Moreover, none of the diet indices was related to reproductive hormone levels (Table III).

Discussion

We found that higher adherence to DASH diet was associated with higher sperm counts. To our knowledge this is the first study evaluating this matter on unselected young men as well as the relationships with reproductive hormone levels. Previously, only two studies have evaluated associations between a priori-defined dietary indices and semen quality (Karayiannis *et al.*, 2017; Efrat *et al.*, 2018). Our results are partially consistent with Efrat et al. (2018) as higher adherence to

Table II **Multivariate adjusted[1] associations of dietary indices and semen quality parameters ($n = 209$) reported as percentage (%) difference or untransformed model coefficients (only for motility), with 95%CI.**

Range for each quartile of index	Sperm concentration (mill/mL)	Total Sperm Count (Mill.)	Total Motile Sperm Count (Mill)	Motility (PR + NP) (%)	Morphologically normal sperm (%)
AHEI-2010					
Q1 (34–50)	Ref.	Ref.	Ref.	Ref.	Ref.
Q2 (51–56)	11.5% (−26.2; 49.2%)	30.1% (−5.2; 65.4%)	34.7% (−2.9; 72.3%)	−0.64 (−4.8; 3.6)	16.6% (−8.4; 41.5%)
Q3 (57–62)	−12.4% (−49.8; 24.9%)	0.50% (−35.2; 34.2%)	2.6% (−34.4; 39.6%)	−0.50 (−4.6; 3.6)	−5.0% (−29.6; 19.5%)
Q4 (63–80)	−6.2% (−43.3; 30.9%)	13.3% (−21.3; 48.0%)	26.7% (−10.5; 63.8%)	1.0 (−3.1; 5.1)	−9.7% (−34.1; 14.7%)
P_{trend}	0.48	0.85	0.42	0.62	0.19
rMED					
Q1 (1–7)	Ref.	Ref.	Ref.	Ref.	Ref.
Q2 (8–9)	−2.7% (−38.5; 33.0%)	12.4% (−20.9; 45.8%)	7.6% (−28.1; 43.2%)	−0.89 (−4.9; 3.1)	−0.60% (−24.5; 23.3%)
Q3 (10–11)	0.50% (−34.5; 35.6%)	27.9% (−4.7; 60.6%)	25.4% (−9.6; 60.3%)	−1.6 (−5.4; 2.4)	−5.6% (−28.8; 17.6%)
Q4 (12–15)	−18.3% (−57.2; 20.6%)	21.9% (−14.5; 58.3%)	28.9% (−9.4; 69.1%)	0.10 (−4.2; 4.3)	5.5% (−20.2; 31.3%)
P_{trend}	0.47	0.11	0.08	0.79	0.88
DASH					
Q1 (10–19)	Ref.	Ref.	Ref.	Ref.	Ref.
Q2 (20–22)	13.3% (−26.5; 53.0%)	24.5% (−12.6; 61.7%)	21.1% (−18.5; 60.6%)	−1.1 (−5.6; 3.3)	23.2% (−3.3; 49.7%)
Q3 (23–27)	32.7% (−7.7; 73.0%)	31.7% (−6.2; 69.7%)	42.2% (1.6; 82.7%)	−0.10 (−4.7; 4.6)	27.5% (0.4; 54.6%)
Q4 (28–37)	47.3% (−7.5; 102%)	64.7% (13.5; 72.0%)	73.8% (19.2; 128%)	−1.5 (−8.0; 5.0)	31.4% (−6.4; 69.2%)
P_{trend}	0.04	0.04	0.02	0.80	0.13

[1] Adjusted for calories intake (kcal/day), physical activity (h/week), presence of varicocele (yes/no), abstinence time (hours) and time to start of analysis (min; for sperm motility only). Percentage (%) change shows natural logarithm values back-transformed to improve interpretability, and untransformed model coefficients shows the mean difference in percentage points between the percentage of motile sperm (PR + NP) in a given dietary index quartile (second, third, or fourth, Q2, Q3, Q4) and the reference group (first quartile, Q1). AHEI-2010, Alternate Healthy Index 2010; rMED, Relative Mediterranean Diet Score; DASH, Dietary Approaches to Stop Hypertension.

