Human Reproduction, Vol.33, No.3 pp. 494–502, 2018
Advanced Access publication on January 30, 2018 doi:10.1093/humrep/dey003

human reproduction

ORIGINAL ARTICLE *Reproductive epidemiology*

Adherence to the Mediterranean diet and IVF success rate among non-obese women attempting fertility

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Submitted on July 19, 2017; resubmitted on December 16, 2017; accepted on January 5, 2018

STUDY QUESTION: Is adherence to the Mediterranean diet (MedDiet) associated with better IVF performance in women attempting fertility?

SUMMARY ANSWER: Greater adherence to the MedDiet, defined using the validated Mediterranean diet score (MedDietScore), was associated with a higher likelihood of achieving clinical pregnancy and live birth among non-obese women <35 years of age.

WHAT IS KNOWN ALREADY: Diet impacts fertility and certain nutrients and food groups appear to have a greater effect on reproductive health, but there are relatively few published data on the role of dietary patterns, and the MedDiet in particular, on assisted reproductive performance.

STUDY DESIGN, SIZE, DURATION: This prospective cohort study included 244 non-obese women (22–41 years of age; BMI < 30 kg/m^2) who underwent a first IVF treatment in an Assisted Conception Unit in Athens, Greece, between November 2013 and September 2016. The study was designed to evaluate the influence of habitual dietary intake and lifestyle on fertility outcomes.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Diet was assessed before the IVF treatment via a validated food-frequency questionnaire. Adherence to the MedDiet was assessed through the MedDietScore (range: 0–55), with higher scores indicating greater adherence. Intermediate outcomes (oocyte yield, fertilization rate and embryo quality measures) and clinical endpoints (implantation, clinical pregnancy and live birth) were abstracted from electronic medical records. Associations between MedDietScore and IVF outcomes were analysed using generalized linear models adjusting for age, ovarian stimulation protocol, BMI, physical activity, anxiety levels, infertility diagnosis, caloric intake and supplements use.

MAIN RESULTS AND THE ROLE OF CHANCE: No association of MedDietScore with any of the intermediate outcomes or with implantation was found. However, compared with women in the highest tertile of the MedDietScore (≥36, $n = 86$), women in the lowest tertile (≤30, $n = 79$) had significantly lower rates of clinical pregnancy (29.1 vs 50.0%, $P = 0.01$) and live birth (26.6 vs 48.8%, $P = 0.01$). The multivariable-adjusted relative risk (95% CI) for clinical pregnancy comparing women in the lowest with women in the highest tertile of the MedDietScore was 0.35 (0.16–0.78; *P*-trend=0.01), and for live birth it was 0.32 (0.14–0.71; *P*-trend = 0.01). These associations were significantly modified by women's age (*P*-interaction <0.01 for both outcomes). MedDietScore was positively related to clinical pregnancy and live birth among women <35 years old ($P \leq 0.01$) but not among women ≥35 years. Among women <35 years, a beneficial 5-point increase in the MedDietScore was associated with ~2.7 times higher likelihood of achieving clinical pregnancy and live birth.

LIMITATIONS, REASONS FOR CAUTION: Our finding cannot be generalized to the whole reproductive population nor to obese women nor to women attending infertility clinics around the world. In addition, due to the observational study design, causal inference is limited.

WIDER IMPLICATIONS OF THE FINDINGS: The results suggest that diet modifications and greater compliance to the Mediterranean diet may help increase the chances of a successful pregnancy and delivering a live baby for women undergoing IVF treatment.

STUDY FUNDING/COMPETING INTEREST(S): This work was partially supported by a grand from Harokopio University (KE321). All authors declare no conflicts of interest.

TRIAL REGISTRATION NUMBER: NCT03050944.

Key words: Mediterranean diet / female fertility / reproduction / IVF / live birth rate

Introduction

Lifestyle factors including diet, smoking, exercise and stress affect reproductive performance, also during assisted reproduction (Klonoff-Cohen, 2005; Hornstein, 2016). Several recent reports have suggested that preconception dietary habits may influence IVF outcome, such as oocyte and embryo quality, implantation and successful completion of pregnancy (Braga *et al.*, 2015; Firns *et al.*, 2015; Hornstein, 2016; Garruti *et al.*, 2017; Machtinger *et al.*, 2017).

