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ORIGINAL CONTRIBUTION

A prospective study of erythrocyte polyunsaturated fatty acid, weight gain, and risk of becoming overweight or obese in middle-aged and older women

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Abstract
Purpose ω3 and ω6 fatty acids (FA) may have divergent effects on the development of obesity. We examined the association of baseline erythrocyte ω3 and ω6 FA composition with body weight change and the risk of becoming overweight or obese in the Women's Health Study (WHS) participants.
Methods We identified 534 women who had baseline erythrocyte FA measured and a baseline body mass index (BMI) of 18.5–<25 kg/m^2. Body weight was updated at a total of six time points during follow-up.
Results Weight gain during a mean of 10.4-year follow-up increased with increasing quartiles of baseline erythrocyte *cis* ω6 FA, ω6/ω3 ratio, and *trans* FA while decreased with increasing *cis* ω3 FA. After multivariable adjustment including total energy intake and physical activity, the weight gain (kg) in the highest versus the lowest quartile was 3.08 versus 2.32 for erythrocyte *cis* ω6 FA (p_{trend} 0.04), 2.07 versus 2.92 for *cis* ω3 FA (p_{trend} 0.08), 2.93 versus 2.05 for ω6/ω3 ratio (p_{trend} 0.046), and 3.03 versus 2.27 for *trans* FA (p_{trend} 0.06). Among individual FA, the associations were significant for 18:2ω6, 18:3ω6, and *trans* 18:1 and marginally significant for 20:3ω6 and *trans* 18:2. The risk of becoming overweight or obese (defined as BMI ≥25 kg/m^2 at any follow-up time point) increased across increasing ω6/ω3 ratio (multivariable model p_{trend} 0.04).
Conclusions In this prospective study, we found suggestive evidence that erythrocyte *cis* ω6 FA may be positively associated, and *cis* ω3 FA inversely associated with weight gain in initially normal-weight women.

Keywords Fatty acids · Prospective study · Women · Obesity · Weight gain

Abbreviation

FA	Fatty acids
PUFA	Polyunsaturated fatty acids
BMI	Body mass index
CVD	Cardiovascular disease
EPA	Eicosapentaenoic acid
DHA	Docosahexaenoic acid
WHS	Women's Health Study
BP	Blood pressure
FFQ	Food frequency questionnaire
GC	Gas chromatograph
HR	Hazard ratio
CI	Confidence interval
AA	Arachidonic acid
PPAR	Peroxisome proliferators-activated receptor
NHS	Nurses' Health Study
WHI	Women's Health Initiative
AHA	American Heart Association

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Introduction

High fat intake has been implicated in the development of obesity [1, 2]. However, evidence from prospective cohort studies [3–6] and randomized trials [7–10] linking total fat intake to body weight gain remains weak and inconsistent. In the past decades, total fat and saturated fat intake (as % of calories) in the typical Western diet has continuously fallen [11, 12], while the intake of ω6 fatty acid (FA) increased and ω3 FA decreased, resulting in a large increase in the ω6/ω3 ratio from 1:1 [13, 14] to 10:1 [14] or even higher [15]. This change in dietary FA composition parallels an alarming increase in the prevalence of overweight and obesity [15].

ω3 and ω6 classes of polyunsaturated FA (PUFA) are distinguished based on location of the first double bond. Parent FA of ω3 and ω6 subclasses, α-linolenic acid (ALA, 18:3ω3) and linoleic acid (LA, 18:2ω6), respectively, are essential for humans because they cannot be synthesized and must be obtained from diet. Longer-chain FA can be desaturated and elongated, to a very low extent, from parent FA of the same class. No conversion can occur between ω3 and ω6 subclasses of PUFA, making them metabolically distinct. Due to their similar chemical structure, ω3 and ω6 FA compete for incorporation into target tissues and metabolism by common enzymes, which may lead to opposing health effects [16]. Intake of ω3 FA, particularly long-chain ω3 FA such as eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3), has demonstrated beneficial effects on multiple cardiometabolic outcomes including hypertension, diabetes, dyslipidemia, and cardiovascular disease (CVD) [17]. The effect of ω6 FA is less clear, with evidence suggesting possible harm [18].

Experimental studies have suggested that ω3 and ω6 FA may elicit divergent effects on body fat gain through mechanisms of adipogenesis [19], lipid homeostasis [20, 21], brain–gut–adipose axis [22], and systemic inflammation [23]. Epidemiologic studies on PUFA intake and either weight gain or the development of obesity are limited, mainly using self-reports from food frequency questionnaires (FFQs) to assess PUFA intake without separate analysis on ω3 and ω6 subclasses [24, 25]. We identified a subgroup of Women's Health Study (WHS) participants who had erythrocyte FA measured as a biomarker of dietary FA and conducted prospective analyses to examine the association of baseline erythrocyte ω3 FA, ω6 FA, ω6/ω3 ratio, and *trans* FA with the longitudinal changes in body weight and the risk of becoming overweight or obese during a mean of 10.4-year follow-up.

