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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— $\omega 3$ and $\omega 6$ fatty acid (FA) may have divergent effects on the development of obesity. We examined the association of baseline erythrocyte $\omega 3$ and $\omega 6$ 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 to $<25 \text{ kg/m}^2$. Body weight was updated at a total of 6 time points during follow-up.

Results—Weight gain during a mean of 10.4-y follow-up increased with increasing quartiles of erythrocyte *cis* $\omega 6$ FAs, $\omega 6/\omega 3$ ratio and *trans* FAs while decreased with increasing *cis* $\omega 3$ FAs. After multivariable adjustment including total energy intake and physical activity, the weight gain (kg) in the highest vs. the lowest quartile was 3.08 vs. 2.32 for erythrocyte *cis* $\omega 6$ FAs (P_{trend} : 0.04), 2.07 vs. 2.92 for *cis* $\omega 3$ FAs (P_{trend} : 0.08), 2.93 vs. 2.05 for $\omega 6/\omega 3$ ratio (P_{trend} : 0.046), and 3.03 vs. 2.27 for *trans* FAs (P_{trend} : 0.06). Among individual FA, the associations were significant

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for 18:2 ω 6, 18:3 ω 6, and *trans* 18:1 and marginally significant for 20:3 ω 6 and *trans* 18:2. The risk of becoming overweight or obese (defined as BMI ≥ 25 kg/m² at any follow-up time point) increased across increasing ω 6/ ω 3 ratio (multivariable model P_{trend} : 0.04).

Conclusions—In this prospective study, we found suggestive evidence that erythrocyte *cis* ω 6 FAs may be positively associated, and *cis* ω 3 FAs inversely associated with weight gain in initially normal-weight women.

Keywords

fatty acids; prospective study; women; obesity; weight gain

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 ω 6 fatty acid (FA) increased and ω 3 FA decreased, resulting in a large increase in the ω 6/ ω 3 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].

ω 3 and ω 6 classes of polyunsaturated FA (PUFA) are distinguished based on location of the first double bond. Parent FA of ω 3 and ω 6 subclasses, α -linolenic acid (ALA, 18: 3 ω 3) and linoleic acid (LA, 18: 2 ω 6) 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 ω 3 and ω 6 subclasses of PUFA, making them metabolically distinct. Due to their similar chemical structure, ω 3 and ω 6 FA compete for incorporation into target tissues and metabolism by common enzymes, which may lead to opposing health effects [16]. Intake of ω 3 FA, particularly long-chain ω 3 FA such as eicosapentaenoic acid (EPA, C20:5 ω 3) and docosahexaenoic acid (DHA, C22:6 ω 3), has demonstrated beneficial effects on multiple cardiometabolic outcomes including hypertension, diabetes, dyslipidemia, and cardiovascular disease (CVD) [17]. The effect of ω 6 FA is less clear, with evidence suggesting possible harm [18].

