Adherence to a Mediterranean diet and plasma concentrations of lipid peroxidation in premenopausal women[1–3]

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ABSTRACT

Background: A Mediterranean diet has been associated with a reduced risk of cardiovascular disease and mortality. A possible mechanism is through a decrease in lipid peroxidation (LPO); however, evidence linking the Mediterranean diet with lower LPO in premenopausal women is sparse.

Objective: We investigated whether adherence to a Mediterranean diet was associated with lower LPO concentrations in premenopausal women.

Design: Two hundred fifty-nine healthy women aged 18–44 y were followed for $\leq$2 menstrual cycles. Plasma concentrations of F_2-isoprostane (8-iso-PGF2α), 9-hydroxyoctadecadieneoic acid (9-HODE), and thiobarbituric acid reactive substances (TBARS) were measured $\leq$8 times per cycle at visits scheduled by using fertility monitors. Diet was assessed $\leq$4 times per cycle by using 24-h dietary recalls. The alternate Mediterranean Diet Score (aMED) (range: 0–9) was calculated on the basis of intake of vegetables, legumes, fruit, nuts, whole grains, red and processed meat, fish, and alcohol and the ratio of monounsaturated to saturated fat.

Results: A 1-unit increase in aMED was associated with a 4.50% decrease in 8-iso-PGF2α concentrations (95% CI: −6.32%, −2.65%) and a 14.01% decrease in 9-HODE concentrations (95% CI: −17.88%, −9.96%) after adjustment for energy intake, age, race, body mass index, plasma ascorbic acid, and serum cholesterol. No significant association was observed between aMED and TBARS. A 1-unit increase in aMED was associated with a 1.39% increase (95% CI: 0.07%, 2.72%) in plasma ascorbic acid concentrations.

Conclusions: Adherence to a Mediterranean diet is associated with lower LPO and higher ascorbic acid concentrations. These results confirm that decreased LPO is a plausible mechanism linking a Mediterranean diet to reduced cardiovascular disease risk. *Am J Clin Nutr* 2010;92:1461–7.

INTRODUCTION

The Mediterranean diet is widely reported as a model of healthy eating and is characterized by a high intake of fruit, vegetables, grains, legumes, olive oil, nuts, and seeds; a low-to-moderate intake of dairy products, fish, poultry, and wine; and a low intake of red meat and eggs (1–3). Adherence to a Mediterranean diet has been associated with reduced risk of cardiovascular disease and total mortality (4–6). One potential mechanism driving this association is a decrease in lipid peroxidation (LPO) concentrations, a type of oxidative stress (OS) (7); however, evidence linking the Mediterranean diet with lower concentrations of these biomarkers is sparse, and to date the populations studied have been limited. In a cohort of male twins, adherence to a Mediterranean diet was associated with more beneficial ratios of reduced-to-oxidized glutathione (GSH/GSSG), a plasma marker of OS (8). In older women, a randomized controlled trial (mean age: 68 y) and a cohort study (mean age: 45 y) found that adherence to a Mediterranean diet was associated with lower oxidized LDL concentrations, a plasma marker of LPO (9, 10). However, no study yet, to our knowledge, has focused on premenopausal women and included several markers of LPO measured on multiple occasions.

We examined the association between adherence to a Mediterranean diet–type dietary pattern and F_2-isoprostane (8-iso-PGF2α), 9-hydroxyoctadecadieneoic acid (9-HODE), and thiobarbituric acid reactive substances (TBARS), which are 3 biomarkers of LPO, in a sample of healthy reproductive-aged women. Because certain markers of LPO fluctuate throughout the menstrual cycle (11), we evaluated markers of LPO at different and specific phases of the menstrual cycle ($\leq$8 times per cycle).