Most of the work on this topic has focused on the role of isolated nutrients (Hammiche *et al.*, 2011) or food groups like dairy (Afeiche *et al.*, 2016) and whole grains (Gaskins *et al.*, 2016). There are also some epidemiological studies considering nutrition in the light of a more holistic approach that focuses on the role of dietary patterns rather than individual nutrients, foods or groups (Vujkovic *et al.*, 2010; Toledo *et al.*, 2011; Twigt *et al.*, 2012; Parisi *et al.*, 2017), as this approach probably better reflects long-term eating habits and behaviours. Among dietary patterns, the Mediterranean diet (MedDiet), a diet rich in vegetables, fruits, whole grains, legumes, nuts and olive oil, and low in red meat, seems to be the most promising and widely accepted for its positive effects on human health (García-Fernández *et al.*, 2014). Previously, Vujkovic *et al.* (2010) have investigated the association between preconception dietary patterns and IVF outcomes among subfertile couples in the Netherlands, and showed that high adherence by the couple to a 'Mediterranean' type pattern (defined using principal component analysis) increased the probability of pregnancy. A comparable effect for the adherence to a healthy diet and the chance of ongoing pregnancy following IVF was subsequently found in a Dutch cohort of couples receiving their first IVF treatment, by calculating a preconception dietary risk score based on dietary recommendations of the Netherlands Nutrition Centre (Twigt *et al.*, 2012). However, more studies are needed to confirm the favourable role of a MedDiet on assisted reproductive performance in other populations. Moreover, given obesity's high impact on female reproductive potential (Maheshwari *et al.*, 2007; Broughton and Moley, 2017; Luke, 2017) it is essential to clarify the role and the potential mechanism(s) by which diet quality, and the MedDiet in particular, may exert beneficial effects on assisted reproduction outcome beyond body weight.

Therefore, the aim of the present study was to explore potential associations between MedDiet and IVF clinical outcomes among non-obese women of infertile couples attempting fertility. We hypothesized that greater adherence to the MedDiet, defined using an a-priori dietary pattern approach and calculation of the validated Mediterranean diet score (MedDietScore) (Panagiotakos *et al.*, 2007), would favourably influence assisted reproductive performance of otherwise healthy women with no obesity problems.

Materials and Methods

Study population

Couples with primary infertility, seeking evaluation and treatment in an 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. Female partners aged ≤41 years, having a BMI of <30 kg/m^2, using their own oocytes, without a previous IVF attempt or pregnancy, and not on a natural cycle attempt, were eligible for the study. Women had also to not have endometriosis, previous ovarian surgery, a history of diabetes mellitus, cardiovascular disease, hypertension, a neoplasm, hypothyroidism or psychiatric disorders, and not to have changed their dietary habits for at least the past 6 months. A total of 244 eligible Greek couples were evaluated from November 2013 until September 2016, and complete data on lifestyle and IVF parameters were obtained. At enrollment, anthropometric measurements were taken for all study participants and a detailed questionnaire was used to collect data on demographics, reproductive health, medical history and lifestyle.

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.

Dietary-lifestyle assessment and evaluation of adherence to the Mediterranean diet

To estimate habitual food and alcohol intake all participants filled out a semi-quantitative 76-item food-frequency questionnaire (FFQ) validated for the Greek population (Bountziouka *et al.*, 2012).

The FFQ included information on all main food groups and beverages usually consumed (i.e. 69 questions regarding consumption of cereals, fruits, vegetables, meat, fish, legumes, dairy products, added fats, alcoholic beverages, stimulants, sweets), as well as seven questions regarding eating behaviours (i.e. eating in restaurants or canteens, consumption of breakfast, number of meals consumed on a daily basis and consumption of organic products or dietary supplements). The amounts of food consumed were expressed either in grams or millilitres or in other common measures, such as slice, tablespoon or cup, representing the standard serving size. Participants were asked to report how often, on average, during the 6-month period before IVF they had consumed each of the foods and beverages included in the FFQ according to a 6-grade scale (never/rarely, 1–3 times/month, 1–2 times/week, 3–6 times/week, 1 times/day or ≥2 times/day). Participants were also asked to report any use of dietary supplements as well as the frequency of their use (daily, weekly, monthly or few times/year). The FFQ used has been previously found to be reproducible and relatively valid for assessing almost all food groups (i.e. dairy products, fruit, vegetables, starchy products, legumes, meat, fish, eggs, sweets, alcohol, and fats and oils), as well as the macronutrients and energy intake. Subgroup analysis has also revealed that the FFQ has good validity regarding food consumption patterns of adults of both sexes during the year preceding its application and that it has a similar validity both in normal weight and overweight/obese subjects (Bountziouka *et al.*, 2012). The FFQ was provided at enrollment and returned by the day of oocyte retrieval.

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 the MedDietScore are presented in Supplementary Table SI. The range of the MedDietScore is 0–55, with higher values indicating greater adherence to the MedDiet.

Assessment of physical activity was performed through a validated Greek version of the 'International Physical Activity Questionnaire' (iPAQ), suitable for assessing population levels of self-reported physical activity, while anxiety was assessed by using the Spielberger State-Trait Anxiety Inventory (STAI-Y), as previously described (Karayiannis et al., 2017).