DASH was associated with higher sperm concentration among men attending a Israeli fertility centre. However, the authors also found that AHEI was positively related to sperm concentration; DASH and AHEI were positively associated with normal sperm morphology; and only AHEI had a significant association with TSC (Efrat *et al.*, 2018). However, our results are not consistent with Karayiannis *et al.* (2017), as they reported that men from couples attending a Greek fertility clinic in the lowest tertile of the MDS had around 2.6 times higher likelihood of having abnormal sperm concentration, sperm count and motility (using WHO reference values), compared to men in the highest tertile of the score. In our study, a relatively low percentage of men presented sperm parameters below the WHO cut-offs, so a similar comparison would not be appropriate.

The DASH eating plan emphasizes increased consumption of fruits and vegetables, whole grains, nuts and low-fat dairy (Rifai and Silver, 2016). These dietary recommendations are nutritionally translated into lower simple carbohydrate, cholesterol, saturated and trans fats intakes and higher protein, fiber, calcium, magnesium and potassium intakes. DASH diet has been associated with a decrease in blood pressure (Rifai and Silver, 2016) as well as improving insulin resistance and diabetes (Shirani *et al.*, 2013) or metabolic syndrome (Calton *et al.*, 2014; Soares *et al.*, 2016; Welty *et al.*, 2016). Some of the pathophysiologic changes occurring in these conditions are related to oxidative stress, and the components of the DASH diet may enhance antioxidant capacity (Lopes *et al.*, 2003; Shirani *et al.*, 2013; Asemi *et al.*, 2014). That may be one of the reasons we observed an association between DASH and sperm counts, as it is known that oxidative stress impairs semen quality (Agarwal *et al.*, 2014).

Similarly, it is recognized that semen quality can be affected by the presence of an inflammatory microenvironment [e.g. interleukins, tumor necrosis factor-α (TNFα)] (La Vignera *et al.*, 2011). The role of diet in reducing inflammation and thereby modulating the risk of non-communicable diseases is supported by numerous studies (Smidowicz and Regula, 2015; Neale, *et al.*, 2016). Actually, DASH diet may be comparable to an anti-inflammatory a posteriori dietary pattern known as prudent dietary pattern (characterized by high intake of fish, chicken, fruit, vegetables, legumes and whole grains) that has been significantly associated with higher sperm motility in young US men (Gaskins *et al.*, 2012) or concentration (Jurewicz *et al.*, 2018) in men who attended a Polish infertility clinic. Our research group also previously found that a Mediterranean pattern was positively associated with TSC in the same population of young men we are reporting on (Cutillas-Tolín *et al.*, 2015).

Moreover, some food groups included in the DASH index may also have a positive influence on semen quality. Fruits was the most important component related to sperm counts in our study population. Indeed, total fruit and vegetable intake has been associated with a lower risk of asthenoteratospermia (Eslamian *et al.*, 2012) or oligoasthenoteratospermia (Mendiola *et al.*, 2009) in men attending assisted reproduction clinics in Iran or Spain, respectively.

No association was found between the indices: AHEI-2010, rMED and DASH and reproductive hormone concentrations in our study