SUBJECTS AND METHODS

Study participants

The BioCycle Study is a prospective cohort study of menstrual cycle function that was conducted between 2005 and 2007 in 259 healthy premenopausal women 18–44 y of age from western New York, who were followed for 1 (*n* = 9) or 2 (*n* = 250) menstrual

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cycles. Exclusion criteria included current use of oral contraceptives, vitamin and mineral supplements, or prescription medications; pregnancy or breastfeeding in the past 6 mo; diagnosis of chronic medical conditions, including metabolic disorders and gastrointestinal diseases associated with malabsorption; self-reported body mass index (BMI; in kg/m^2) at screening <18 or >35; or current dietary restrictions for weight loss or medical reasons. Details of the study have been published elsewhere (12). The University at Buffalo Health Sciences Institutional Review Board approved the study, and all participants provided written informed consent.

Blood collection

Fasting blood specimens were collected between 0700 and 0830 at ≤8 clinic visits per cycle. Visits were timed by using fertility monitors (Clear Blue Easy Fertility Monitor; Inverness Medical, Waltham, MA) and occurred on approximately the second day of menstruation, during the mid and late follicular phase, at 2 d around expected ovulation, and during the early, mid, and late luteal phase (13). Collection and handling protocols were designed to minimize variability in preanalytic factors and have been previously described (14). All samples were frozen at −80°C within 90 min of phlebotomy. They were later shipped on dry ice to analytic laboratories as a complete cycle, and measured simultaneously, within a single analytic run, to limit analytic variability. Ninety-four percent of participants completed ≥7 clinic visits per cycle, and 100% completed 5 visits per cycle, with fewer visits typically a result of shorter cycles.

Measurements of lipid peroxidation and antioxidant status

All LPO measurements were conducted by using anticoagulated blood plasma in EDTA-coated tubes. Free 8-iso-PGF2α was the primary marker of LPO used because it is currently considered the gold standard (15). Isoprostanes are prostaglandin-like eicosanoids formed in vivo by nonenzymatic, free radical–initiated peroxidation of arachidonic acid–derived eicosanoids (16). Plasma 8-iso-PGF2α was measured with a gas chromatography–mass spectrometry–based method by the Molecular Epidemiology and Biomarker Research Laboratory (University of Minnesota, Minneapolis, MN) (9.4% CV). 9-HODE was used as an additional marker of LPO. It is formed from the oxidation of linoleic acid, which is the major polyunsaturated fatty acid (PUFA) in serum, and is considered an excellent marker for LPO (17). Total plasma 9-HODE (9.0% CV) was determined by HPLC with diode array detection at 234 nanometers (nm) (18). All solvents were HPLC grade from Fischer Scientific (Fair Lawn, NJ). Malondialdehyde in the form of thiobarbituric acid reactive substances (TBARS) was used as the third measure of LPO. Plasma TBARS (8.3% CV) were measured at the University at Buffalo by using OxiTech reagent kits from ZeptoMetrix Corporation (Buffalo, NY) and are expressed in nmol/mL of malondialdehyde equivalents (19). TBARS pigment was measured at an excitation of 535 nm and an emission of 552 nm by using a RF-5000U spectrofluorometer (Shimadzu Scientific Instruments Inc, Columbia, MD).

Vitamin A (retinol), vitamin E (α-, γ-, and δ-tocopherol), and vitamin C (ascorbic acid) were measured in blood to assess antioxidant status. Retinol (6.1% CV) and tocopherols (α-, δ-, and γ-tocopherol; 2.3%, 2.2%, and 21.2% CVs, respectively) were measured simultaneously in serum by using HPLC (20). Total ascorbic acid (9.6% CV) was determined by the dinitrophenylhydrazine (DNPH) method in heparin plasma stabilized at collection in 6% meta-phosphoric acid (21). The absorbance of each DNPH-derivatized sample was determined at 520 nm by using a Shimadzu 160U spectrophotometer (Shimadzu Scientific Instruments Inc). All antioxidants were measured at the University at Buffalo (Buffalo, NY).

Assessment of dietary intake

Dietary intake was assessed by 24-h dietary recalls on the same days as blood sample collection and were conducted ≤4 times per cycle (corresponding to menses, mid-follicular phase, ovulation, and mid-luteal phase), for a total of 8 possible recalls over 2 cycles. The 24-h recalls were conducted by trained research staff and were done both in person and by phone. Dietary data were analyzed by using the Nutrition Data System for Research software version 2005 developed by the Nutrition Coordinating Center of the University of Minnesota, Minneapolis, MN. The majority of women completed 4 dietary recalls per cycle (87%), and all participants completed ≥2 recalls per cycle.