IVF procedure and outcome assessment

Before IVF, women underwent ovarian reserve testing and were assigned into one of three ovarian stimulation protocols as clinically indicated: (i) GnRH antagonist protocol (Cetrotide, Orgalutran); (ii) follicular-phase GnRH agonist/Flare protocol (Daronda, Arvekap); or (iii) mild ovarian stimulation with clomiphene citrate. Subcutaneous recombinant FSH (Gonal-F, Puregon, Altrmon) and/or hMG (Menopur, Merional, Pergonal) were administered in all three regimens with a maximum combined daily dose of 450 IU. Women were monitored during ovarian stimulation for serum estradiol (E2) and follicle size measurements and counts. hCG was administered ~36 h before the scheduled oocyte retrieval procedure to induce ovulation. Oocyte retrieval was performed when follicle sizes reached 16–18 mm and the E2 level reached at least 1800 pmol/l.

ICSI was carried out for all IVF cycles in this study. Embryologists classified oocytes as germinal vesicle or metaphase-I or metaphase-II (by the presence of a polar body) and determined fertilization 17–20 h after insemination as the number of oocytes with two pronuclei. Fertilization rate was defined as the total number of fertilized oocytes divided by the number of metaphase-II oocytes. The resulting embryos were monitored for cell number and morphological quality (1 = best to 5 = worst) on Day 3. Embryos that had reached 6–8 cells on Day 3 were considered to be cleaving at a normal rate, whereas embryos with ≤5 cells and ≥9 were considered to be slow cleaving or to have accelerated cleavage, respectively. In our analysis, we classified embryos as high quality if they had at least eight cells on Day 3 and a morphological quality score of 1 or 2. A maximum of four embryos was transferred, according to the Greek National Legislation for embryo transfer guidelines (Greek National Authority of Assisted Reproduction; Law 3305/01/2005; http://eaiya.gov.gr/en/law-fek/).

We defined successful implantation as a serum β-hCG level >20 IU/l measured at 14–21 days after oocyte retrieval. Clinical pregnancy was defined as the presence of an intrauterine pregnancy confirmed by ultrasound (presence of at least one gestational sac and cardiac activity at 6 weeks estimated gestational age), and live birth was defined as the birth of a neonate on or after 24 weeks of gestation. All clinical information, including infertility diagnosis, hormone levels and protocol type, was abstracted from the patient's electronic medical records. The primary outcomes were implantation, clinical pregnancy and live birth, whereas oocyte yield, fertilization rate and embryo quality measures were used as intermediate outcomes.

Statistical analysis

Continuous variables are presented as median (interquartile range, IQR), and categorical variables are presented as absolute and relative frequencies. Associations between categorical variables were tested by χ^2 tests or Fisher's exact tests (when one or more cell counts were ≤5), while differences between categorical and several clinical and nutritional variables were tested using the non-parametric Mann–Whitney-*U* test. Comparisons between various variables and tertiles of the MedDietScore were performed using Kruskal–Wallis test, and the Bonferroni correction was used to account for the increase in Type-I error. We used generalized linear models to test associations between the MedDietScore and IVF outcomes. A Poisson distribution with log link function were used to test association of number of total and mature oocytes, fertilized oocytes, and high-quality embryos (all count data), while a binomial distribution with logit link function was used for fertilization rate and clinical endpoints. The results are presented as relative risk (RR) and 95% CIs. Tests for trend across tertiles were conducted by modelling the median value of MedDietScore in each tertile as a single continuous variable and assessing significance using Wald test.

Confounding was evaluated using prior knowledge regarding biological relevance as well as descriptive statistics from our study population. Covariates considered in full models included age (years), ovarian stimulation protocol (antagonist, agonist or clomiphene), BMI (kg/m^2), physical activity (MET-min/week), state/trait-anxiety (score value), total energy intake (kcal/day), cause of infertility (male factor, female factor or unexplained infertility) and use of dietary supplements [by adjusting both for the frequency of use (ordinal) and the type of supplement (as dummy variables)]. We further assessed effect modification of MedDietScore with primary outcomes by age (<35 vs ≥35 years), infertility type (male/female/unexplained infertility) and BMI (<25 vs ≥25 kg/m^2) using cross product terms in the final multivariable models.

Statistical Package for Social Sciences software (SPSS, version 21.0, Chicago, IL, USA) was used for all the statistical calculations. All reported *P*-values are based on two-sided tests and compared with a significance level of 5%.

Results

Our study population consisted of 244 women (median age: 35.0 years; range: 22–41 years) who received their first IVF/ICSI treatment within 2 months after dietary assessment. None of the women were obese as this was an exclusion criterion from the study (median BMI: 22.8 kg/m^2; range: 18.0–29.9 kg/m^2). Women were either physically inactive (38.5%) or minimally active, and the majority had never smoked (77.5%). Approximately half of the women reported making use of dietary supplements (45.9%), mainly multivitamins and folate, but none reported a change in dietary habits over a period of 6-month preceding evaluation. Overall, 229 women (93.9%) had an embryo transfer, 138 (56.5%) had a successful implantation, 104 (42.6%) achieved a clinical pregnancy and 99 (40.5%) had a live birth. Women with a clinical pregnancy and live birth did not differ in age, BMI, smoking habits, physical activity or stress levels, compared to those who did not achieve pregnancy (all $P > 0.05$).