Subjects and methods

Study population

The WHS was a randomized, double-blind, placebo-controlled, 2 × 2 factorial trial evaluating the risks and benefits of low-dose aspirin and vitamin E in the primary prevention of CVD and cancer [26, 27]. A third component, β-carotene, was initially included in the trial but terminated after a median treatment of 2.1 years [28]. From 1992 to 1995, 39,876 female US health professionals, aged ≥39 years and free from CVD and cancer (except non-melanoma skin cancer), were randomized into the WHS. Baseline blood samples were collected from 28,345 participants and stored in liquid nitrogen freezers. During the course of the trial, the participants received study agents and follow-up questionnaires by mail and reported the occurrence of major cardiovascular and cancer end points and risk factor information every 6 months for the first year and annually thereafter. Blinded treatment of aspirin and vitamin E ended as scheduled on March 31, 2004, after which the cohort follow-up continued for willing women as an observational study. Written informed consent was obtained from all participants. The study was approved by the institutional review board at Brigham and Women's Hospital, Boston, MA.

We previously conducted a study of 516 incident hypertension cases and 516 matched controls nested within the WHS. Hypertension was defined by meeting any of the following four criteria: a physician diagnosis of hypertension, self-reported systolic blood pressure (BP) ≥140 mmHg, diastolic BP ≥90 mmHg, or use of antihypertensive treatment. Incident hypertension was identified as women who had no hypertension at baseline but reported newly developed hypertension during follow-up. For each incident hypertension case, one control was randomly selected from women who remained free of hypertension until the case was identified. Each case and the respective control were matched on age (±1 year) and follow-up time (±3 months). The FA composition in baseline erythrocyte membrane was measured in all cases and controls.

For the current study, we included 551 women in this nested case–control study who reported a baseline body mass index (BMI) ranging from 18.5 to <25 kg/m^2. We then excluded 14 women who insufficiently completed the FFQ, defined as >70 items left blank, or an implausible mean energy intake of <600 or ≥3500 kcal/d. We also excluded two women with baseline diabetes or pre-randomization CVD or cancer, and one woman who did not update her weight during follow-up. As a result, 534 women remained for analysis.

Blood assays of erythrocyte fatty acid composition

The FA profile in erythrocyte membrane was measured at the Department of Laboratory Medicine and Pathology, University of Minnesota, using the method by Cao et al. [29]. Previous studies have shown that long-term storage of frozen blood samples did not influence FA profiles [30, 31]. After thawing and adding 50 μL of 17:0 internal standard, FA were extracted from erythrocyte membranes with a mixture of chloroform and methanol (2:1, v/v), dissolved in heptane, and injected onto a capillary Varian CP7420 100-m column with a Hewlett Packard 5890 gas chromatograph (GC) equipped with a HP6890A autosampler. The GC was configured for a single capillary column with a flame ionization detector and interfaced with HP chemstation software. Adequate separation of FA methyl esters was obtained over a 50-min period with an initial temperature of 190 °C followed by subsequent temperature gradually increased to 240 °C. FA from 12:0 through 24:1ω9 were separated, identified, and expressed as percent of total FA. FA subtypes, including ω3 FA, ω6 FA, and *trans* FA, were calculated as sum of the respective individual FA. The ratio of ω6 to ω3 FA was calculated. The coefficients of variation on 51 blind triplicates from 17 individual samples were 5.1 % for ω3 FA, 3.0 % for ω6 FA, and 3.6 % for *trans* FA.

Ascertainment of body weight change and incident case of becoming overweight or obese

On the baseline questionnaire, WHS participants reported height and weight. Every 6 months during the first year and annually thereafter, participants completed mailed follow-up questionnaires, with weight updated in the 2-, 3-, 5-, 6-, 9-year, and at the end of intervention. BMI was calculated as weight (kg) divided by the square of height (m^2) at a total of seven time points (baseline plus six follow-up), and then categorized as <25 kg/m^2 (normal weight) and ≥25 kg/m^2 (overweight or obese). Women who had normal BMI at baseline but subsequently reported a BMI ≥25 kg/m^2 at any follow-up time point were defined as incident cases that became overweight or obese. For each case, the 'time-of-event' was estimated as the time point when BMI crossed the cutoff for overweight or obese (i.e., 25 kg/m^2) through a regression line from the last-reported BMI of <25 kg/m^2 to the first-reported BMI of ≥25 kg/m^2 over time. Women who did not become overweight or obese were censored on the last day when a BMI <25 kg/m^2 was reported. Women who developed intermediate diabetes, the management of which often involves weight control, were censored on the day of diabetes diagnosis. In a similar population of female health professionals, self-reported weights were highly correlated with clinic measured weights (Pearson $r = 0.97$) [32]. Studies across different populations also have found that self-reported overweight and obesity status is accurate [33, 34].