Calculation of the alternate Mediterranean Diet Score

The alternate Mediterranean Diet Score (aMED), a modification of the traditional score (tMED) developed by Trichopoulou et al (4), was calculated for each dietary recall day (22). The score is based on dietary intake of 9 items: vegetables (excluding potatoes), legumes, fruit, nuts, whole grains, red and processed meat, fish, alcohol, and the ratio of monounsaturated to saturated fat. In most cases, intakes above the median received 1 point, whereas other intakes received 0 points. Exceptions to this were the red- and processed-meat category, in which consumption of less than the median received 1 point, and the alcohol category, in which alcohol intake between 5 and 15 g/d received 1 point. Possible scores on the aMED range from 0 to 9; higher scores correspond to greater adherence. We also compared results to the tMED (4). This score is based on the dietary intake of the same 9 items, except that the tMED combines fruit and nuts into 1 group, creates a dairy group, and includes all grain products in the cereal category and all meats in the meat category. Like the aMED, the tMED also yields a range of scores from 0 to 9.

Covariate assessment

At baseline, height (in m) and weight (in kg) were measured by using standardized techniques, and BMI was calculated. Participants also completed surveys on physical activity (23), lifestyle, and reproductive health history. High, moderate, and low physical activity categories were created on the basis of standard International Physical Activity Questionnaire cutoffs. Intake of medications or supplements and/or vitamin use were recorded in daily diaries across the cycle; however, intake was minimal. Total cholesterol (CV <5.0%) was measured in serum by an autochemistry analyzer at the Kaleida Center for Laboratory Medicine (Buffalo, NY). Triglycerides were measured by an enzymatic, single-reagent procedure on a Beckman LX20

automated chemistry analyzer at Kaleida Health Systems (Buffalo, NY) (24). Estradiol was measured by radioimmunoassay at Specialty Laboratories (Valencia, CA).

Statistical analyses

Repeated-measures analysis of variance was used to compare aMED and other dietary components across the cycle. Because no significant differences were observed, the average aMED score and average daily intake for other dietary variables were calculated per cycle for this analysis. Descriptive statistics were calculated for demographic characteristics, diet components, and measures of LPO and antioxidants according to categories of average aMED: ≤2, 2.01–3, 3.01–4, and >4. Linear mixed models were used to test for associations between demographic variables, dietary components, LPO markers, and antioxidant vitamins, and category of aMED (25).

Associations between aMED and log LPO and antioxidant concentrations were assessed by using linear mixed models, which account for correlation both between and within women (25). Random intercepts were included to account for variability in average LPO and antioxidant concentrations between women. Because the outcome variables were log transformed, we expressed the results as a percentage difference of the nontransformed values as follows:

$$[(\exp^{\beta}) - 1] \times 100\ (\%)] \qquad (1)$$

where β is the regression coefficient. The median value of each category of aMED was used as a continuous variable to test for linear trends.

The choice of covariates in the adjusted models was determined by a review of the prior literature. Important a priori confounders considered were as follows: age (continuous); BMI

TABLE 1

Demographic and dietary characteristics according to mean alternate Mediterranean Diet Score (aMED) across the menstrual cycle in premenopausal women (n = 259)[1]