Table 1 shows the clinical and reproductive characteristics of the women according to tertiles of the MedDietScore. Compared with women in the highest tertile of MedDietScore (≥36, $n = 86$), those in the lowest tertile (≤30, $n = 79$) had higher BMI and waist circumference, were more frequent physically inactive, and exhibited higher

Table I **Characteristics of the 244 women participating in the study by tertiles of the MedDietScore.**

	MedDietScore tertile			
	First (≤30)	Second (31–35)	Third (≥36)	*P*-value
N	79	79	86	
Age, y	35 (32–37)	36 (32–39)	36 (34–38)	0.370
<35, *n* (%)	38 (48.1)	27 (34.2)	31 (36.0)	0.350
35–37, *n* (%)	22 (27.8)	26 (32.9)	25 (29.1)	
38–41, *n* (%)	19 (24.1)	26 (32.9)	30 (34.9)	
Educational level, *n* (%)				0.054
Primary/secondary school	29 (36.7)	19 (24.1)	16 (18.6)	
University degree	48 (63.3)	60 (75.9)	70 (81.3)	
Individual income level, *n* (%)				0.603
Low (<10.000 euros/annually)	23 (29.1)	18 (22.8)	18 (20.9)	
Moderate(10–30.000 euros)	49 (62.0)	51 (64.6)	61 (70.9)	
High (>30.000 euros)	7 (8.9)	10 (12.7)	7 (8.1)	
Waist circumference, cm	85.0 (79.0–93.0)[a]	78.0 (74.0–82.0)[b]	78.0 (72.0–81.0)[b]	<0.001
Body mass index, kg/m^2	24.1 (22.6–27.2)[a]	22.7 (21.2–23.6)[b]	22.2 (20.7–23.4)[b]	<0.001
<25 kg/m^2, *n* (%)	50 (63.3)	65 (82.3)	73 (84.9)	
≥25 kg/m^2, *n* (%)	29 (36.7)	14 (17.7)	13 (15.1)	
Smoking status, *n* (%)				0.686
Never	59 (74.7)	64 (81.0)	66 (76.8)	
Former	7 (8.9)	5 (6.3)	10 (11.6)	
Current	13 (16.5)	10 (12.7)	10 (11.6)	
Physical activity, MET-min/week	594 (372–870)[a]	870 (447–1236)[b]	870 (590–1127)[b]	0.002
Inactive, *n* (%)	41 (51.9)	27 (34.2)	26 (30.2)	0.011
Minimally active, *n* (%)	38 (48.1)	52 (65.8)	60 (69.8)	
S-Anxiety (score range 20–80)[a]	48.0 (39.0–56.0)[a]	43.0 (35.0–50.0)[b]	42.0 (34.0–48.0)[b]	0.001
T-Anxiety (score range 20–80)[a]	45.0 (35.0–51.0)[a]	38.0 (32.0–46.0)[b]	37.5 (32.0–43.0)[b]	<0.001
Total energy intake, kcal/day	1870 (1658–2162)[a]	1754 (1582–1995)[b]	1757 (1460–2011)[b]	0.007
Supplements use, *n* (%)				0.002
Never	58 (73.5)	40 (50.6)	34 (39.5)	
Monthly or few times/year	11 (14.9)	13 (16.5)	16 (18.6)	
Weekly	3 (3.8)	12 (15.2)	20 (23.3)	
Daily	7 (8.9)	14 (17.7)	16 (18.6)	
Type of supplements, *n* (%)				0.008
Multivitamins	7 (8.9)	13 (16.5)	17 (19.8)	
Folate	4 (5.1)	7 (8.9)	15 (17.5)	
Vitamin C	4 (5.1)	6 (7.6)	11 (14.0)	
Iron	5 (6.3)	11 (13.9)	7 (8.2)	
Other[b]	1 (1.3)	2 (2.5)	5 (5.9)	
MedDietScore (male partners)	30 (29–33)	34 (30–36)	36 (32–39)	<0.001
Reproductive characteristics and IVF outcome				
Family subfertility history, *n* (%)	20 (25.3)	24 (30.4)	20 (23.3)	0.568
Cause of infertility, *n* (%)				0.011
Male factor	38 (48.1)	32 (40.5)	19 (22.1)	
Female factor	5 (6.3)	7 (8.9)	10 (11.6)	
Unexplained	36 (45.6)	40 (50.6)	57 (66.3)	
Infertility duration, y	3.0 (2.0–4.0)	3.0 (1.5–4.0)	2.2 (1.5–4.0)	0.549