Experimental studies have suggested that ω 3 and ω 6 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 (FFQ) to assess PUFA intake without separate analysis on ω 3 and ω 6 subclasses [24, 25]. We identified a subgroup of Women Health Study (WHS) participants who had erythrocyte FA measured as biomarker of dietary FA and conducted prospective analyses to examine the association of baseline erythrocyte ω 3 FA, ω 6 FA, ω 6/ ω 3 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 years 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 y [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 4 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 y) and follow-up time (± 3 mo). 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/m². 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 2 women with baseline diabetes or pre-randomization CVD or cancer, and 1 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 μ L of 17:0 internal standard, FAs 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 methylesters was obtained over a 50-min period with an initial temperature of 190°C followed by subsequent temperature gradually increased to 240°C. FAs from 12:0 through 24:1 ω 9 were separated, identified and expressed as percent of total FA. FA subtypes, including ω 3 FA, ω 6 FA, and *trans* FA, were calculated as sum of the respective individual FA. The ratio of ω 6 to ω 3 FA was calculated. The coefficients of variation on 51 blind triplicates from 17 individual samples were 5.1% for ω 3 FA, 3.0% for ω 6 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 (m²) at a total of 7 time points (baseline plus 6 follow-up), and then categorized to <25 kg/m² (normal weight) and $\geq$ 25 kg/m² (overweight or obese). Women who had normal BMI at baseline but subsequently reported a BMI $\geq$ 25 kg/m² 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/m²) through a regression line from the last reported BMI of <25 kg/m² to the first reported BMI of $\geq$ 25 kg/m² over time. Women who did not become overweight or obese were censored on the last day when a BMI <25 kg/m² 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 for chronic disease [37] and includes greater intake of vegetables (excluding potatoes), fruits (excluding juices), whole grains, nuts, legumes, vegetable proteins, long-chain ω 3 FAs, other PUFAs (excluding long-chain ω 3 FAs), 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 (nonadherence) 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 2-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 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/m² at any follow-up time, we assigned the body weight at subsequent follow-up as missing, due to 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 $\omega 3$ and $\omega 6$ FA, we included $\omega 3$ and $\omega 6$ FA in the same model and also examined the joint categories of $\omega 3$ and $\omega 6$ FA that were each dichotomized at the median. Finally, we stratified all analyses by baseline age (< 55 vs. ≥ 55 y) and race/ethnicity (whites vs. non-whites) because of the variations in body composition and by baseline BMI (18.5 – < 23 , 23 – < 25 kg/m²) 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 $\pm$ SD baseline age of 53.8 ± 6.4 years, BMI of 22.4 ± 1.6 kg/m², 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) were 6.2% for *cis* $\omega 3$ FA, 27.0% for *cis* $\omega 6$ FA, and 2.0% for *trans* FA. The corresponding mean dietary FA

(presented as percent of total fat intake) were 2.7% for *cis* ω 3 FA, 19.2% for *cis* ω 6 FA, and 3.7% for *trans* FA. Spearman correlation coefficients between erythrocyte and dietary FA ranged from 0.073 ($p=0.09$, for 22: 5 ω 3) to 0.41 ($p<0.0001$, for 22: 6 ω 3). Among 534 women who initially had normal BMI, 186 women became overweight or obese during a mean of 10.4 years 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 versus those who did not, no significant difference in *cis* ω 3 FA, *cis* ω 6 FA, ω 6/ ω 3 ratio, and *trans* FA was found. (**Table 1**)

The mean $\pm$ SD of body weight change from baseline to 2-, 3-, 5-, 6-, 9- years of follow-up in the 534 women were 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 erythrocyte PUFA was 2.59, 2.14, 2.29, 1.62 kg (p , trend: 0.04) for *cis* ω 3 FA, 1.95, 1.51, 2.44, and 2.63 kg (p , trend: 0.06) for *cis* ω 6 FA, and 1.58, 2.20, 2.39, 2.58 kg (p , trend: 0.02) for ω 6/ ω 3 ratio. (**Table 2**) After additional adjustment for lifestyle and dietary factors including total energy intake and exercise, the associations for *cis* ω 6 FA and ω 6/ ω 3 ratio were statistically significant, while the association for *cis* ω 3 FA was marginally significant. For Individual FAs, LA (18: 2 ω 6), γ -linoleic acid (GLA, 18: 3 ω 6), and dihomo- γ -linolenic acid (DGLA, 20: 3 ω 6) was 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 erythrocyte *cis* ω 3 FA, *cis* ω 6 FA, but significantly increased across increasing quartiles of ω 6/ ω 3 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 ω 3 and ω 6 FA did not change when both were simultaneously included in the same model. There was also no interaction between ω 3 and ω 6 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).

Discussion

In this prospective analysis, we found suggestive evidence that erythrocyte *cis* ω 3 FA is inversely associated and *cis* ω 6 FA 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* FAs also tended to be positively associated with weight gain. The

associations of erythrocyte *cis* ω 3 and ω 6 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 ω 3 and ω 6 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. ω 3 and ω 6 FA compete for common metabolic enzymes and incorporation into plasma lipids and cell membranes. In the past half century, ω 6/ ω 3 ratio in US diet has substantially increased [14]. Some research groups recommend a reduction of ω 6 FA intake to lower ω 6/ ω 3 ratio [42, 43]. However, American Heart Association suggests ω 6 FA intake comprising at least 5-10% of total energy [44]. The optimal intake of ω 3 and ω 6 FA and the target ω 6/ ω 3 ratio remains to be determined.