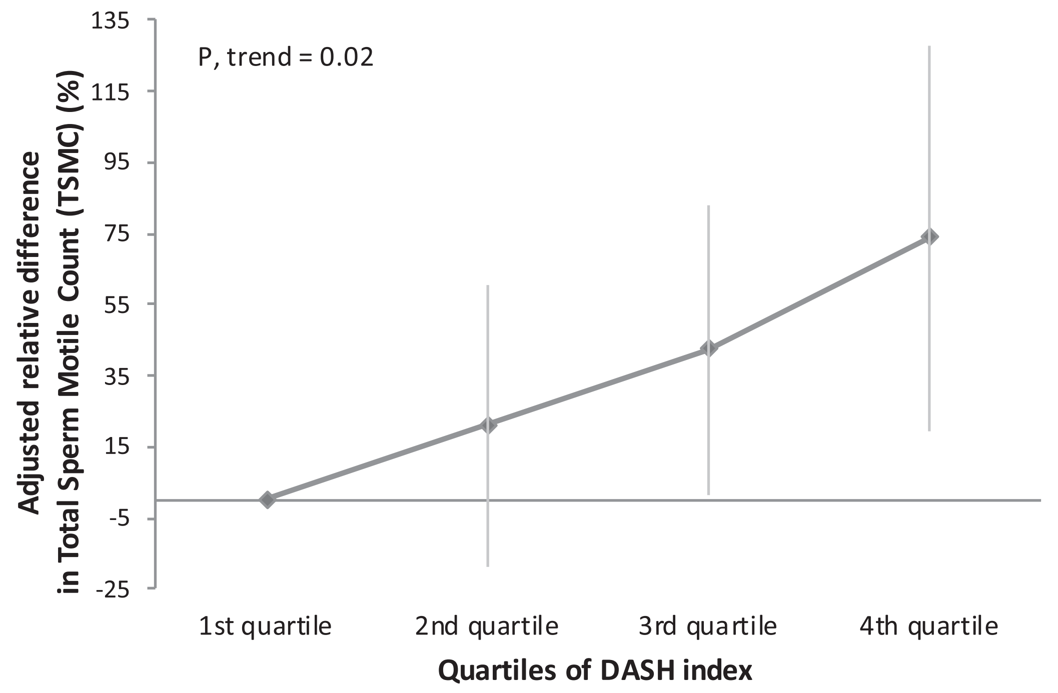

Figure 1 **Adjusted relative difference (%) and 95% confidence intervals in TMSC for men in increasing quartiles of adherence to the DASH diet.**

population. The literature about nutrition intake and hormone levels in humans is very scarce. Cutillas-Tolín et al. (2015) found that neither Mediterranean nor Western dietary pattern was related to hormone levels in the same study population employed here. Similarly, in a young US male population, weak or no association between dairy food (Afeiche *et al.*, 2013), meat (Afeiche *et al.*, 2014) or sugar-sweetened beverage intake (Chiu *et al.*, 2014) and reproductive hormone levels have been reported. However, recently, Mínguez-Alarcón et al. (2017) showed that *trans*, monounsaturated and polyunsaturated fatty acids intake were associated with serum blood levels of several reproductive hormones (testosterone, inhibin or LH) in young Mediterranean men, showing that they may influence testicular function.

This study has some limitations. As with all observational studies, causal inference is restricted, therefore prevents causal interpretations of the observed associations. Adjustment for known and potential confounders and covariates was carried out. However, there is always the possibility of residual or unmeasured confounding, and chance findings must always be contemplated. Moreover, it cannot be precluded that type II error might explain the negative results regarding other sperm parameters or reproductive hormones. There is no perfect method to assess diet, so bias due to measurement errors may also occur, and therefore calculating the prior dietary indices from a FFQ. Nonetheless, the FFQ employed in this work was previously validated in an adult Spanish population of the same area and it has been utilized in other populations (Guxens *et al.*, 2012; Castelló *et al.*, 2014). Many foods, particularly those that are plant-based, have nutrient contents that change substantially depending on the growing environments and preparation methods. However, any bias in assessing diet should not be differential which should strengthen our findings. Only one semen sample was obtained per subject, nevertheless, several studies have reported that one sample is adequate to evaluate semen quality in epidemiological studies (Stokes-Riner *et al.*, 2007; Chiu *et al.*, 2017). Equally, a single sample can be employed to categorize male reproductive hormones (Vermeulen and Verdonck, 1992). It is also worth mentioning the limitation that dietary assessment and time from previous ejaculation were both self-reported. Our population may have been potentially exposed to pesticides in fruit and vegetables and this may have an impact on the observed associations as shown elsewhere (Chiu *et al.*, 2015, 2016). Unfortunately, there is not pesticide residues surveillance data in Spain to construct and validate these equivalent indices previously reported (Chiu *et al.*, 2015, 2016). Consequently, we were not able to explore this potential issue in our study population. Finally, multiple comparisons might be of concern in statistical tests. However, in our study, this might be ameliorated since specific objectives about specific dietary indices and male reproductive outcomes were defined a priori. This study was carried out on healthy young men with unknown fertility; therefore, it is difficult to predict the external validity or generalizability of the obtained results to other male populations. Consequently, future studies in more diverse populations (e.g. fertile or other young men populations), as well as other epidemiological designs providing more evidence of causality are recommended.

In conclusion, higher adherence to DASH index was associated with higher TSC and TMSC. On balance, our data support the hypothesis that a potential diet with high intake of low-fat dairy products, vegetables, fruits and fish and low intake of sugar-sweetened beverages, processed meat and salt, such as DASH diet, might have positive effects

Table III Multivariate adjusted[1] associations of dietary indices and serum reproductive hormone concentrations ($n = 209$) reported as percentage (%) difference (only for FSH and estradiol) or untransformed model coefficients, with 95%CI.

