PEDIATRICOBESITY
doi:10.1111/ijpo.12191

Adherence to the Mediterranean diet during pregnancy and offspring adiposity and cardiometabolic traits in childhood

L. Chatzi[1], S. L. Rifas-Shiman[2], V. Georgiou[1], K. E. Joung[3], S. Koinaki[1], G. Chalkiadaki[1], A. Margioris[4], K. Sarri[1], M. Vassilaki[1], M. Vafeiadi[1], M. Kogevinas[5], C. Mantzoros[3], M. W. Gillman[2] and E. Oken[2]

[1]Department of Social Medicine, University of Crete, Heraklion, Greece; [2]Obesity Prevention Program, Department of Population Medicine, Harvard Medical School and Harvard Pilgrim Health Care Institute, Boston, MA, USA; [3]Division of Endocrinology, Diabetes, & Metabolism, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA; [4]Department of Laboratory Medicine, University of Crete, Heraklion, Greece; [5]Centre for Research in Environmental Epidemiology, CREAL, Barcelona, Spain

Address for correspondence: L Chatzi, Department of Social Medicine, Faculty of Medicine, University of Crete, PO Box 2208, Heraklion, 71003, Crete, Greece. E-mail: lchatzi@med.uoc.gr

Received 3 September 2015; revised 21 July 2016; accepted 26 August 2016

Summary

Background: In adults, adherence to the Mediterranean diet has been inversely associated with cardiovascular risk, but the extent to which diet in pregnancy is associated with offspring adiposity is unclear. We aimed to investigate the association between adherence to Mediterranean diet in pregnancy and offspring cardiometabolic traits in two pregnancy cohorts.

Methods: We studied 997 mother–child pairs from Project Viva in Massachusetts, USA, and 569 pairs from the Rhea study in Crete, Greece. We estimated adherence to the Mediterranean diet with an *a priori* defined score (MDS) of nine foods and nutrients (0 to 9). We measured child weight, height, waist circumference, skin-fold thicknesses, blood pressure, and blood levels of lipids, c-reactive protein and adipokines in mid-childhood (median 7.7 years) in Viva, and in early childhood (median 4.2 years) in Rhea. We calculated cohort-specific effects and pooled effects estimates with random-effects models for cohort and child age.

Results: In Project Viva, the mean (SD, standard deviation) MDS was 2.7 (1.6); in Rhea it was 3.8 (1.7). In the pooled analysis, for each 3-point increment in the MDS, offspring BMI *z*-score was lower by 0.14 units (95% CI, −0.15 to −0.13), waist circumference by 0.39 cm (95% CI, −0.64 to −0.14), and the sum of skin-fold thicknesses by 0.63 mm (95% CI, −0.98 to −0.28). We also observed lower offspring systolic (−1.03 mmHg; 95% CI, −1.65 to −0.42) and diastolic blood pressure (−0.57 mmHg; 95% CI, −0.98 to −0.16).

Conclusion: Greater adherence to Mediterranean diet during pregnancy may protect against excess offspring cardiometabolic risk.

Keywords: Blood pressure, cohort study, diet, lipids, obesity, pregnancy.

Introduction

Early life is a critical period of developmental plasticity (1). Metabolic programming is the phenomenon whereby a nutritional stress/stimulus applied during critical periods of early development permanently alters an organism's physiology and metabolism, the consequences of which are often observed much later in life (2). Although the foetal origins hypothesis, proposed by Barker, has been well documented in animal studies, data from human studies on maternal diet quality during pregnancy in association with offspring cardiometabolic risk factors are scarce with disparate results (3,4). Prior studies have examined associations of specific nutrients, foods, or food groups during pregnancy with offspring health, and these approaches may not take into consideration that some nutrients are interrelated. Moreover, they all refer to a specific population group, and their results cannot be easily generalized because of cultural and socio-economic population differences associated with diet.

The traditional Mediterranean diet is characterized by a high intake of olive oil, fruits, vegetables, legumes, nuts, and whole grain products; a moderate intake of fish; and only small amounts of red and processed meat. This dietary pattern is low in saturated fat intake and high in monounsaturated fat intake from

olive oil; it is rich in fibre and glutathione, provides a balanced ratio of n-6/n-3 essential fatty acids, and high amounts of antioxidants (especially polyphenols from olive oil, vitamins E and C) (5). Several epidemiological studies and clinical trials support the role of the Mediterranean diet in preventing obesity, type 2 diabetes mellitus and metabolic syndrome in adults (6,7), while some recent studies suggest a protective role against obesity development in children (8,9). In pregnancy, a higher adherence to the Mediterranean diet has been associated with lower risk of preterm birth (10,11), higher birth weight (12,13) and lower offspring waist circumference at preschool age (14).

The objective of this study was to investigate associations of maternal adherence to the Mediterranean diet in early pregnancy with offspring obesity and cardiometabolic risk in two cohorts with different socio-economic characteristics and different geographic locations: Project Viva, a prospective mother–child cohort that was established in Massachusetts, USA in 1999 (15) and the Rhea study, a population based mother-child cohort initiated in Crete, Greece in 2007 (16).