Continued

Table I *Continued*

	MedDietScore tertile			
	First (≤30)	**Second (31–35)**	**Third (≥36)**	***P*-value**
PCOS, *n* (%)	13 (16.5)	14 (17.7)	12 (14.0)	0.797
Day three FSH, IU/l[c]	7.0 (5.0–8.3)	7.0 (5.0–9.0)	7.0 (5.4–8.0)	0.761
Day three LH, IU/l[c]	5.0 (4.0–7.7)	6.0 (5.0–7.3)	5.0 (4.0–6.8)	0.192
Day three Estradiol, pmol/l[d]	130.5 (92.4–199.0)	118.3 (82.6–164.4)	128.6 (97.6–159.7)	0.868
Baseline AMH, nmol/l[e]	16.5 (9.4–31.3)	14.1 (8.3–27.6)	14.3 (7.8–24.2)	0.746
Ovarian stimulation protocol, *n* (%)				0.547
Antagonist	47 (59.5)	51 (64.6)	48 (55.8)	
Agonist (flare protocol)	28 (35.4)	24 (30.4)	36 (41.9)	
Clomiphene citrate	4 (5.1)	3 (3.8)	2 (2.3)	
Oocytes produced, *n*	11 (7–15)	10 (6–14)	11 (7–15)	0.668
Oocytes-MII, *n*	7 (4–10)	7 (4–11)	8 (5–12)	0.275
Fertilization rate, %	81.8 (70.0–100.0)	86.6 (66.6–100.0)	83.3 (68.1–94.2)	0.884
Embryos produced, *n*	6 (3–9)	5 (3–9)	6 (4–10)	0.342
High-quality embryos, %	33.3 (14.2–54.5)	33.3 (15.7–55.5)	33.3 (17.2–57.1)	0.917
Number of embryos transferred, *n* women (%)				0.475
No embryos transferred	8 (10.1)	4 (5.0)	3 (3.5)	
1 embryo	16 (20.3)	12 (15.2)	11 (12.8)	
2 embryos	31 (39.2)	37 (46.8)	38 (44.2)	
3+ embryos	24 (30.4)	26 (33.0)	34 (39.5)	
Successful implantation, *n* (%)	40 (50.6)	46 (58.2)	52 (60.5)	0.416
Clinical pregnancy, *n* (%)	23 (29.1)[a]	38 (48.1)[a,b]	43 (50.0)[b]	0.012
Live birth, *n* (%)	21 (26.6)[a]	36 (45.6)[a,b]	42 (48.8)[b]	0.008

Values represent median (IQR) or number of subjects (%). Differences in variables across tertiles of the MedDietScore were tested using Kruskal–Wallis test for continuous variables and χ^2 test or Fisher's exact test for categorical variables. Values with different superscript letter are statistically significant different (Bonferroni correction, $P < 0.015$).
Abbreviations used: MedDietScore = Mediterranean diet score; MET = metabolic equivalent of task; PCOS = polycystic ovary syndrome; FSH = follicle-stimulating hormone; LH = luteinizing hormone; AMH = anti-mullerian hormone; Oocytes-MII = metaphase-II stage oocytes.
[a]State (S)—Anxiety evaluates the current emotional state; Trait (T)—Anxiety evaluates relatively stable aspects of anxiety (how the respondent usually feels), with higher values suggesting higher levels of anxiety.
[b]Other: *n*−3 fatty acids, calcium, vitamin D, zinc, plant sterols.
[c]Sample size by tertile of MedDietScore *n* = 58, 53 and 70.
[d]Sample size by tertile of MedDietScore *n* = 32, 23 and 25.
[e]Sample size by tertile of MedDietScore *n* = 33, 30 and 35.

levels of current and long-term anxiety. In addition, they had a higher total energy intake and reported less frequent use of dietary supplements (all $P < 0.01$). Unexplained infertility was more common in the group of women with the highest MedDietScores, but there were no differences across tertiles of MedDietScore in women's age distribution, duration of infertility, ovarian reserve biomarkers (Day 3 FSH/LH levels, anti-mullerian hormone levels), and in the ovarian stimulation protocol used. Moreover, oocyte yield, fertilization rates and embryo quality measures were similar across tertiles of MedDietScore (Table I). Implantation was also similar across tertiles. However, the rate of clinical pregnancy and live birth was significantly lower in women in the lowest compared with women in the highest tertile of MedDietScore (29.1 vs 50.0%, $P = 0.01$ and 26.6 vs 48.8%, $P = 0.01$, respectively).