	Total cohort	Mean aMED				*P* value[2]
		0–2	2.01–3	3.01–4	4.01–9	
No. of cycles	509	132	185	126	66	
Demographic characteristics						
Age (y)	27.3 ± 8.2[3]	27.6 ± 8.5	27.0 ± 8.1	28.1 ± 8.4	26.9 ± 8.1	0.70
BMI (kg/m^2)	24.1 ± 3.9	25.1 ± 4.1	23.8 ± 3.6	24.3 ± 4.0	22.5 ± 3.6	0.007
Physical activity [*n* (%)]						0.11
Low	48 (9.5)	12 (9.1)	19 (10.2)	12 (9.5)	5 (7.6)	
Moderate	181 (36.0)	43 (32.6)	58 (31.2)	45 (35.7)	35 (53.0)	
High	279 (54.5)	77 (58.3)	107 (58.6)	69 (54.8)	26 (39.4)	
Race [*n* (%)]						0.004
White	302 (59.4)	71 (53.8)	100 (54.1)	83 (66.9)	48 (72.7)	
African American	101 (19.8)	38 (28.8)	42 (22.7)	13 (10.3)	8 (12.1)	
Other	106 (20.8)	23 (17.4)	43 (23.2)	30 (23.8)	10 (15.2)	
Past or current smoker [*n* (%)]	92 (18.1)	27 (20.5)	32 (17.3)	21 (16.7)	12 (18.2)	0.74
Less than high school education [*n* (%)]	64 (12.6)	20 (15.2)	26 (14.1)	15 (11.9)	3 (4.5)	0.17
Age at menarche (y)	12.4 (1.2)	12.3 (1.2)	12.4 (1.3)	12.6 (1.3)	12.7 (1.1)	0.03
Nulliparous [*n* (%)]	367 (73.6)	94 (73.4)	133 (73.9)	90 (72.0)	50 (75.8)	0.94
Nulligravida [*n* (%)]	347 (69.5)	91 (71.1)	125 (69.4)	83 (66.4)	48 (72.7)	0.83
aMED components						
Vegetables (except potatoes) (servings/d)	2.24 ± 1.26	1.59 ± 0.77	1.99 ± 1.04	2.58 ± 1.13	3.60 ± 1.61	<0.001
Legumes (peas, beans, and tofu) (servings/d)	0.14 ± 0.29	0.03 ± 0.09	0.09 ± 0.19	0.15 ± 0.25	0.45 ± 0.52	<0.001
Fruit (whole and juices) (servings/d)	1.17 ± 1.06	0.79 ± 0.87	1.11 ± 1.12	1.34 ± 0.96	1.77 ± 1.11	<0.001
Nuts (and nut butters) (servings/d)	0.33 ± 0.73	0.05 ± 0.19	0.21 ± 0.49	0.42 ± 0.73	1.05 ± 1.30	<0.001
Whole grains (servings/d)	0.85 ± 1.03	0.35 ± 0.56	0.74 ± 0.73	1.03 ± 1.10	1.78 ± 1.55	<0.001
Red and processed meats (servings/d)	1.55 ± 1.48	2.12 ± 1.56	1.61 ± 1.39	1.29 ± 1.44	0.74 ± 1.08	<0.001
Fish (servings/d)	0.42 ± 0.75	0.16 ± 0.43	0.41 ± 0.71	0.61 ± 0.93	0.59 ± 0.82	<0.001
MUFA:SFA ratio	1.19 ± 0.31	1.07 ± 0.23	1.13 ± 0.23	1.24 ± 0.32	1.47 ± 0.44	<0.001
Alcohol[4] (g/d)	2.66 ± 6.02	2.57 ± 6.88	2.18 ± 4.96	3.57 ± 7.10	2.44 ± 4.34	0.12
Other dietary components						
Total energy (kcal)	1608.1 ± 405.0	1576.0 ± 399.5	1569.0 ± 386.3	1634.2 ± 435.8	1732.1 ± 386.5	0.08
PUFA (%)	7.03 ± 1.98	6.50 ± 1.78	6.93 ± 1.92	7.35 ± 2.02	7.78 ± 2.18	<0.001
MUFA (%)	12.56 ± 2.82	12.93 ± 2.52	12.53 ± 2.88	12.29 ± 2.86	12.43 ± 3.13	0.29
SFA (%)	11.54 ± 2.94	12.95 ± 2.65	11.83 ± 2.84	10.73 ± 2.75	9.43 ± 2.46	<0.001
MUFA (g/d)	23.06 ± 8.44	22.91 ± 7.40	22.48 ± 8.14	23.39 ± 10.28	24.37 ± 7.29	0.43
Omega-3 fatty acids (g/d)	1.37 ± 0.64	1.18 ± 0.53	1.28 ± 0.54	1.50 ± 0.68	1.73 ± 0.82	<0.001
Omega-6 fatty acids (g/d)	11.42 ± 4.88	10.33 ± 4.30	10.94 ± 4.18	12.27 ± 6.09	13.34 ± 4.43	<0.001

[1] PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid.