Subjects and methods

Study population

Project viva

We recruited women at their first prenatal visit from Atrius Harvard Vanguard Medical Associates, a multi-specialty group practice in Massachusetts, from 1999 to 2003 (15). Of 2128 live singleton births in Project Viva, 1784 pregnant women provided information on first trimester diet via a validated semi-quantitative food frequency questionnaire (17). We excluded seven women with implausible values of energy intake (<600 or >6000 cal d^{-1}). Of the 1777 remaining mother–child pairs, 997 attended an in-person mid-childhood visit at 6–10 years (median 7.7 years; IQR: 7.3–8.3), during which study staff measured anthropometry and collected fasting blood samples.

The Rhea cohort

The Rhea project is a population-based cohort of pregnant women and their children in the prefecture of Heraklion Crete (16). Of 1363 singleton live births in the Rhea study, we included 905 pregnant women who provided information on first trimester diet via a validated semi-quantitative food frequency, after excluding 11 women with implausible values of energy intake (<600 or >6000 cal d^{-1}). Of the remaining 895 mother–child pairs, 570 participated at the 4-year follow-up visit (median 4.2 years, IQR: 4.1–4.3), during which we measured anthropometry and collected non-fasting blood samples from 569 children, who comprised the population included in this analysis.

In both study populations, all procedures were in accordance with the ethical standards for human experimentation established by the Declaration of Helsinki, and all women provided written informed consent. Institutional review boards of participating institutions approved each study.

Dietary intake during pregnancy

In Project Viva, mothers reported their diet since the time of their last menstrual period at study enrolment (median 9.9 weeks gestation), using a validated semi-quantitative food frequency questionnaire (FFQ) (17). Rhea participants completed a validated FFQ at mean 14.6 weeks gestation (18). To evaluate adherence to the Mediterranean diet during pregnancy, we used a score modified from one applied in a large cohort study in adults (Mediterranean diet score, MDS) (5). To use the same thresholds for both cohorts, we applied cut-offs based where possible on current recommendations for pregnant women (Table S1) (19). For components presumed to be beneficial (vegetables, fruits, fish, dairy products, legumes, whole grain products, nuts and monounsatured fatty acids), women whose consumption was above recommendations were assigned a value of 1, otherwise they were assigned 0 points for intake equal or below the threshold. For components presumed to be detrimental (red and processed meat), we assigned 1 point for women whose consumption was below or equal to the threshold, and 0 for women whose consumption was above the threshold. The MDS thus ranged from 0 (minimal adherence to the Mediterranean diet) to 9 (maximal adherence). We have presented our results per 3 points increment on the MDS as conventionally a score of 0–3 represents low adherence, a score of 4–6 represents moderate adherence and a score of 7–9 represents high adherence to the Mediterranean diet (5).

Child adiposity measures

In both cohorts, trained research assistants measured children's weight, height, waist circumference and subscapular (SS) and triceps (TR) skinfold thicknesses (Supplementary Methods).

Child blood pressure and cardiometabolic biomarkers

In both cohorts, trained research assistants measured systolic and diastolic blood pressure using a Dinamap

automated oscillometric recorder (Supplementary Methods).

We collected blood via venipuncture and measured lipids [total cholesterol, and high-density lipoprotein cholesterol (HDL)], plasma leptin and adiponectin concentrations and C-reactive protein concentrations following standard protocols (Supplementary Methods).

Statistical analysis

We used linear regression to estimate associations of adherence to the Mediterranean diet during pregnancy with adiposity or cardiometabolic outcomes in childhood. Generalized additive models were applied to explore the shape of the relationships between MDS and outcomes under study.

To select the confounders for adjustment in multivariable models, we used a directed acyclic graph approach based on prior knowledge about parental and child covariates that may be related to child adiposity and/or adherence to the Mediterranean diet in pregnancy. According to this graph (Fig. S1), we included the following variables in multivariable models: The first model was adjusted for the child's sex and age at outcome measurement (crude model); the second model (confounder model) was additionally adjusted for maternal age at recruitment (years), education (high level: university or technical college degree), ethnicity (Greek/non-Greek; USA/non-USA citizen), race (Black people, Asian, Hispanic, White people and others), pre-pregnancy body mass index (based on measured height at recruitment and pre-pregnancy self-reported weight (BMI, $kg\,m^{-2}$), smoking in pregnancy (never, quit before pregnancy and smoked during pregnancy) and parity (nulliparous; multiparous). In a third model (mediation model), we additionally adjusted for birth weight for gestational length *z*-score and breastfeeding duration (months); and in a fourth model (also mediation), we additionally adjusted for child lifestyle characteristics [fast food intake (times/week and questionnaire based), TV viewing (hours per day and questionnaire based)] and child's anthropometry at age of outcome assessment [height (for waist circumference and SS + TR) and child's BMI (for cardiometabolic outcomes)] as potential mediators. We considered the confounder model as the main model.

We used a two-stage approach to assess the association of adherence to the Mediterranean diet during pregnancy with adiposity and cardiometabolic traits in children. First, we analysed associations separately for each cohort. Second, we calculated pooled effect estimates using mixed models, including cohort and child age at outcome assessment as random effects and all other covariates as fixed effects. Finally, the overall summary effect of the individual cohorts was estimated using random effects meta-analysis to check the consistency with the pooled analysis. We presented results as combined estimates from the random-effect models with their 95% confidence intervals (CIs).