In Table II, the results of the fully adjusted multivariable models for study's primary outcomes are presented. Compared with women in the highest tertile (highest adherence) of the MedDietScore, women in the lowest tertile (lowest adherence) had at least a 65% lower RR (95% CI) of achieving clinical pregnancy [0.35 (0.16–0.78), *P*-trend = 0.01] and live birth [0.32 (0.14–0.71), *P*-trend = 0.01]. To reduce potential of confounding by dietary supplementation, we also performed a sensitivity analysis restricted to women not taking supplements ($n = 132$), using the same covariates as in full models except supplements use (model 2 in Table II), and found similar results to those within the full sample. The multivariable-adjusted RR (95% CI) for clinical pregnancy comparing women in the lowest with women in the highest tertile of the MedDietScore was 0.29 (0.10–0.82), and for live birth it was 0.25 (0.09–0.73). No significant association of MedDietScore with study intermediate outcomes (ovarian stimulation outcomes, fertilization rate and embryo quality measures) was observed (data not shown).

We also found a significant MedDietScore × age interaction in the association with clinical pregnancy (*P*-interaction = 0.007) and live birth (*P*-interaction = 0.008). MedDietScore (continuous) was positively associated with clinical pregnancy and live birth ($P = 0.001$)

Table II Associations between MedDietScore and clinical end-points of IVF (n = 244 women/cycles).

	MedDietScore tertile			
	First	Second	Third	*P*-trend[a]
Successful implantation (yes vs no)				
Model 1	0.49 (0.24–0.98)*	0.81 (0.42–1.58)	1 (ref)	0.049
Model 2	0.52 (0.25–1.08)	0.81 (0.41–1.60)	1	0.090
Model 3	0.62 (0.28–1.36)	0.81 (0.39–1.65)	1	0.257
Clinical pregnancy (yes vs no)				
Model 1	0.30 (0.15–0.62)*	0.83 (0.43–1.56)	1 (ref)	0.002
Model 2	0.35 (0.17–0.75)*	0.85 (0.45–1.64)	1	0.010
Model 3	0.35 (0.16–0.78)*	0.81 (0.41–1.59)	1	0.013
Live birth (yes vs no)				
Model 1	0.28 (0.13–0.57)*	0.77 (0.40–1.47)	1 (ref)	0.001
Model 2	0.31 (0.14–0.66)*	0.81 (0.42–1.55)	1	0.004
Model 3	0.32 (0.14–0.71)*	0.78 (0.39–1.54)	1	0.007

All analyses were conducted using generalized linear models with binomial distribution and logit link function. Data represent relative risk (95% CI). Model 1 was adjusted for age, ovarian stimulation protocol and body mass index. Model 2 was adjusted as for model 1 and for physical activity, state and trait anxiety, infertility diagnosis and total energy intake. Model 3 was adjusted as for model 2 and for dietary supplements use (frequency and type of supplement).

*$P < 0.05$.

Abbreviations used: MedDietScore = Mediterranean diet score; ref = referent.

[a]Test for trend were performed using the median level of MedDietScore in each tertile as a continuous variable in the model.

among women <35 years old but not among older women. Among women <35 years, the multivariable-adjusted RR (95% CI) for clinical pregnancy for increasing MedDietSore was 1.22 (1.05–1.43) and for women ≥35 years, it was 1.00 (0.92–1.09). The corresponding values for live birth were 1.25 (1.07–1.45) and 1.01 (0.93–1.11), respectively (Supplementary Table SII). Additional adjustment for the male partner's MedDietScore yielded almost identical results (Supplementary Table SII). Among women <35 years a beneficial 5-point increase in the MedDietScore associates with ~2.7 times higher likelihood of achieving clinical pregnancy and live birth. No evidence of effect modification by infertility type or BMI was observed (*P* for interaction >0.05 in all cases).

Discussion

In this study among non-obese women undergoing a first autologous IVF treatment, we assessed adherence to the MedDiet using the validated MedDietScore and found that greater adherence to this healthy diet during the 6-month period before IVF was associated with higher chance of clinical pregnancy and live birth. Specifically, we found that a beneficial 5-point increase in the MedDietScore was associated with ~2.7 times higher likelihood of clinical pregnancy and live birth among women <35 years old. The MedDietScore was not related to ovarian stimulation outcomes, embryo quality measures or implantation.

Surprisingly, the favourable effect of MedDiet was evident among women <35 years but not among the older women of our study. Women's age is the most important risk factor for infertility and data from a large-scale study have recently confirmed the significant effect of age also on assisted reproduction outcomes (Grøndahl *et al.*, 2017). In our cohort, compared with women <35, women ≥35 years had lower yield of total and mature oocytes [median (IQR): 10 (6–14) vs 13 (8–16), $P = 0.003$ and 7 (4–10) vs 9 (5–13), $P = 0.02$, respectively] and a lower rate of successful implantation (51.4 vs 64.6%, $P = 0.04$), but there were no differences in clinical pregnancy and live birth or in lifestyle factors between the two age-groups. Women experience major physiological changes in reproductive functions as they age, including major endocrine alterations (Diamanti-Kandarakis *et al.*, 2017), and it is possible that such profound changes could mask any influences from other environmental factors, such as diet quality, that otherwise impact fertility and IVF success rate also during the later reproductive years. In line with this, it has been found that a high BMI has a pronounced negative influence on IVF success at younger ages, but this effect diminishes as women reach their mid-30s (Sneed *et al.*, 2008). These authors postulated that in older women, the dominating effect of age on fertility and IVF outcomes becomes more important than the effects of BMI.