[2] Two-sided *P* values were calculated by using generalized linear mixed models. All comparisons take repeated measures and correlations between cycles into account.

[3] Mean ± SD (all such values).

[4] One standard alcohol drink = 12 g alcohol.

(continuous); race (white, black, other); physical activity (low, moderate, high); smoking status (never, current/past); reproductive history [gravidity (0, ≥1), parity (0, ≥1), age at menarche (continuous)]; total energy intake (continuous); serum concentrations of total cholesterol (continuous), triglycerides, and estradiol; and several antioxidants, in particular E vitamins and ascorbic acid (continuous). These a priori confounders were included in the fully adjusted model if they changed the exposure coefficient by >15% (26). We adjusted for serum cholesterol concentrations because differences in blood lipid concentrations can confound the interpretation of antioxidant concentrations, and because lipoproteins are carriers of vitamin E and have been associated with oxidation (27–29). Interactions between aMED and lifestyle factors such as smoking, BMI, and chronic alcohol consumption were tested by using cross-product terms in the linear mixed models. Analyses were conducted by using SAS version 9.1 (SAS Institute, Cary, NC), and all comparisons took repeated measures and correlations between cycles into account.

RESULTS

Baseline characteristics

Overall, this cohort of women was young (mean age: 27.3 y), of normal weight (mean BMI: 24.1), had moderate to high physical activity (90.8%), had never smoked (81.9%), and had low adherence to the aMED (mean: 2.9; range: 0.7–6.8) (**Table 1**). Women with a higher average aMED were more likely to be white and have lower BMIs; these women also had significantly higher concentrations of ascorbic acid and lower concentrations of 8-iso-PGF2α and 9-HODE (**Table 2**).

Adherence to the aMED in relation to concentrations of lipid peroxidation and antioxidants

The aMED was inversely and significantly associated with plasma 8-iso-PGF2α and 9-HODE concentrations in the fully adjusted models (**Table 3**). On average (after adjustment for total energy intake, race, BMI, age, plasma ascorbic acid, and total serum cholesterol), a 1-unit increase in aMED was associated with a 4.50% decrease in concentrations of 8-iso-PGF2α (95% CI: −6.32%, 2.65%) and a 14.01% decrease in concentrations of 9-HODE (95% CI: −17.88%, 9.96%). Slightly attenuated but statistically significant results were also observed for adherence to the traditional (tMED) score and 8-iso-PGF2α and 9-HODE concentrations (3.6% and 10.2% reduction, respectively, per 1-unit increase; data not shown). Women with an average aMED >4 (mean score: 4.7) had, on average, 10.4% lower plasma 8-iso-PGF2α and 36.8% lower plasma 9-HODE concentrations than did women with an average aMED <2 (mean score: 1.6) ($P < 0.05$). TBARS was not significantly associated with adherence to aMED (or tMED). With regard to the antioxidant vitamin profile, the effect of aMED was minimal; only plasma ascorbic acid was significantly associated with adherence to an aMED. Specifically, a 1-unit increase in aMED score was associated with a 1.39% increase (95% CI: 0.07%, 2.72%) in plasma ascorbic acid concentrations.

DISCUSSION

Greater adherence to a Mediterranean diet, as measured by the aMED, was associated with lower concentrations of LPO, specifically plasma 8-iso-PGF2α and 9-HODE concentrations, and with higher concentrations of plasma ascorbic acid in premenopausal women even after adjustment for demographic and biological confounders.