We assessed effect modification by maternal pre-pregnancy BMI (≥ 25 vs. $< 25\,kg\,m^{-2}$), maternal smoking during pregnancy (yes vs. no), and breastfeeding duration (>3 vs. ≤ 3 months) through inclusion of the interaction terms in the models (statistically significant effect modification if p-value < 0.05). We also performed further adjustment for energy intake in pregnancy ($kcal\,d^{-1}$), gestational diabetes (questionnaire based) and gestational weight gain (questionnaire based) for mother–child pairs with available information on these variables.

We performed analyses with SAS version 9.3 software (SAS Institute, Cary, NC) and R version R3.1.

Results

Participant characteristics and compliance with Mediterranean diet

Intake of all food groups in the MDS during pregnancy differed between the two cohorts except nut intake (Fig. S2). Pregnant women in Rhea cohort had higher intakes of almost all protective components of the MDS except legumes. On the other hand, they also reported a higher intake of red and processed meat products than Project Viva mothers. The MDS was higher in the Rhea cohort (Mean 3.8, SD 1.7) than in Project Viva (Mean 2.7, SD 1.6, $p < 0.001$, Table 1).

At the time of outcome assessment, Viva children were approximately 3.5 years older than those in Rhea; accordingly, BMI and fat measurements were higher for the Viva children (Mean BMI, (SD): Viva; 17.1, (2.9); Rhea; 16.4 (1.9), $p < 0.01$). Table S2 shows that in both cohorts, mothers without offspring follow-up data were more likely to be younger, smokers, less educated and of non-white or non-Greek race/ethnicity accordingly.

Mediterranaean diet adherence and offspring obesity

Generalized additive models examining the shape of the relationships of MDS with child *z*-BMI showed no departures from linearity overall and separately in Project Viva and Rhea cohorts (Fig. S3).

Associations of MDS in pregnancy with offspring adiposity were broadly similar in each of the two

Table 1 Maternal and child characteristics by Mediterranean diet score in project viva and rhea cohorts

	Project Viva		Rhea	
	MDS		MDS	
	No. (%)	Mean (SD)	No. (%)	Mean (SD)
All	997 (100)	2.7 (1.6)	569 (100)	3.8 (1.7)
Maternal characteristics				
Age (years)				
<25	25 (2.5)	1.7 (1.3)	13 (2.3)	4.4 (1.8)
25–35	655 (65.7)	2.7 (1.6)	461 (81.4)	3.7 (1.7)
≥35	317 (31.8)	2.9 (1.6)	92 (16.3)	3.9 (1.6)
Pre-pregnancy BMI ≥25 kg m^{-2}				
No	651 (65.5)	2.8 (1.6)	377 (67.0)	3.8 (1.6)
Yes	343 (34.5)	2.5 (1.5)	186 (33.0)	3.5 (1.8)
College graduate				
No	289 (29.0)	2.3 (1.4)	381 (67.4)	3.7 (1.7)
Yes	708 (71.0)	2.9 (1.6)	184 (32.6)	3.8 (1.7)
Race/ethnicity				NA
Black	126 (12.6)	2.3 (1.6)		
Hispanic	61 (6.1)	2.8 (1.8)		
Asian	53 (5.3)	2.8 (1.6)		
White	713 (71.5)	2.8 (1.6)	569 (100)	3.8 (1.7)
Other	44 (4.4)	2.4 (1.7)		
Married or cohabitating				
No	71 (7.1)	2.1 (1.6)	11 (1.9)	3.8 (1.9)
Yes	925 (92.9)	2.8 (1.6)	558 (98.1)	3.7 (1.7)
Nulliparous				
No	516 (51.8)	2.6 (1.6)	316 (57.8)	3.6 (1.7)
Yes	481 (48.2)	2.8 (1.6)	231 (42.2)	3.9 (1.7)
Smoking status				
Never	693 (69.6)	2.8 (1.6)	364 (65.8)	3.8 (1.7)
Quit before pregnancy	209 (21.0)	2.7 (1.5)	97 (17.5)	3.7 (1.6)
Smoked during pregnancy	93 (9.3)	2.2 (1.4)	92 (16.6)	3.6 (1.7)
Child characteristics				
Sex				
Male	491 (49.2)	2.7 (1.6)	308 (54.1)	3.6 (1.7)
Female	506 (50.8)	2.7 (1.6)	261 (45.9)	3.9 (1.7)
Gestation length				
<34 weeks	15 (1.5)	2.7 (1.4)	8 (1.4)	3.6 (1.9)
≥34 weeks	982 (98.5)	2.7 (1.6)	553 (98.6)	3.8 (1.7)
Breastfeeding duration				
<3 months	243 (25.7)	2.4 (1.5)	272 (50.0)	3.7 (1.8)
≥3 months	701 (74.3)	2.9 (1.6)	272 (50.0)	3.8 (1.6)

MDS, mediterranean diet score.

cohorts studied separately (Table 2), although the magnitude and precision of estimates differed slightly. For example, in the covariate-adjusted model, each 3-point increment in MDS was associated with 0.13 units lower BMI *z*-score (95% CI: −0.24, −0.02) in Viva and 0.15 units lower in Rhea (95% CI: −0.29, 0.00). In the pooled analysis, for each 3-point increment in the MDS in pregnancy, offspring BMI *z*-score was lower by 0.14 units (95% CI, −0.15 to −0.13), waist circumference was lower by 0.39 cm (95% CI,