Our results are consistent with findings of previous studies showing that high adherence to a healthy diet may improve the chance of pregnancy after IVF/ICSI treatment. Vujkovic and collaborators compared two dietary patterns defined using principal component analysis, a 'health conscious-less processed' dietary pattern (consisting of high intakes of fruits, vegetables, fish and whole grains; and low intakes of snacks, meats and mayonnaise) and a 'Mediterranean' style dietary pattern (high intakes of vegetable oils, vegetables, fish, and legumes, and low intakes of snacks). Although the two dietary patterns showed remarkable overlap in foods, only the 'Mediterranean' dietary pattern was associated with a borderline significant improvement in IVF pregnancy rates [odds ratio (OR): 1.4; 95% CI: 1.0–1.9], which may be attributed to a higher beneficial effect of this dietary pattern on vitamin B6 and folate levels and to the high intake of vegetable oils (Vujkovic *et al.*, 2010). In a case–control study nested in a Spanish cohort of university graduates, Toledo *et al.* (2011) used a similar dietary pattern approach and found that women in the highest quartile of adherence to a 'Mediterranean-type' pattern had a lower risk of difficulty getting pregnant, compared with women in the lowest quartile (OR: 0.56, 95% CI: 0.35–0.95). More recently, in a study involving 199 Dutch couples with a first IVF/ICSI treatment, a preconception dietary risk score (PDR) was assigned giving 1-point to each of six healthy food groups (fruits, vegetables, meat, fish, whole wheat products and fats) as determined by the Netherlands Nutrition Centre. There was a higher pregnancy rate among women with a higher PDR score (OR: 1.65; 95% CI: 1.08–2.52), suggesting that increasing adherence to Dutch dietary recommendations in women undergoing IVF treatment increases the chance of ongoing pregnancy (Twigt *et al.*, 2012).

Central to the effort to link MedDiet with IVF success rate is the notion that it is a pattern characterized by high consumption of foods and food groups that have been positively associated with fertility and treatment outcomes, such as whole grains (Gaskins *et al.*, 2016). In line with this, the consumption of cereals, vegetables and fruits has been reported to positively influence embryo quality in women undergoing IVF/ICSI treatment, while increased consumption of red meat appears to have a negative effect on implantation and clinical pregnancy rates (Braga *et al.*, 2015). Interestingly, increased fruit and vegetable consumption in male partners has been associated with increased fertilization rates (Firns et al., 2015), suggesting a possible relationship between male diet and some IVF outcome parameters. In our cohort, MedDietScore was not related to any of the intermediate outcomes, but a positive association with clinical pregnancy and live birth was observed even after adjusting for the male partners' MedDietScore.

The MedDiet has been favourably related to glycemic control (Rossi et al., 2013), which may be an important parameter affecting pregnancy outcome in IVF patients (Wei et al., 2008). In addition, the MedDiet is characterized by an optimal ratio of macronutrients and a high intake of beneficial fatty acids, such as mono- and polyunsaturated fatty acids (from olive oil, fish and nuts). Although it is unclear which component of the MedDiet mostly contributes to its favourable effects, a particular feature of this diet is the use of olive oil, which leads to a high ratio of monounsaturated to saturated fatty acids. Data from a large prospective cohort study among premenopausal women without a history of infertility have suggested that replacing unsaturated fats, commonly found in vegetable oils, with trans fatty acids may increase the risk of ovulatory infertility (Chavarro et al., 2007) and is likely to also impact on IVF clinical outcome. Interestingly, periconceptional maternal adherence to a 'high fish and olive oil, low meat' dietary pattern has recently been positively associated with embryonic growth in spontaneous pregnancies, although no significant association was observed in IVF/ICSI pregnancies (Parisi et al., 2017). Similar effects for the quality of protein and carbohydrate in the diet and the risk of infertility due to anovulation have been reported (Chavarro et al., 2008, 2009); hence, apart from lipids, other components of MedDiet may be equally important.