Other baseline covariates

On the baseline questionnaire, women also provided self-reports of age, smoking status, alcohol use, recreational exercise, menopausal status, postmenopausal hormone use, multivitamin use, history of diabetes, and history of hypercholesterolemia. Diet was assessed from a 131-item validated semiquantitative FFQ. A commonly used unit or portion size was specified for each food item, and participants reported how often they had consumed that amount, on average, during the previous year. Nutrient intake including FA was computed by multiplying the intake frequency of each unit of food by the nutrient content of the specified portion size according to food composition tables from the US Department of Agriculture and Harvard School of Public Health database sources. The FFQ used in the WHS has demonstrated reasonable validity and reproducibility as a measure of long-term dietary intake [35]. We also used the FFQ to calculate the Alternative Healthy Eating Index-2010 (AHEI-2010) scores. The original HEI was based on the Dietary Guidelines for Americans [36]. The AHEI-2010 further incorporates new knowledge on foods and nutrients predictive of risk of chronic disease [37] and includes greater intake of vegetables (excluding potatoes), fruits (excluding juices), whole grains, nuts, legumes, vegetable proteins, long-chain ω3 FA, other PUFA (excluding long-chain ω3 FA); lower intake of sugar-sweetened beverages or fruit juices, red or processed meats, *trans* fats, and sodium; and moderate intake of alcohol. Each AHEI-2010 component was scored from 0 (worst) to 10 (best) according to component-specific criteria reflecting either the current dietary guidelines or associations reported in the literature. Total AHEI-2010 scores range from 0 (non-adherence) to 110 (perfect adherence). The rationale for component selection and methodology to derive the AHEI-2010 score has been described previously [37].

Statistical analysis

Statistical analyses were performed using SAS, version 9.1 (SAS Institute, Cary, NC, USA). All statistical tests were two-sided, with $p < 0.05$ considered statistically significant and $p > 0.05$ but <0.10 marginally significant. The correlation of erythrocyte FA with dietary FA was assessed by Spearman r^2. Erythrocyte PUFA composition was first compared between women who became overweight or obese and those who maintained normal weight, along with major lifestyle and dietary factors. We then divided erythrocyte FA into quartiles based on their distribution in controls. We calculated body weight change

from baseline to each follow-up time point and used PROC MIXED models with an unstructured covariance matrix for repeated measures to compare the longitudinal changes in body weight across the quartiles of erythrocyte FA. For women who reported a BMI ≥25 kg/m^2 at any follow-up time point, we assigned the body weight at subsequent follow-up as missing, due to the concern that lifestyle and diet may change in response to the weight gain. Basic models controlled for age, race, randomized treatment, and hypertension case–control status. Multivariable models additionally adjusted for total energy intake, physical activity, smoking, alcohol use, menopausal status, postmenopausal hormone use, multivitamin use, history of hypercholesterolemia, AHEI, and energy-adjusted intake of protein, carbohydrates, and cholesterol. Adjustment for baseline BMI attenuated the magnitude, but did not change direction, of the associations. Because adjustment for baseline status may induce biased statistical association in analysis of change [38], we did not show the results with baseline BMI adjustment. We further used Cox regression to calculate hazard ratios (HRs) and 95 % confidence intervals (CIs) of becoming overweight or obese according to the quartiles of erythrocyte FA. Linear trend across the increasing quartiles was tested using the median value in each quartile as an ordinal variable. To evaluate the independent associations for ω3 and ω6 FA, we included ω3 and ω6 FA in the same model and also examined the joint categories of ω3 and ω6 FA that were each dichotomized at the median. Finally, we stratified all analyses by baseline age (<55 vs. ≥55 years) and race/ethnicity (whites vs. non-whites) because of the variations in body composition, and by baseline BMI (18.5–<23, 23–<25 kg/m^2) out of concern for misclassification of borderline overweight. Interactions were tested using Wald Chi-square tests.

Results

Women included in the current study had a mean ± SD baseline age of 53.8 ± 6.4 years, BMI of 22.4 ± 1.6 kg/m^2, 27.5 % of non-whites (8.9 % of African Americans and 17.7 % of Asian Americans), and were free of CVD, cancer, diabetes, and hypertension at baseline. The overall mean of erythrocyte FA (presented as percent of total FA) was 6.2 % for *cis* ω3 FA, 27.0 % for *cis* ω6 FA, and 2.0 % for *trans* FA. The corresponding mean dietary FA (presented as percent of total fat intake) were 2.7 % for *cis* ω3 FA, 19.2 % for *cis* ω6 FA, and 3.7 % for *trans* FA. Spearman correlation coefficients between erythrocyte and dietary FA ranged from 0.073 ($p = 0.09$, for 22:5ω3) to 0.41 ($p < 0.0001$, for 22:6ω3). Among 534 women who initially had normal BMI, 186 women became overweight or obese during a mean of 10.4-year follow-up. Compared with women who maintained normal BMI, those who became overweight or obese were younger and had higher baseline BMI (Table 1). Smoking status, alcohol use, total energy intake, exercise, postmenopausal hormone use, and multivitamin use did not differ according to whether or not the woman became overweight or obese. When comparing erythrocyte FA in women who became overweight or obese with those who did not, no significant difference in *cis* ω3 FA, *cis* ω6 FA, ω6/ω3 ratio, and *trans* FA was found (Table 1).