Despite differences in study design (eg, demographic characteristics of study populations, measures of OS examined, and type of Mediterranean diet score used), most of the studies have consistently reported that adherence to a Mediterranean diet is associated with lower concentrations of OS (8–10). A randomized controlled trial from Spain showed that a 3-mo Mediterranean diet intervention was associated with significant reductions in LDL oxidation in older men (n = 162; mean age: 67 y)

TABLE 2
Plasma concentrations of lipid peroxidation and serum antioxidants according to mean alternate Mediterranean Diet Score (aMED) across the menstrual cycle in premenopausal women (n = 259)[1]

	Total cohort	Mean aMED				P value[2]
		0–2	2.01–3	3.01–4	4.01–9	
No. of cycles	509	128	187	126	68	
Lipid peroxidation						
8-iso-PGF2α (pg/mL)	46.2 (36.9, 59.0)[3]	52.0 (39.6, 68.6)	46.5 (37.0, 58.7)	45.5 (37.3, 57.2)	39.3 (33.0, 47.5)	0.01
9-HODE (μmol/L)	0.15 (0.09, 0.24)	0.16 (0.09, 0.26)	0.15 (0.09, 0.25)	0.14 (0.10, 0.23)	0.12 (0.08, 0.21)	<0.001
TBARS (nmol/mL)	0.85 (0.72, 1.00)	0.81 (0.70, 0.98)	0.87 (0.74, 1.02)	0.84 (0.73, 0.99)	0.87 (0.75, 0.99)	0.15
Antioxidants						
α-Tocopherol (μg/mL)	8.0 (6.8, 9.3)	7.5 (6.6, 8.8)	7.9 (6.7, 9.1)	8.2 (7.0, 9.6)	8.3 (7.2, 10.3)	0.19
δ-Tocopherol (μg/mL)	0.15 (0.11, 0.19)	0.14 (0.11, 0.19)	0.15 (0.12, 0.20)	0.14 (0.11, 0.19)	0.14 (0.10, 0.18)	0.80
γ-Tocopherol (μg/mL)	1.7 (1.4, 2.2)	1.8 (1.5, 2.2)	1.8 (1.4, 2.2)	1.6 (1.2, 2.0)	1.5 (1.1, 2.0)	0.65
Retinol (μg/mL)	0.37 (0.32, 0.42)	0.36 (0.30, 0.42)	0.36 (0.32, 0.41)	0.38 (0.32, 0.43)	0.38 (0.34, 0.43)	0.85
Ascorbic acid[4] (mg/dL)	1.7 (1.4, 2.0)	1.6 (1.3, 1.9)	1.6 (1.4, 2.0)	1.8 (1.5, 2.1)	1.9 (1.6, 2.3)	0.04

[1] 8-iso-PGF2α, F_2-isoprostane; 9-HODE, 9-hydroxyoctadecadieneoic acid; TBARS, thiobarbituric acid reactive substances.

[2] Two-sided P values were calculated by using generalized linear mixed models. All comparisons take repeated measures and correlations between cycles into account.

[3] Median; 25th, 75th percentile in parentheses (all such values).

[4] Ascorbic acid was measured in plasma.

TABLE 3
Association between adherence to the alternate Mediterranean Diet Score (aMED) and adjusted geometric mean concentrations of plasma lipid peroxidation and serum antioxidants[1]

Outcome and model	Difference per 1-unit increase in aMED	95% CI	Mean aMED[2]				P value[3]
			0–2	2.01–3	3.01–4	4.01–9	
	%						
No. of cycles	—	—	128	187	126	68	—
Lipid peroxidation							
8-iso-PGF2α (pg/mL)							
Energy adjusted	−4.48	(−6.35, −2.58)	50.57	48.67	46.61	45.06	0.01
Fully adjusted[4]	−4.50	(−6.32, −2.65)	54.49	52.33	49.98	48.85	0.003
9-HODE (μmol/L)							
Energy adjusted	−13.45	(−17.21, −9.53)	0.19	0.16	0.13	0.11	0.05
Fully adjusted[4]	−14.01	(−17.88, −9.96)	0.19	0.15	0.13	0.12	<0.001
TBARS (nmol/mL)							
Energy adjusted	0.87	(−0.21, 1.96)	0.84	0.84	0.83	0.88	0.19
Fully adjusted[4]	1.01	(−0.10, 2.22)	0.83	0.83	0.82	0.86	0.12
Antioxidants							
α-Tocopherol (μg/mL)							
Energy adjusted	−0.04	(−0.90, 0.83)	8.00	7.91	8.06	7.97	0.69
Fully adjusted[5]	0.16	(−0.67, 0.99)	8.25	8.19	8.33	8.24	0.75
δ-Tocopherol (μg/mL)							
Energy adjusted	0.64	(−0.35, 1.64)	0.17	0.17	0.18	0.19	0.48
Fully adjusted[5]	0.64	(−0.41, 1.64)	0.15	0.15	0.16	0.17	0.39
γ-Tocopherol (μg/mL)							
Energy adjusted	−1.39	(−3.04, 0.32)	1.67	1.67	1.66	1.60	0.27
Fully adjusted[5]	−1.29	(−2.75, 0.67)	1.65	1.65	1.64	1.59	0.44
Retinol (μg/mL)							
Energy adjusted	−0.12	(−0.98, 0.76)	0.37	0.37	0.37	0.37	0.60
Fully adjusted[5]	−0.08	(−0.94, 0.79)	0.38	0.38	0.38	0.38	0.89
Ascorbic acid[6] (mg/dL)							
Energy adjusted	1.44	(0.14, 2.77)	1.69	1.67	1.73	1.73	0.04
Fully adjusted[5]	1.39	(0.07, 2.72)	1.67	1.65	1.71	1.70	0.07