Since MedDietScore was positively associated with study final outcome only, we can assume that MedDiet affects key elements in the endometrial environment which have a positive influence on pregnancy maintenance. Using data form the Nurses' Health Study-II, Gaskins et al. (2014) have found that prepregnancy adherence to well-characterized dietary patterns, including the Fertility Diet and the alternate MedDiet pattern, was not associated with risk of pregnancy loss, although in subanalyses restricted to pregnancies occurring shortly after diet assessment, the Fertility Diet pattern was inversely related to pregnancy loss. We also performed a subanalysis restricted to women with positive β-hCG and found that lower adherence to MedDiet was associated with lower relative risk of clinical pregnancy and live birth (Supplementary Table SIII), which suggests that the beneficial effect of MedDiet could be through increasing embryo survival. Several mechanisms could explain the observed association, and antioxidants is a possible candidate. The MedDiet is rich in antioxidants due to the high consumption of fruit, vegetables and whole grains. Recent data suggest that oxidative stress and low antioxidant status may lead to known or unexplained infertility (Agarwal et al., 2012); however, studies examining the effects of antioxidants administration in fertility treatment have concluded that taking antioxidants in the form of supplements does not improve fertility outcomes (Showell et al., 2013). More recently, it has been shown that administration of antioxidants 3 months before IVF cycles improves oocyte quality (Luddi et al., 2016), while increased intake of antioxidants through diet has been associated with shorter time for successful conception, although this effect varied with the women's BMI and age (Ruder et al., 2014). Unlike supplements, diet contains antioxidants in ideal proportions and combinations that dispose, scavenge, or suppress the formation of reactive oxygen species and contribute against oxidative damage to the endometrium (Agarwal et al., 2012). The favourable effect of the MedDiet observed in our study was independent of dietary supplementation, and also confirmed among women not taking supplements.

There are several limitation in our study. First, a single diet assessment was used and FFQ is susceptible to measurement error. However, due to the study's prospective character and since one of the study prerequisites was that the participants should have had stable eating habits during the last 6 months, any measurement error would likely be non-differential with respect to the results of IVF treatment. Secondly, study participants were Greek infertile non-obese women selected from an IVF clinic, so our finding cannot be equally generalized to the whole reproductive population nor obese women nor to non-Europeans or women attending infertility clinics around the world. Thirdly, the possibility of residual confounding by other factors that were not measured or poorly measured in our study remains, although we have adjusted for multiple maternal covariates (including age, BMI, physical activity and stress levels), and additionally for the male partners' dietary habits. Alcohol consumption also increases the risk of IVF failure and a recent review of the literature has concluded that couples undergoing IVF should be advised to abstain from alcohol prior to and during their procedures (Nicolau et al., 2014). In our analysis, we did not adjust for alcohol intake because alcohol consumption was considered in the construction of the MedDietScore (Karayiannis et al., 2017). In addition, we did not control for number of embryos transferred, because embryo and transfer outcomes could be considered to be downstream consequences of dietary exposure that could mediate in part any observed relation with clinical end-points (implantation, clinical pregnancy and live birth), and therefore it would be inappropriate to adjust for this intermediate (Messerlian and Gaskins, 2017). Finally, due to the observational study design, causal inference is limited.

A main strength of our study is the evaluation of compliance to the MedDiet by implementing an a-priori dietary pattern approach (MedDietScore) that captures pre-defined healthy habits and has the advantage of relying on the current scientific data concerning nutrition, health and disease. Of note, median MedDietScore values in our cohort of infertile couples are comparable to values estimated for healthy adults in corresponding studies of Greek population (Chrysohoou et al., 2004). Another strength of this study is that only women with a BMI $< 30\,kg/m^2$ receiving their first IVF treatment were included. Obese women undergoing IVF require higher gonadotrophin doses and longer stimulation duration, have lower implantation, clinical intrauterine gestation and live birth rates, and increased rates of pregnancy loss (Luke, 2017). In regard to this, in a study involving >4600 women with a fresh autologous IVF, women with a BMI $> 30\,kg/m^2$ had up to 68% lower odds of having a live birth compared with those with BMI $< 30\,kg/m^2$ (Moragianni et al., 2012). Consequently, given the high impact of obesity on assisted reproductive performance, it is likely that any influences of diet quality would be more difficult to detect among women with obesity problems. Finally, the long follow-up period of couples (up to giving live birth) allowed us to evaluate not only intermediate but also final IVF outcomes.

In conclusion, we have shown that higher adherence to the MedDiet is associated with increased chance of clinical pregnancy and live birth after IVF/ICSI treatment in non-obese women <35 years of age. More research and intervention studies are warranted to investigate the role of diet quality in assisted reproductive performance, to reveal underlying mechanisms, and for developing nutritional guidelines for women to further improve fertility treatment and success rates.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Acknowledgements

The authors would like to thank the Embryogenesis Assisted Conception Unit clinic staff for their assistance in data collection. We would also like to thank Prof. D.B. Panagiotakos for his valuable advice regarding the statistical processing of the data.

Authors' roles

D.K., M.K. and N.Y. were involved in the study concept and design. D.K., C.M. and M.M. contributed to the acquisition of data. D.K. analysed the data and drafted the article. M.K. and N.Y. supervised the analysis and critically revised the article. N.Y. had the primary responsibility for final content. All authors read and approved the final article.

Funding

Partially supported by a grant from Harokopio University (KE321).

Conflict of interest

None declared.

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