The mean ± SD of body weight change from baseline to 2, 3, 5, 6, and ≥9 years of follow-up in the 534 women was 1.23 ± 3.10, 1.17 ± 2.92, 1.34 ± 3.41, 1.52 ± 3.29, and 1.88 ± 4.21 kg, respectively. In the model that adjusted only for age, race, and randomized treatment, longitudinal weight gain during the overall follow-up across increasing quartiles of baseline erythrocyte PUFA was 2.59, 2.14, 2.29, and 1.62 kg (p_{trend} 0.04) for *cis* ω3 FA; 1.95, 1.51, 2.44, and 2.63 kg (p_{trend} 0.06) for *cis* ω6 FA; and 1.58, 2.20, 2.39, and 2.58 kg (p_{trend} 0.02) for ω6/ω3 ratio (Table 2). After additional adjustment for lifestyle and dietary factors including total energy intake and exercise, the associations for *cis* ω6 FA and ω6/ω3 ratio were statistically significant, while the association for *cis* ω3 FA was marginally significant. For individual FA, LA (18:2ω6), γ-linoleic acid (GLA, 18:3ω6), and dihomo-γ-linolenic acid (DGLA, 20:3ω6) were each positively associated with weight gain in the basic model. In the multivariable model, the association remained significant for LA and GLA and was marginally significant for DGLA. Total *trans* FA, *trans* 18:1, and *trans* 18:2 were all significantly and positively associated with weight gain in the basic model, but only the association for *trans* 18:1 remained significant after multivariable adjustment.

The risk of becoming overweight or obese did not significantly differ by quartiles of baseline erythrocyte *cis* ω3 FA and *cis* ω6 FA, but significantly increased across increasing quartiles of ω6/ω3 ratio (HR [95 % CI] 1.00, 1.37 [0.84, 2.22], 1.57 [1.01, 2.43], and 1.58 [1.01, 2.46], respectively; p_{trend} 0.046) (Table 3). Additional adjustment for lifestyle and dietary factors in the multivariable model did not attenuate this association. The associations for ω3 and ω6 FA did not change when both were simultaneously included in the same model. There was also no interaction between ω3 and ω6 FA dichotomized at the median in association with weight gain or the risk of becoming overweight or obese. Erythrocyte *trans* FA was not associated with the risk of becoming overweight or obese. None of the associations differed by age, race/ethnicity, or baseline BMI categories (data not shown).

Table 1 Baseline characteristics[a] of women who had normal body mass index at baseline and remained normal weight during follow-up compared with those who became overweight or obese

	Remaining normal weight $N = 348$	Becoming overweight or obese $N = 186$	p[b]
N of hypertension case/control	127/221	91/95	0.005
Age, years	54.5 ± 6.8	52.5 ± 5.5	0.0003
Body mass index, kg/m^2	21.7 ± 1.4	23.6 ± 1.1	<0.0001
Total energy intake, kcal/d	1746.2 ± 553.1	1706.5 ± 529.6	0.42
Exercise, kcal/week	1084.1 ± 1171.0	977.5 ± 1086.2	0.30
Race, % of white	70.9	75.4	0.27
Smoking, %			0.17
Current	10.3	13.4	
Past	27.6	32.8	
Never	62.1	53.8	
Alcohol intake, %			0.40
Never	42.0	40.3	
>0–< 5 g/day	29.6	35.5	
5–<15 g/day	20.4	15.6	
≥15 g/day	8.05	8.60	
Postmenopausal, %	54.2	46.8	0.32
Postmenopausal hormone use, %	44.7	44.6	0.99
Multivitamin use, %	29.4	30.4	0.80
History of hypercholesterolemia, %	22.4	26.3	0.31
Erythrocyte fatty acid composition, %			
cis Polyunsaturated fatty acid	33.2 ± 3.6	33.2 ± 3.6	0.90
cis ω6 polyunsaturated fatty acid	26.9 ± 2.9	27.1 ± 2.9	0.45
cis ω3 polyunsaturated fatty acid	6.31 ± 1.64	6.07 ± 1.56	0.10
ω6/ω3 ratio	4.59 ± 1.42	4.78 ± 1.44	0.14
trans fatty acid	1.98 ± 0.61	2.03 ± 0.58	0.34

BMI body mass index

[a] Values were mean ± standard deviation (SD) for continuous variables and % for categorical variables

[b] p for t test for continuous variables and Chi-square test for categorical variables

Discussion

In this prospective analysis, we found suggestive evidence that baseline erythrocyte *cis* ω3 FA is inversely associated and *cis* ω6 FA is positively associated with longitudinal weight gain in initially normal-weight women. The associations remained borderline significant after controlling for potential confounding factors, including total energy intake and physical activity. Erythrocyte *trans* FA also tended to be positively associated with weight gain. The associations of erythrocyte *cis* ω3 and ω6 FA and *trans* FA with the risk of becoming overweight or obese followed similar patterns, with a lower magnitude of effect.