Table 2 Associations of Mediterranean diet score in pregnancy (per 3-point increment on a 0–9 scale) with offspring adiposity outcomes in project viva ($N = 997$) and rhea ($N = 569$) cohorts, separately and in pooled analysis

		Model 1 Basic model[†]	Model 2 Confounder model[‡]	Model 3 Mediator model – birth & infant characteristics[§]	Model 4 Mediator model – child lifestyle[¶]
	Mean (SD)		β (95% CI)		
Project Viva ($N = 997$)					
BMI *z*-score	0.36 (1.0)	−0.24 (−0.35,−0.12)	−0.13 (−0.24,−0.02)	−0.13 (−0.24,−0.01)	−0.13 (−0.24,−0.01)
Waist circumference (cm)	59.7 (7.9)	−0.95 (−1.82,−0.07)	−0.22 (−1.08, 0.64)	−0.23 (−1.08, 0.63)	−0.42 (−1.21, 0.38)
SS + TR (mm)	19.5 (9.2)	−1.40 (−2.43,−0.36)	−0.39 (−1.41, 0.63)	−0.22 (−1.24, 0.81)	−0.41 (−1.42, 0.61)
Rhea ($N = 569$)					
BMI *z*-score	−0.16 (1.0)	−0.13 (−0.27, 0.02)	−0.15 (−0.29, 0.00)	−0.12 (−0.27, 0.03)	−0.13 (−0.28, 0.02)
Waist circumference (cm)	53.5 (4.9)	−0.44 (−1.17, 0.29)	−0.58 (−1.32, 0.17)	−0.45 (−1.23, 0.33)	−0.34 (−1.00, 0.33)
SS + TR (mm)	17.0 (5.0)	−0.69 (−1.43, 0.05)	−0.86 (−1.62,−0.10)	−0.80 (−1.59,−0.01)	−0.88 (−1.63,−0.12)
Combined effect-Pooled analysis					
BMI *z*-score		−0.19 (−0.27,−0.12)	−0.14 (−0.15,−0.13)	−0.13 (−0.13,−0.12)	−0.12 (−0.13,−0.12)
Waist circumference (cm)		−0.73 (−1.08,−0.38)	−0.39 (−0.64,−0.14)	−0.34 (−0.49,−0.18)	−0.40 (−0.46,−0.34)
SS + TR (mm)		−1.13 (−1.59,−0.67)	−0.63 (−0.98,−0.28)	−0.53 (−0.97,−0.09)	−0.61 (−0.91,−0.31)

[†]Basic model includes child sex and age at outcome. [‡]Confounder model is basic model additionally adjusted for maternal age, pre-pregnancy body mass index, race/ethnicity, education level, parity, and smoking during pregnancy. [§]Mediator model – birth and infant characteristics is confounder model additionally adjusted for birth weight for gestation age *z*- score and breastfeeding duration. [¶]Mediator model – Child lifestyle characteristics is confounder model additionally adjusted for fast food intake, TV viewing and child's height (only for waist circumference and SS + TR) at age of outcome assessment. [††]SS + TR, sum of subscapular and triceps skin fold thickness.

−0.64 to −0.14), and the sum of SS and TR skin-fold thicknesses was lower by 0.63 mm (95% CI, −0.98 to −0.28) (Table 2).

Mediterranean diet adherence and offspring cardiometabolic traits

In the pooled analysis, for each 3-point increment in the MDS in pregnancy, offspring systolic blood pressure was lower by 1.03 mmHg (95% CI, −1.65 to −0.42), and diastolic blood pressure was lower by 0.57 mmHg (95% CI, −0.98 to −0.16) (Table 3). Higher adherence to Mediterranean diet was also associated with lower offspring serum (log) leptin levels (% change −6.0; 95% CI −8.5 to -3.5).

Stratified and sensitivity analyses

The study-level meta-analysis showed similar effect estimates, although with wider confidence intervals as expected (Tables S3 and S4; Fig. S4). We further performed additional analyses to explore the extent to which observed associations differed according to pre-specified maternal and child characteristics. We saw no evidence for a multiplicative interaction of adherence to the Mediterranean diet during pregnancy with pre-pregnancy BMI, maternal smoking during pregnancy and breastfeeding (*p* for interaction 0.10 to 0.99). Further adjustment for birth characteristics and infant feeding did not substantively alter any of adjusted models for childhood outcomes (Tables 2 and 3, Model 3). Results were also similar when we additionally adjusted for child TV watching, fast food intake and child anthropometry at age of the outcome assessment (Tables 2 and 3; Model 4) though with wider confidence intervals (data not shown). Adjustment for gestational weight gain, energy intake and gestational diabetes did not modify the direction of associations, although effect estimates were slightly attenuated (data not shown). A sensitivity analysis using the original MDS (based on cohort-specific median intake of nine food groups) gave similar results with the presented analysis (data not shown).