[1] 8-iso-PGF2α, F_2-isoprostane; 9-HODE, 9-hydroxyoctadecadieneoic acid; TBARS, thiobarbituric acid reactive substances. Analyses used linear mixed models with random intercepts.
[2] Values are geometric means.
[3] P values were calculated by using a test for trend across aMED category.
[4] Adjusted for energy intake (kcal, continuous), age (continuous), race (white, black, other), BMI (continuous), plasma ascorbic acid (continuous), and serum cholesterol (continuous).
[5] Adjusted for energy intake (kcal, continuous), age (continuous), race (white, black, other), BMI (continuous), and serum cholesterol (continuous).
[6] Ascorbic acid was measured in plasma.

and women (n = 210; mean age: 68 y) at high risk of cardiovascular disease (9). Similarly, a cohort study in male twins (n = 297; mean age: 55 y) showed that a 1-unit within-pair difference in tMED was associated with a 10% higher GSH/GSSG ratio (corresponding to lower concentrations of OS) (8). A cohort study from Greece in older men (n = 322; mean age: 46 y) and women (n = 252; mean age: 45 y) also showed that greater adherence to a Mediterranean diet was inversely associated with oxidized LDL concentrations ($\beta = -0.34$, $P < 0.001$) (10). Similar to prior research, our results showed a robust association between adherence to a Mediterranean diet and lower OS as measured by markers of LPO. In addition, our study extends the inverse association between a Mediterranean diet and LPO to young, healthy women and includes 3 markers of LPO (8-iso-PGF2α, 9-HODE, and TBARS) that have not previously been evaluated.

Because the Mediterranean diet is characterized by abundant plant foods rich in antioxidants, vitamins, minerals, and phytochemicals (7), the observed associations between adherence to a Mediterranean diet and decreased LPO can be explained by several biological mechanisms. Higher intake of tocopherols, in foods including nuts and vegetable oils, is thought to have one of the strongest protective effects as it is the most abundant antioxidant in LDL particles. α-Tocopherol has been shown to inhibit radical chain propagation by scavenging highly reactive lipid peroxyl and alkoxyl radicals, which can promote propagation of LPO (30, 31). Similarly, higher intakes of ascorbic acid may prevent LPO by scavenging free radicals and other reactive species in an aqueous milieu (31). Ascorbate may also inhibit the prooxidant activity of α-tocopherol by reducing α-tocopheroxyl radical to α-tocopherol and thus regenerating its antioxidant activity (31). The high intake of monounsaturated fatty acids (olive oil) rather than PUFAs (vegetable oil) might also confer LPO protection because lipoproteins enriched in oleate (monounsatured fatty acid) were markedly more resistant to LPO than were those enriched with linoleate (PUFA) (32). Finally, the high flavonoid intake (from fruit, vegetables, olive oil, and red wine) may offer LPO protection by directly scavenging some radical species and thus breaking the chain reaction of LPO and inhibiting cellular enzymes responsible for the cell-

mediated oxidation of LDL particles (33, 34). Flavanoids may also suppress LPO by recycling other antioxidants such as α-tocopherol and by preserving the activity of the HDL-associated antioxidant enzyme paraoxonase 1 (34, 35). The components found collectively in a Mediterranean diet may work synergistically to protect lipids from oxidation.