Our study is the first to prospectively examine PUFA and *trans* FA in erythrocyte membrane in relation to the weight gain and the risk of becoming overweight or obese. In epidemiologic studies, FA in plasma lipid (reflecting intake in weeks) [39, 40] and erythrocyte membrane (reflecting intake in months) [41] have been measured as biomarkers of dietary fat. Our study findings suggest that dietary ω3 and ω6 FA may have divergent effects in the development of obesity, and FA composition, in addition to absolute amount of intake, may be important for the prevention of obesity. ω3 and ω6 FA compete for common metabolic enzymes and incorporation into plasma lipids and cell membranes. In the past half century, ω6/ω3 ratio in US diet has substantially increased [14]. Some research groups recommend a reduction in ω6 FA intake to lower ω6/ω3 ratio [42, 43]. However, American Heart Association suggests ω6 FA intake comprising at least 5–10 % of total energy [44]. The optimal intake of ω3 and ω6 FA and the target ω6/ω3 ratio remain to be determined.

ω3 and ω6 FA may elicit contrasting effects in adipogenesis [19] and lipid homeostasis [20, 21]. Metabolites

Table 2 Body weight change (kg)[a] over a mean of 10.4-year follow-up according to baseline erythrocyte polyunsaturated fatty acids

	Quartiles				
	1st	2nd	3rd	4th	p^{b}_{trend}
cis ω6 fatty acid					
Median, range	25.0, 14.3–<26.56	27.2, 26.56–<27.7	28.3, 27.7–<28.8	29.4, 28.8–32.5	
Basic model[c]	1.95 ± 0.28	1.51 ± 0.31	2.44 ± 0.31	2.63 ± 0.31	0.061
Multivariable model[d]	2.32 ± 0.50	1.91 ± 0.54	2.84 ± 0.55	3.08 ± 0.55	0.043
18:2ω6 (linoleic acid)					
Median, range	10.8, 6.32–<11.45	11.9, 11.45–<12.3	12.8, 12.3–<13.4	14.1, 13.4–16.6	
Basic model	1.88 ± 0.29	1.68 ± 0.29	2.38 ± 0.31	2.60 ± 0.30	0.032
Multivariable model	2.38 ± 0.52	2.13 ± 0.51	2.90 ± 0.54	3.14 ± 0.54	0.022
18:3ω6 (gamma-linolenic acid)					
Median, range	0.038, 0.01–<0.049	0.06, 0.049–<0.07	0.08, 0.07–<0.09	0.11, 0.09–0.26	
Basic model	1.40 ± 0.30	2.45 ± 0.32*	2.39 ± 0.32*	2.65 ± 0.30*	0.016
Multivariable model	1.71 ± 0.57	2.68 ± 0.54*	2.61 ± 0.54*	2.78 ± 0.51*	0.045
20:3ω6 (dihomo-gamma-linolenic acid)					
Median, range	1.14, 0.15–<1.28	1.35, 1.28–<1.43	1.54, 1.43–<1.67	1.85, 1.67–2.86	
Basic model	1.75 ± 0.29	1.84 ± 0.31	2.40 ± 0.31	2.64 ± 0.30*	0.015
Multivariable model	2.17 ± 0.52	2.21 ± 0.54	2.75 ± 0.53	2.87 ± 0.53	0.054
20:4ω6 (arachidonic acid)					
Median, range	11.3, 2.74–<12.4	12.9, 12.4–<13.3	13.7, 13.3–<14.3	14.9, 14.3–16.4	
Basic model	1.68 ± 0.27	2.55 ± 0.30*	2.47 ± 0.30*	1.85 ± 0.32	0.43
Multivariable model	1.96 ± 0.51	2.99 ± 0.52*	2.77 ± 0.52*	2.17 ± 0.55	0.38
cis ω3 fatty acid					
Median, range	4.87, 1.18–<5.40	5.81, 5.40–<6.21	6.64, 6.21–<7.16	7.96, 7.16–13.2	
Basic model	2.59 ± 0.30	2.14 ± 0.31	2.29 ± 0.31	1.62 ± 0.31*	0.038
Multivariable model	2.92 ± 0.53	2.55 ± 0.54	2.68 ± 0.54	2.07 ± 0.53	0.083
18:3ω3 (alpha-linolenic acid)					
Median, range	0.11, 0.05–<0.12	0.14, 0.12–<0.15	0.17, 0.15–<0.19	0.22, 0.19–0.36	
Basic model	1.77 ± 0.30	2.45 ± 0.30	1.88 ± 0.30	2.39 ± 0.29	0.30
Multivariable model	2.18 ± 0.53	2.93 ± 0.54	2.28 ± 0.52	2.78 ± 0.52	0.34
20:5ω3 (eicosapentaenoic acid, EPA)					
Median, range	0.32, 0.13–<0.39	0.43, 0.39–<0.48	0.55, 0.48–<0.66	0.81, 0.66–2.57	
Basic model	2.53 ± 0.31	2.45 ± 0.31	2.13 ± 0.30	1.58 ± 0.30*	0.02
Multivariable model	2.80 ± 0.56	2.83 ± 0.55	2.52 ± 0.52	2.17 ± 0.53	0.11
22:6ω3 (docosahexaenoic, DHA)					
Median, range	2.49, 0.39–<2.88	3.25, 2.88–<3.61	3.96, 3.61–<4.41	4.98, 4.41–8.35	
Basic model	2.43 ± 0.30	2.17 ± 0.31	2.32 ± 0.32	1.80 ± 0.29	0.17
Multivariable model	2.77 ± 0.52	2.64 ± 0.55	2.76 ± 0.55	2.22 ± 0.53	0.24
ω6/ω3 ratio					
Median, range	3.36, 1.81–<3.89	4.17, 3.89–<4.38	4.76, 4.38–<5.15	5.69, 5.15–13.9	
Basic model	1.58 ± 0.29	2.20 ± 0.34	2.39 ± 0.30	2.58 ± 0.30*	0.018
Multivariable model	2.05 ± 0.53	2.58 ± 0.55	2.72 ± 0.53	2.93 ± 0.53*	0.046
trans fatty acid					
Median, range	1.29, 0.71–<1.60	1.81, 1.60–<2.01	2.19, 2.01–<2.38	2.70, 2.38–3.81	
Basic model	1.70 ± 0.28	2.14 ± 0.29	2.39 ± 0.32	2.52 ± 0.31*	0.039
Multivariable model	2.27 ± 0.50	2.68 ± 0.54	2.87 ± 0.57	3.03 ± 0.57	0.060
trans 16:1					
Median, range	0.02, 0.003–<0.03	0.04, 0.03–<0.05	0.06, 0.05–<0.07	0.08, 0.07–0.35	
Basic model	2.04 ± 0.30	1.82 ± 0.29	2.41 ± 0.31	2.38 ± 0.31	0.25
Multivariable model	2.40 ± 0.53	2.25 ± 0.51	2.81 ± 0.54	2.75 ± 0.55	0.24