Discussion

In this analysis of prospective data from two cohorts with different levels and predictors of adherence to the Mediterranean diet, women with a greater adherence to the Mediterranean diet in early pregnancy had offspring who were less adipose and had lower blood pressure in childhood. The results were broadly similar in each of the two cohorts studied separately after controlling for a variety of confounders, remained robust in the pooled analysis and were not mediated by several birth, infant and child characteristics.

Few human studies have evaluated the role of maternal diet on childhood obesity risk. A pregnancy cohort study from Spain showed that higher adherence to the Mediterranean diet in pregnancy was associated with lower waist circumference but not with BMI in preschool children (14), suggesting a specific effect on programming body fat distribution leading to a lower abdominal obesity risk without influencing general obesity. Two other pregnancy cohorts investigated the association between maternal dietary patterns and child body composition and showed no significant associations (20,21). Previous studies have focused on specific food groups and showed that the consumption of a high-meat, low-carbohydrate diet (22) and high-fish intake during pregnancy (23) were associated with increased offspring adiposity.

In contrast with food groups or macronutrient analysis, the study of dietary patterns accounts for cumulative and interactive effects among nutrients, reflect real-world-dietary preferences and may be particularly suitable for analysis in epidemiology of childhood obesity where many dietary components could be related with the outcome of interest (24). A large cross-sectional study of 16 220 children aged 2–9 years in eight different European countries showed that a high adherence to the Mediterranean diet was inversely associated with overweight, obesity and fat mass (9). Suggested mechanisms include the low glycaemic effect of Mediterranean diet and its high antioxidant contain, which may lead to better foetal glucose metabolism and metabolic function and finally influence individual susceptibly to weight gain later in life (4).

We also found that a greater adherence to the Mediterranean diet during pregnancy was associated with lower levels of systolic and diastolic blood pressure in childhood. A recent meta-analyses of clinical trials and prospective cohort studies in adults showed that a high adherence to the Mediterranean diet was associated with lower systolic and diastolic blood pressure (7). Further, two observational cohort studies have showed that the consumption of a high-meat, low-carbohydrate diet in pregnancy was associated higher adult blood pressure in the offspring (25,26). These results were supported by animal studies suggesting that maternal high-fat diet can program rat offspring hypertension by activating the adipose renin-angiotensin system (27).

The pooled analysis revealed that higher adherence to the Mediterranean diet in early pregnancy was associated with lower leptin levels in childhood, likely reflecting lower offspring fat mass and lower triglyceride levels. A previous Project Viva analysis did not

Table 3 Associations of Mediterranean diet score in pregnancy (per 3-point increment on a 0–9 scale) with offspring cardiometabolic risk factors in project viva ($N = 997$) and rhea ($N = 569$) cohorts, separately, and in pooled analysis

		Model 1 Basic model[†]	Model 2 Confounder model[‡]	Model 3 Mediator model – birth & infant characteristics[§]	Model 4 Mediator model – child lifestyle[¶]
	Mean (SD)		β (95% CI)		
Project Viva ($N = 997$)					
SBP (mmHg)	94.5 (8.7)	−1.58 (−2.59,−0.57)	−1.38 (−2.42,−0.35)	−1.53 (−2.60,−0.46)	−1.17 (−2.18,−0.17)
DBP (mmHg)	54.4 (5.7)	−0.83 (−1.50,−0.16)	−0.81 (−1.50,−0.13)	−0.91 (−1.62,−0.20)	−0.77 (−1.48,−0.05)
	Mean (SD)		% Change (95% CI)		
CRP (mL dL^{-1})	0.8 (2.1)	−8.0 (−28.9, 19.0)	−2.9 (−21.2, 34.5)	−1.2 (−23.1, 33.2)	−1.0 (−23.5, 28.1)
HDL-cholesterol (mg dL^{-1})	57.1 (13.7)	0.9 (−2.9, 4.8)	0.5 (−3.4, 4.5)	0.3 (−3.7, 4.5)	0.5 (−3.5, 4.6)
Total cholesterol (mg dL^{-1})	159.8 (27.0)	2.8 (0.0, 5.6)	2.3 (−0.6, 5.2)	2.0 (−1.0, 5.0)	2.4 (−0.6, 5.5)
Adiponectin (uL mL^{-1})	15.6 (8.8)	5.3 (−3.5, 14.9)	1.7 (−7.1, 11.4)	3.9 (−5.3, 13.9)	3.5 (−5.7, 13.7)
Leptin (ng mL^{-1})	5.8 (6.8)	−7.5 (−18.7, 5.3)	−4.2 (−16.3, 9.7)	−2.3 (−14.8, 12.0)	−2.9 (−14.2, 10.0)
Rhea ($N = 569$)	Mean (SD)		β (95% CI)		
SBP (mmHg)	90.7 (7.9)	−0.35 (−1.70, 1.00)	−0.50 (−1.88, 0.88)	−0.85 (−2.25, 0.55)	−0.38 (−1.71, 0.95)
DBP[†](mmHg)	53.9 (5.3)	−0.12 (−1.02, 0.79)	−0.26 (−1.20, 0.68)	−0.39 (−1.35, 0.57)	−0.14 (−1.12, 0.83)
	Mean (SD)		% Change (95% CI)		
CRP (mL dL^{-1})	0.2 (0.7)	−13.4 (−31.1, 8.8)	−7.6 (−27.0, 17.0)	−10.1 (−29.5, 14.6)	−8.6 (−28.6, 16.9)
HDL-cholesterol (mg dL^{-1})	46.6 (10.5)	1.3 (−2.4, 5.1)	0.6 (−3.2, 4.6)	0.4 (−3.6, 4.5)	0.5 (−3.4, 4.7)
Total cholesterol (mg dL^{-1})	156.7 (28.7)	0.0 (−2.9, 2.9)	−1.1 (−4.1, 2.0)	−1.0 (−4.0, 2.1)	−1.1 (−4.1, 2.1)
Adiponectin (uL mL^{-1})	15.0 (8.6)	−6.6 (−15.2, 2.8)	−5.7 (−14.8, 4.5)	−6.8 (−16.1, 3.6)	−3.7 (−13.3, 7.0)
Leptin (ng mL^{-1})	2.8 (3.7)	−5.7 (−17.4, 7.8)	−7.7 (−19.5, 5.9)	−9.4 (−21.4, 4.6)	−4.0 (−14.1, 7.3)
Combined effect-pooled analysis			β (95% CI)		
SBP (mmHg)		−1.13 (−1.93,−0.33)	−1.03 (−1.65,−0.42)	−1.22 (−1.72,−0.71)	−0.82 (−1.40,−0.25)
DBP (mmHg)		−0.56 (−1.04,−0.07)	−0.57 (−0.98,−0.16)	−0.66 (−1.06,−0.27)	−0.49 (−0.96,−0.02)
			% Change (95% CI)		
CRP (mL/dL^{-1})		−11.9 (−15.8,−7.8)	−4.0 (−11.2, 3.8)	−6.4 (−13.9, 1.9)	−4.7 (−9.5, 0.3)
HDL-cholesterol (mg dL^{-1})		1.1 (0.8, 1.4)	0.6 (0.4, 0.8)	0.6 (0.3, 0.8)	0.2 (0.1, 0.2)
Total cholesterol (mg dL^{-1})		1.4 (−0.5, 3.4)	0.9 (−1.5, 3.3)	0.9 (−1.4, 3.2)	1.0 (−1.7, 3.7)
Adiponectin (uL mL^{-1})		−0.9 (−8.7, 7.5)	−1.6 (−6.5, 3.4)	−0.9 (−8.0, 6.7)	−0.1 (−5.2, 5.2)
Leptin (ng mL^{-1})		−6.4 (−7.5,−5.3)	−6.0 (−8.5,−3.5)	−5.9 (−11.0,−0.5)	−3.7 (−4.3,−3.1)