Range for each quartile of index	LH (IU/L)	FSH (IU/L)	Estradiol (pmol/L)	Calculated Free testosterone (pmol/L)	Total testosterone (nmol/L)	Inhibin B (pg/mL)	SHBG (nmol/L)
AHEI-2010							
Q1 (34–50)	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Q2 (51–56)	−0.03 -(0.69; 0.64)	−18.7% (−40.3; 2.8%)	2.0% (−9.3; 13.3%)	−6.2 (−74.7; 62.2)	−0.61 (−3.3; 2.0)	15.4 (−14.8; 45.6)	−1.2 (−5.4; 3.0)
Q3 (57–62)	0.15 (−0.50; 0.80)	−0.2% (−20.9; 21.3%)	4.9% (−6.2; 15.9%)	−18.6 (−86.3; 49.1)	0.02 (−2.6; 2.6)	11.1 (−18.4; 40.7)	1.2 (−2.9; 5.3)
Q4 (63–80)	0.11 (−0.55; 0.76)	2.0% (−19.2; 23.1%)	−0.5% (−11.6; 10.5%)	−4.1 (−71.3; 63.1)	0.06 (−2.6; 2.5)	7.4 (−22.2; 37.0)	0.34 (−3.8; 4.5)
P_{trend}	0.64	0.48	0.94	0.82	0.88	0.70	0.61
rMED							
Q1 (1–7)	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Q2 (8–9)	0.28 (−0.35; 0.90)	10.1% (−10.0; 30.2%)	−6.1% (−16.6; 4.4%)	−39.1 (−103; 24.9)	−1.7 (−4.2; 0.8)	−7.2 (−35.6; 21.1)	−1,3 (−5.2; 2.7)
Q3 (10–11)	−0.22 (−0.83; 0.39)	−3.2% (−22.9; 16.5%)	−9.5% (−19.8; 0.80%)	−47.7 (−110; 14.6)	−1.2 (−3.6; 1.3)	0.35 (−27.4; 28.1)	1.5 (−2.3; 5.4)
Q4 (12–15)	0.43 (−0.25; 1.1)	29.1% (7.2; 51.0%)	4.3% (−7.1; 15.7%)	39.4 (−29.7; 109)	0.93 (−1.8; 3.6)	−26.1 (−56.9; 4.7)	−1.3 (−5.6; 3.0)
P_{trend}	0.54	0.07	0.97	0.69	0.77	0.20	0.93
DASH							
Q1 (10–19)	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Q2 (20–22)	−0.24 (−0.95; 0.47)	5.4% (−18.0; 28.8%)	−12.3% (−24.3; −0.30%)	−59.4 (−133; 13.8)	−2.2 (−5.0; 0.63)	−6.0 (−38.2; 26.4)	0.68 (−3.8; 5.2)
Q3 (23–27)	0.15 (−0.58; 0.88)	3.0% (−21.0; 26.9%)	−0.70% (−13.0; 11.5%)	−30.4(−105; 44.6)	−1.1 (−3.6; 1.8)	−19.0 (−52.1; 14.1)	0.34 (−4.2; 5.0)
Q4 (28–37)	−0.06 (−1.1; 0.95)	0.5% (−32.7; 33.8%)	−5.4% (−22.4; 11.7%)	−13.5 (−117; 90.4)	−0.56 (−4.6; 3.5)	−30.0 (−76.0; 16.0)	0.12 (−6.3; 6.6)
P_{trend}	0.82	0.94	0.89	0.77	0.78	0.17	0.96

[1] Adjusted for calories intake (kcal/day), physical activity (h/week) and time to blood sampling (min). Percentage (%) change shows natural logarithm values back-transformed to improve interpretability, and untransformed model coefficients shows the mean difference in percentage points between the hormone values in a given dietary index quartile (second, third, or fourth) and the reference group (first quartile). SHBG, sex hormone binding globulin; AHEI-2010, Alternate Healthy Index 2010; rMED, Relative Mediterranean Diet Score; DASH, Dietary Approaches to Stop Hypertension.

on sperm counts. Further research is warranted to confirm these findings and extend these results to other male populations.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

AMTC, NJ and JM were involved in study conception and study design. JM, NJ and AMTC were involved in study execution and acquisition of data. EA, ACT, JM, EMNM, JV, MMG, NJ, JEC and AMTC contributed to data analysis and interpretation. ACT, EA, MMG, EMNM, JV and JM drafted the manuscript. All authors provided substantial intellectual contributions and approved the final version of the manuscript.

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Conflict of interest

Authors have no competing interests to declare.

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