Table 2 continued

	Quartiles				
	1st	2nd	3rd	4th	p^{b}_{trend}
trans 18:1					
Median, range	1.11, 0.49–<1.39	1.59, 1.39–<1.73	1.91, 1.73–<2.07	2.34, 2.07–3.45	
Basic model	1.74 ± 0.28	2.04 ± 0.30	2.36 ± 0.32	2.63 ± 0.31*	0.024
Multivariable model	2.30 ± 0.49	2.58 ± 0.54	2.83 ± 0.57	3.18 ± 0.57*	0.032
trans 18:2					
Median, range	0.15, 0.08–<0.17	0.19, 0.17–<0.21	0.22, 0.21–<0.24	0.27, 0.24–0.80	
Basic model	1.85 ± 0.29	1.95 ± 0.30	2.13 ± 0.31	2.70 ± 0.30*	0.03
Multivariable model	2.37 ± 0.53	2.35 ± 0.52	2.54 ± 0.57	3.00 ± 0.53	0.093

[a] Values were mean ± standard error

[b] Linear trends were tested using the median value in each quartile of erythrocyte fatty acids as an ordinal variable

[c] Model adjusted for age (continuous), race (white, non-white), randomized treatment (vitamin E, aspirin, β-carotene, or placebo), hypertension case/control status (case, control)

[d] Model additionally adjusted for total energy intake (continuous), physical activity (continuous), smoking (never, former, current), alcohol use (0, >0–<5, 5–<15, ≥15 g/d), postmenopausal status (yes, no, uncertain), postmenopausal hormone use (never, former, current), multivitamin use (never, former, current), history of hypercholesterolemia (yes, no), intake of energy-adjusted protein, carbohydrates, cholesterol, and alternative healthy eating index (all continuous)

* $p < 0.05$ compared with the lowest quartile

of arachidonic acid (AA, 20:4ω6) play important roles in the terminal differentiation of pre-adipocyte to mature adipocyte [45]. Such effect can be inhibited by ω3 FA at multiple steps [46–49]. ω6 FA also increase cellular triglyceride content by increasing membrane permeability [50], while ω3 FA reduce fat deposition in adipose tissues by suppressing lipogenic enzymes and increasing β-oxidation [51]. In addition, ω3 and ω6 FA differentially modulate the brain–gut–adipose axis [22] and the inflammatory properties of downstream eicosanoids, which ultimately affect pre-adipocyte differentiation and fat mass growth [52]. Epidemiologic studies on dietary FA and changes in body weight and body fat remain limited, with only two known prospective studies [24, 25]. Both studies used self-reported FFQs to assess dietary fat intake and did not examine subtype or individual PUFA. Some intervention studies showed that ω3 FA supplementation reduced body weight and obesity in lean [53], overweight [54, 55], and obese [56] individuals. Comparable data on ω6 FA are lacking. One small trial in 17 healthy, normal-weight men and women found that a 10-week diet intervention to improve ω6/ω3 ratio, with no change in intake of total energy and other macronutrients, did not change body weight, waist/hip ratio, and fat mass but significantly increased plasma adiponectin and decreased plasma inflammatory markers [57]. Large-scale, controlled trials with longer duration are needed to further elucidate the effects of dietary PUFA composition change on obesity and obesity-related morbidities.