Lipids, leptin, adiponectin and CRP were log transformed to normalize their distributions. We calculated percent change by exponentiating beta coefficients, subtracting by 1 and multiplying by 100. [†]Basic model includes child sex and age at outcome. [‡]Confounder model also includes maternal age, pre-pregnancy body mass index, race/ethnicity, education level, parity, smoking during pregnancy and child sex and age at outcome assessment. [§]Mediator model – birth and infant characteristics is confounder model additionally adjusted for birth weight for gestation age z-score and breastfeeding duration. [¶]Mediator model – child lifestyle characteristics is confounder model additionally adjusted for fast food intake, TV viewing and child's BMI at age of outcome assessment. SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; CRP, C-reactive protein.

show association between Mediterranean diet adherence in pregnancy and leptin in cord blood (28), but we have previously reported that leptin in cord blood appears inversely associated with adiposity, whereas leptin in childhood predicts later excess weight gain.

Strengths of our study include the population-based prospective design, the fairly large sample size, the harmonized exposure estimates in the two cohorts, the detailed childhood body fat and cardiometabolic measurements and the centralized statistical analysis following a consensus protocol. While, in some respects, the Rhea and Viva cohorts were ideally suited for pooling because of their similar data collection tools, there was also variability in confounders (such as maternal ethnicity and education level), age of outcome assessment and interpretation of 'serving' size between the two cohorts. In spite of all these sources of variability, we found fairly homogeneous effects across the different cohorts, indicating that our results are robust. We included women living in a Mediterranean country and in the USA therefore, our results can be generalized to other than Mediterranean settings.

This study also had some limitations. First, imperfections in dietary assessment are always a concern in nutritional epidemiology. However, in both cohorts, FFQs were validated, were only asking about a relatively short time period, which results in less recalling, estimation and abstraction for the participants, and the frequency scales used were almost identical in the two cohorts. When we further adjusted the final models for energy intake, results remained in the same direction, although attenuated. Indices like the MDS have inherent limitations such as assuming equal contribution from each component and variability in choosing cut-off points for each component. To minimize this variability, we used the same absolute cut-off point for each MDS component in both cohorts so as to allow direct comparisons between the two cohorts. The levels of attrition in the Project Viva and Rhea cohorts are similar to those found in other birth cohort studies. We do not know obesity status of children lost to follow up. However, assuming that lost to follow up mother–child pairs, characterized by low socio-economic status, may have a worse quality of diet during pregnancy and higher BMI in childhood, our estimates may be underestimated. We assessed several adiposity outcomes and cardiometabolic risk factors, raising concern about multiple testing. However, an application of Bonferroni correction to take into account multiple comparisons will be inappropriate in this case given that we are studying outcomes that are highly correlated (29). We observed small effects for adiposity outcomes, while the results for leptin and blood pressure were more powerful. Although a small decrement of 1 cm in child waist circumference might not seem substantial at the individual level, the aggregative effect at the population level, as measured by a leftward shift in the distribution of abdominal obesity, may translate into a substantively large increase in the number of healthy children. Finally, it is important to note that this was an observational study and thus lacks the ability to conclude causality.