The effect of a Mediterranean diet on antioxidant status remains unclear, because we observed a significant association only between adherence to a Mediterranean diet and plasma ascorbic acid. Many previous studies have found little correlation between dietary intake and serum α-tocopherol concentrations and low to no correlation with serum γ-tocopherol and vitamin A concentrations (36). One potential explanation for this is that lipid-soluble vitamins, such as E vitamins, are carried in serum with lipoproteins, so their concentrations are often more reflective of serum lipid concentrations than recent dietary intake. Serum concentrations of vitamin E may also have been affected by the increased intake of fish or other foods rich in PUFAs. Because PUFAs are easily oxidized, they have been hypothesized to increase the expenditure of vitamin E (37). The inverse association observed between α- and γ-tocopherol is biologically plausible because α-tocopherol is preferentially incorporated into lipoproteins in the liver to the exclusion of γ-tocopherol (38). Serum retinol may have shown a weak relation with intake because it has strict homeostatic control, except in cases of deficiency (39). Finally, ascorbic acid is water-soluble, so plasma measurements reflect recent dietary intake. This increase is often not dose dependent due to efficient ascorbic acid excretion in the urine (40); however, the increased intake of dietary vitamin C in a Mediterranean diet, as observed in our study, resulted in higher plasma concentrations and was similar to magnitudes seen in other studies (41).

To our knowledge, our study is the first study in a US cohort to evaluate a Mediterranean diet pattern and LPO in premenopausal women. Strengths include the use of multiple 24-h dietary recalls and the use of multiple markers of OS measured multiple times during 2 menstrual cycles. This rigorous approach reduced the probability of misclassification and took into consideration fluctuations that occur throughout the menstrual cycle (11). Furthermore, by using the aMED, we considered the whole diet rather than the effect of a single nutrient on LPO, which allowed us to potentially observe synergistic and antagonistic effects of foods. Similar results were obtained in analyses evaluating the tMED and OS and in analyses excluding women who completed $<$4 recalls per cycle. Because LPO has been implicated in the development of $>$100 diseases (42), including heart disease and cancer (the top 2 killers of women) (43), studying modifiable factors such as the Mediterranean diet in relation to lipid peroxidation in young women is critical because this is a prime age group to target prevention efforts.

Our study had some limitations. The BioCycle cohort may not be generalizable to all American women due its the strict inclusion criteria; however, the dietary patterns of the participants were similar to women in the United States (44). Although we did observe an inverse association between a Mediterranean diet and plasma 8-iso-PGF2α and 9-HODE concentrations, we did not observe a significant association between the aMED and TBARS. The lack of association may be due to the lack of specificity of TBARS as a marker of LPO because, in addition to being a product of lipid peroxide breakdown, malondialdehyde is also a secondary decomposition product of oxidative injury to DNA (and possibly other nonlipid biomolecules) (45, 46). Furthermore, in a subsample of BioCycle women (n = 9), the analytic performance of TBARS was found to have minimal analytic acceptability, with an imprecision of 8% CV, and a high degree of intraindividual variability (47). Finally, although we relied on 3 measures of LPO, we lacked other markers of OS such as biomarkers of protein and nucleic acid damage to confirm our results.

In conclusion, a stronger adherence to a Mediterranean diet was associated with lower concentrations of LPO among healthy premenopausal women. These results provide confirmation that decreased LPO, as a result of adherence to a Mediterranean diet, is a plausible mechanism linking a Mediterranean diet to reduced cardiovascular disease risk in women. Because antioxidant supplementation trials have mostly failed to show positive results in the prevention of disease (48), our findings provide reassurance that changes in dietary pattern, such as stronger adherence to a Mediterranean diet, could possibly have beneficial, preventative effects on disease development even in young healthy women. Further studies are needed to confirm these findings and elucidate the role of a Mediterranean diet on other markers of OS in young women.

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