trans FA are unsaturated FA with at least one double bond in *trans* configuration [58]. The *trans* bonds alter not only the physical properties but also the biological effects of the unsaturated FA. Studies in rats showed that *trans* FA consumption raised hepatic fat contents [59]. In monkeys, *trans* FA diet resulted in larger weight gain compared with *cis* monounsaturated FA diet, and the differential weight gain was largely attributed to higher visceral fat accumulation [60]. The association of *trans* FA intake with weight gain in epidemiologic studies has been weak to date [24]. The similarly weak associations observed in the current study may be partly due to the fact that WHS participants were health professionals with largely favorable dietary and behavior patterns including relatively low *trans* fat intake.

In our study, the direction of associations for individual FA was generally consistent with their respective classes, but the magnitude of associations varied. After multivariable adjustment, significant relations with weight gain were found only for DGLA (20:3ω6), LA (18:2ω6), and GLA (18:3ω6) among ω6 FA; EPA (20:5ω3) among ω3 FA; and *trans* 18:1 among *trans* FA. The variations by individual FA may be due to unknown and uncontrolled factors involved in the conversion and metabolism of each FA, and should be interpreted cautiously given the multiple comparisons. Moreover, the current study included only women who had normal BMI at baseline to minimize potential confounding and address the risk of becoming overweight or obese. To further evaluate the impact of baseline BMI on the results, we stratified analyses by baseline BMI levels (18.5–≤23, 23–≤25 kg/m^2) and also included women

Table 3 Hazard ratios[a] of becoming overweight or obese according to quartiles of baseline erythrocyte polyunsaturated fatty acid

	Quartiles				
	1st	2nd	3rd	4th	p^{b}_{trend}
cis ω6 fatty acid					
N of becoming overweight	51	34	53	48	
Basic adjusted[c]	1.00 (reference)	0.77 (0.49–1.21)	1.12 (0.75–1.67)	1.19 (0.79–1.77)	0.31
Multivariable adjusted[d]	1.00 (reference)	0.77 (0.49–1.23)	1.13 (0.74–1.73)	1.20 (0.79–1.82)	0.32
18:2ω6 (linoleic acid)					
N of becoming overweight	48	40	58	40	
Basic adjusted	1.00 (reference)	0.73 (0.48–1.12)	1.30 (0.88–1.91)	0.86 (0.56–1.32)	0.92
Multivariable adjusted	1.00 (reference)	0.74 (0.48–1.14)	1.30 (0.87–1.95)	0.82 (0.52–1.29)	0.89
18:3ω6 cc (gamma-linolenic acid)					
N of becoming overweight	32	44	51	59	
Basic adjusted	1.00 (reference)	1.39 (0.86–2.25)	1.45 (0.89–2.38)	1.40 (0.85–2.30)	0.32
Multivariable adjusted	1.00 (reference)	1.29 (0.79–2.12)	1.40 (0.85–2.32)	1.36 (0.81–2.29)	0.33
20:3ω6 (dihomo-gamma-linolenic acid)					
N of becoming overweight	39	39	48	60	
Basic adjusted	1.00 (reference)	1.12 (0.71–1.76)	1.29 (0.83–2.00)	1.56 (1.03–2.36)	0.025
Multivariable adjusted	1.00 (reference)	1.16 (0.73–1.85)	1.32 (0.84–2.07)	1.56 (1.00–2.43)	0.041
20:4ω6 (arachidonic acid)					
N of becoming overweight	48	47	51	40	
Basic adjusted	1.00 (reference)	1.10 (0.73–1.66)	1.13 (0.76–1.70)	1.02 (0.67–1.57)	0.82
Multivariable adjusted	1.00 (reference)	1.17 (0.77–1.80)	1.15 (0.76–1.75)	1.03 (0.67–1.60)	0.79
cis ω3 fatty acid					
N of becoming overweight	61	49	40	36	
Basic adjusted	1.00 (reference)	0.90 (0.61–1.31)	0.76 (0.51–1.13)	0.71 (0.45–1.10)	0.089
Multivariable adjusted	1.00 (reference)	0.86 (0.58–1.28)	0.71 (0.46–1.08)	0.69 (0.43–1.11)	0.082
18:3ω3 (alpha-linolenic acid)					
N of becoming overweight	50	43	46	47	
Basic adjusted	1.00 (reference)	0.90 (0.60–1.36)	0.92 (0.61–1.38)	0.94 (0.63–1.42)	0.83
Multivariable adjusted	1.00 (reference)	0.91 (0.60–1.39)	0.89 (0.59–1.34)	0.89 (0.58–1.35)	0.60
20:5ω3 (eicosapentaenoic acid, EPA)					
N of becoming overweight	52	56	47	31	
Basic adjusted	1.00 (reference)	1.05 (0.72–1.55)	0.93 (0.62–1.40)	0.68 (0.42–1.09)	0.082
Multivariable adjusted	1.00 (reference)	1.02 (0.68–1.52)	0.94 (0.60–1.45)	0.75 (0.46–1.24)	0.23
22:6ω3 (docosahexaenoic, DHA)					
N of becoming overweight	55	50	42	39	
Basic adjusted	1.00 (reference)	0.92 (0.63–1.36)	0.99 (0.66–1.49)	0.71 (0.46–1.11)	0.17
Multivariable adjusted	1.00 (reference)	0.98 (0.66–1.47)	0.93 (0.60–1.42)	0.69 (0.42–1.12)	0.13
ω6/ω3 ratio					
N of becoming overweight	37	34	55	60	
Basic adjusted	1.00 (reference)	1.37 (0.84–2.22)	1.57 (1.01–2.43)	1.58 (1.01–2.46)	0.046
Multivariable adjusted	1.00 (reference)	1.27 (0.77–2.09)	1.50 (0.95–2.38)	1.63 (1.01–2.63)	0.040
trans Fatty acid					
N of becoming overweight	43	51	44	48	
Basic adjusted	1.00 (reference)	1.24 (0.83–1.87)	1.19 (0.78–1.84)	1.17 (0.76–1.79)	0.53
Multivariable adjusted	1.00 (reference)	1.20 (0.78–1.84)	1.07 (0.68–1.70)	1.14 (0.72–1.81)	0.69
trans 16:1					
N of becoming overweight	43	46	50	47	
Basic adjusted	1.00 (reference)	0.97 (0.63–1.49)	1.15 (0.76–1.74)	1.02 (0.67–1.55)	0.79
Multivariable adjusted	1.00 (reference)	0.96 (0.62–1.50)	1.09 (0.71–1.68)	0.95 (0.62–1.47)	0.92