Conclusion

In conclusion, our results from two pregnancy cohort studies in the USA and Greece support the hypotheses that maternal adherence to the Mediterranean diet during pregnancy was associated with lower child adiposity, leptin and blood pressure levels. While intervention trials are needed to confirm these associations, it seems reasonable for healthcare providers to recommend healthy dietary patterns such as the Mediterranean diet for pregnant women.

Contributors

LC and EO designed the research; SR, SK, GC, AM, KS, MV collected the data and provided essential materials (cohort specific databases necessary for research); SR and VG, analysed the data and performed statistical analysis; LC, KEJ, and EO wrote the paper; and LC, MK, CM, MG, EO contributed to study supervision. All authors had primary responsibility for final content and read and approved the final manuscript.

Conflict of interest statement

No conflict of interest was declared.

Acknowledgements

We are grateful to all the participating families in the USA and Greece who take part in these ongoing cohort studies. The Rhea project was financially supported by European projects (EU FP6-2003-Food-3-NewGeneris, EU FP6. STREP Hiwate, EU FP7 ENV.2007.1.2.2.2. Project No 211250 Escape, EU FP7-2008-ENV-1.2.1.4 Envirogenomarkers, EU FP7-HEALTH-2009- single stage CHICOS, EU FP7 ENV.2008.1.2.1.6. Proposal No 226285 ENRIECO, EU- FP7- HEALTH-2012 Proposal No 308333 HELIX, and the Greek Ministry of Health (Program of Prevention of obesity and neurodevelopmental disorders in preschool children, in Heraklion district, Crete, Greece: 2011–2014; 'Rhea Plus': Primary Prevention Program of Environmental Risk Factors for

Reproductive Health, and Child Health: 2012–2015). Dr Chatzi was additionally supported by Fulbright institution. Project Viva was supported by grants from the US National Institutes of Health (R01 HL075504, R37 HD 034568, R01 ES016314). Dr. Oken was additionally funded by K24 HD069408, P30 DK040561, and P30 DK092924.

References

1. Gluckman PD, Hanson MA, Cooper C, Thornburg KL. Effect of *in utero* and early-life conditions on adult health and disease. *N Engl J Med* 2008; 359: 61–73.
2. Symonds ME, Sebert SP, Hyatt MA, Budge H. Nutritional programming of the metabolic syndrome. *Nat Rev Endocrinol* 2009; 5: 604–10.
3. Murrin C, Shrivastava A, Kelleher CC. Maternal macronutrient intake during pregnancy and 5 years postpartum and associations with child weight status aged five. *Eur J Clin Nutr* 2013; 67: 670–9.
4. Donnelly JM, Walsh JM, Byrne J, Molloy EJ, McAuliffe FM. Impact of maternal diet on neonatal anthropometry: a randomized controlled trial. *Pediatr Obes* 2015; 10: 52–6.
5. 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: 2599–608.
6. Huo R, Du T, Xu Y, *et al.*. Effects of Mediterranean-style diet on glycemic control, weight loss and cardiovascular risk factors among type 2 diabetes individuals: a meta-analysis. *Eur J Clin Nutr* 2015; 69: 1200–8.
7. Kastorini CM, Milionis HJ, Esposito K, *et al.*. The effect of Mediterranean diet on metabolic syndrome and its components: a meta-analysis of 50 studies and 534,906 individuals. *J Am Coll Cardiol* 2011; 57: 1299–313.
8. Romaguera D, Norat T, Vergnaud AC, *et al.*. Mediterranean dietary patterns and prospective weight change in participants of the EPIC-PANACEA project. *Am J Clin Nutr* 2010; 92: 912–21.
9. Tognon G, Hebestreit A, Lanfer A, *et al.*. Mediterranean diet, overweight and body composition in children from eight European countries: cross-sectional and prospective results from the IDEFICS study. *Nutr Metab Cardiovasc Dis* 2014; 24: 205–13.
10. Saunders L, Guldner L, Costet N, *et al.*. Effect of a Mediterranean diet during pregnancy on fetal growth and preterm delivery: results from a French Caribbean Mother-Child Cohort Study (TIMOUN). *Paediatr Perinat Epidemiol* 2014; 28: 235–44.
11. Mikkelsen TB, Osterdal ML, Knudsen VK, *et al.*. Association between a Mediterranean-type diet and risk of preterm birth among Danish women: a prospective cohort study. *Acta Obstet Gynecol Scand* 2008; 87: 325–30.
12. Timmermans S, Steegers-Theunissen RP, Vujkovic M, *et al.*. The Mediterranean diet and fetal size parameters: the Generation R Study. *Br J Nutr* 2012; 108: 1399–409.
13. Chatzi L, Mendez M, Garcia R, *et al.*. Mediterranean diet adherence during pregnancy and fetal growth: INMA (Spain) and RHEA (Greece) mother-child cohort studies. *Br J Nutr* 2012; 107: 135–45.
14. Fernandez-Barres S, Romaguera D, Valvi D, *et al.*. Mediterranean dietary pattern in pregnant women and offspring risk of overweight and abdominal obesity in early childhood: the INMA birth cohort study. *Pediatr Obes* 2016. [Epub ahead of print] doi: 10.1111/ijpo.12092.
15. Oken E, Baccarelli AA, Gold DR, *et al.*. Cohort profile: project viva. *Int J Epidemiol* 2015; 44: 37–48.
16. Chatzi L, Plana E, Daraki V, *et al.*. Metabolic syndrome in early pregnancy and risk of preterm birth. *Am J Epidemiol* 2009; 170: 829–36.
17. Fawzi WW, Rifas-Shiman SL, Rich-Edwards JW, Willett WC, Gillman MW. Calibration of a semi-quantitative food frequency questionnaire in early pregnancy. *Ann Epidemiol* 2004; 14: 754–62.
18. Chatzi L, Melaki V, Sarri K, *et al.*. Dietary patterns during pregnancy and the risk of postpartum depression: the mother-child 'Rhea' cohort in Crete. *Greece Public Health Nutr* 2011; 14: 1663–70.
19. McGuire S. U.S. Department of Agriculture and U.S. Department of Health and Human Services, Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U. S. Government Printing Office, January 2011. *Adv Nutr* 2011; 2: 293–4.
20. Poon AK, Yeung E, Boghossian N, Albert PS, Zhang C. Maternal dietary patterns during third trimester in association with birthweight characteristics and early infant growth. *Scientifica (Cairo)* 2013; 2013: 786409.
21. van den Broek M, Leermakers ET, Jaddoe VW, *et al.*. Maternal dietary patterns during pregnancy and body composition of the child at age 6 y: the Generation R Study. *Am J Clin Nutr* 2015; 102: 873–80.
22. Yin J, Quinn S, Dwyer T, Ponsonby AL, Jones G. Maternal diet, breastfeeding and adolescent body composition: a 16-year prospective study. *Eur J Clin Nutr* 2012; 66: 1329–34.
23. Stratakis N, Roumeliotaki T, Oken E, *et al.*. Fish intake in pregnancy and child growth: a pooled analysis of 15 European and US birth cohorts. *JAMA Pediatr* 2016; 170: 381–90.
24. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* 2002; 13: 3–9.
25. Campbell DM, Hall MH, Barker DJ, *et al.*. Diet in pregnancy and the offspring's blood pressure 40 years later. *Br J Obstet Gynaecol* 1996; 103: 273–80.
26. Shiell AW, Campbell-Brown M, Haselden S, *et al.*. High-meat, low-carbohydrate diet in pregnancy: relation to adult blood pressure in the offspring. *Hypertension* 2001; 38: 1282–8.
27. Guberman C, Jellyman JK, Han G, Ross MG, Desai M. Maternal high-fat diet programs rat offspring hypertension and activates the adipose renin-angiotensin system. *Am J Obstet Gynecol* 2013; 209: 262 e1–8.
28. Mantzoros CS, Sweeney L, Williams CJ, *et al.*. Maternal diet and cord blood leptin and adiponectin concentrations at birth. *Clin Nutr* 2010; 29: 622–6.
29. Rothman KJ. No adjustments are needed for multiple comparisons. *Epidemiology* 1990; 1: 43–6.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Description of food groups in the Mediterranean Diet Score for pregnant women in project Viva and Rhea cohorts

Table S2. Maternal and child characteristics of participants and non-participants in the childhood follow up in Project Viva and Rhea cohorts

Table S3. Adjusted associations (meta-analysis) of Mediterranean diet score in pregnancy with offspring adiposity outcomes

Table S4. Adjusted associations (meta-analysis) of Mediterranean diet score in pregnancy with offspring cardiometabolic risk factors

Figure S1. Directed acyclic graph for assessing the association between maternal adherence to the Mediterranean diet in pregnancy and child adiposity. Green node, exposure of interest; blue node: outcome of interest; and white nodes: the adjustment set used in the study.

Figure S2. Food groups intake during pregnancy according to Mediterranean Diet score in Project Viva and Rhea pregnancy cohort studies. Ratio MUFA: SFA; Ratio of monounsaturated to saturated fatty acids

Figure S3. Association of Mediterranean diet score in pregnancy with child BMI z score in pooled analysis (A), Project Viva (B) and Rhea (C) pregnancy cohort studies. Generalized additive models adjusted for maternal age, pre-pregnancy body mass index, race/ethnicity, education level, parity, smoking during pregnancy, and child sex and age at outcome assessment.

Figure S4. Adjusted associations (meta-analysis) of Mediterranean diet score in pregnancy with offspring adiposity and blood pressure measurements. *B* coefficients (95% CIs) by cohort were obtained by using linear regression models adjusted for maternal age, pre-pregnancy body mass index, race/ethnicity, education level, parity, smoking during pregnancy, child sex, and age at outcome assessment. Combined estimates were obtained by using a random-effects meta-analysis. SS+TR, sum of subscapular and triceps skin fold thickness; *p*-heter values estimated by using Cochran's *Q* test; I^2 = percentage of the total variability due to between-areas heterogeneity.