Table 3 continued

	Quartiles				
	1st	2nd	3rd	4th	p^{b}_{trend}
trans 18:1					
N of becoming overweight	45	47	45	49	
Basic adjusted	1.00 (reference)	1.18 (0.78–1.78)	1.21 (0.79–1.85)	1.18 (0.77–1.79)	0.44
Multivariable adjusted	1.00 (reference)	1.11 (0.72–1.71)	1.07 (0.68–1.69)	1.15 (0.73–1.82)	0.58
trans 18:2					
N of becoming overweight	40	45	46	55	
Basic adjusted	1.00 (reference)	1.12 (0.73–1.73)	1.18 (0.76–1.81)	1.30 (0.85–1.98)	0.22
Multivariable adjusted	1.00 (reference)	1.13 (0.72–1.76)	1.12 (0.72–1.76)	1.22 (0.79–1.90)	0.40

[a] Values were hazard ratio (95 % confidence intervals)

[b] Linear trends were tested using the median value in each quartile of erythrocyte fatty acids as an ordinal variable

[c] Model adjusted for age (continuous), race (white, non-white), randomized treatment (vitamin E, aspirin, β-carotene, or placebo), hypertension case/control status (case, control)

[d] Model additionally adjusted for total energy intake (continuous), physical activity (continuous), smoking (never, former, current), alcohol use (0, >0–<5, 5–<15, ≥15 g/d), postmenopausal status (yes, no, uncertain), postmenopausal hormone use (never, former, current), multivitamin use (never, former, current), history of hypercholesterolemia (yes, no), intake of energy-adjusted protein, carbohydrates, cholesterol, and alternative health eating index (all continuous)

who were already overweight or obese at baseline (baseline BMI ≥ 25 kg/m^2) in sensitivity analyses. Similar patterns of associations were found in these additional analyses (data not shown).

Several limitations of the current study deserve comments. First, self-reported body weight, though showing excellent validity in health professionals [32], remains subject to random misclassification and may lead to underestimation of the true associations. Second, our study is limited in a single baseline measurement of erythrocyte FA without assessment of any change over time. Third, our study used a convenient sample from a previous study, but was not a priori designed, to test our hypotheses. Nevertheless, we do not anticipate substantial bias to our reported associations due to how this study population was selected. Fourth, although we have adjusted for a broad range of dietary, lifestyle, and clinical factors in analysis, residual confounding cannot be ruled out as in all observational studies. Since adjustment for baseline levels of body weight to control for residual confounding may also induce biased statistical association with the change in body weight [38], we have examined the associations with and without adjustment for baseline BMI. Fifth, because the WHS did not collect data on waist and hip circumference at baseline, we cannot assess abdominal obesity. Finally, WHS participants were predominantly white female health professionals, which limited the generalizability of our study results to other populations.

In conclusion, this prospective study provided suggestive evidence that erythrocyte *cis* ω3 FA may be inversely associated, while *cis* ω6 FA, ω6/ω3 ratio, and *trans* FA positively associated, with longitudinal weight gain. Future studies are needed to further elucidate the role of dietary FA composition in the development of obesity and the underlying mechanisms.

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Conflict of interest None.

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