ω 3 and ω 6 FA may elicit contrasting effects in adipogenesis [19] and lipid homeostasis [20, 21]. Metabolites of arachidonic acid (AA, 20: 4 ω 6) play important roles in the terminal differentiation of preadipocyte to mature adipocyte [45]. Such effect can be inhibited by ω 3 FA at multiple steps [46-49]. ω 6 FA also increase cellular triglyceride content by increasing membrane permeability [50] while ω 3 FA reduce fat deposition in adipose tissues by suppressing lipogenic enzymes and increasing β -oxidation [51]. In addition, ω 3 and ω 6 FA differentially modulate the brain-gut-adipose axis [22] and the inflammatory properties of downstream eicosanoids, which ultimately affect preadipocyte 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 PUFAs. Some intervention studies showed that ω 3 FA supplementation reduced body weight and obesity in lean [53], overweight [54, 55], and obese [56] individuals. Comparable data on ω 6 FA are lacking. One small trial in 17 healthy, normal weight men and women found that a 10-week diet intervention to improve ω 6/ ω 3 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 FAs are unsaturated FAs 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 unsaturated FAs. Studies in rats showed that *trans* FAs 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 FAs 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: 3 ω 6), LA (18: 2 ω 6), and GLA (18: 3 ω 6) among ω 6 FAs, EPA (20: 5 ω 3) among ω 3 FAs, and *trans* 18:1 among *trans* FAs. The variations by individual FAs 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/m²) and also included women who were already overweight or obese (BMI $\geq$ 25 kg/m²) at baseline 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 convenience sample from a previous study, but was not *a priori* designed, to test our hypotheses. However, we don't 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* ω 3 FA may be inversely associated, while *cis* ω 6 FA, ω 6/ ω 3 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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Abbreviation list

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

References

1. Hooper L, Abdelhamid A, Moore HJ, Douthwaite W, Skeaff CM, Summerbell CD. Effect of reducing total fat intake on body weight: systematic review and meta-analysis of randomised controlled trials and cohort studies. *Bmj*. 2012; 345:e7666. doi 10.1136/bmj.e7666. [PubMed: 23220130]
2. Tremblay A. Dietary fat and body weight set point. *Nutrition reviews*. 2004; 62:S75–77. [PubMed: 15387471]
3. Carmichael HE, Swinburn BA, Wilson MR. Lower fat intake as a predictor of initial and sustained weight loss in obese subjects consuming an otherwise ad libitum diet. *J Am Diet Assoc*. 1998; 98:35–39. [PubMed: 9434648]
4. Heitmann BL, Lissner L, Sorensen TI, Bengtsson C. Dietary fat intake and weight gain in women genetically predisposed for obesity. *The American journal of clinical nutrition*. 1995; 61:1213–1217. [PubMed: 7762519]
5. Lissner L, Heitmann BL, Bengtsson C. Low-fat diets may prevent weight gain in sedentary women: prospective observations from the population study of women in Gothenburg, Sweden. *Obes Res*. 1997; 5:43–48. [PubMed: 9061715]
6. Sherwood NE, Jeffery RW, French SA, Hannan PJ, Murray DM. Predictors of weight gain in the Pound of Prevention study. *Int J Obes Relat Metab Disord*. 2000; 24:395–403. [PubMed: 10805494]
7. Sheppard L, Kristal AR, Kushi LH. Weight loss in women participating in a randomized trial of low-fat diets. *The American journal of clinical nutrition*. 1991; 54:821–828. [PubMed: 1951152]

8. Westerterp-Plantenga MS, Wijckmans-Duijsens NE, Verboeket-van de Venne WP, de Graaf K, van het Hof KH, Weststrate JA. Energy intake and body weight effects of six months reduced or full fat diets, as a function of dietary restraint. *Int J Obes Relat Metab Disord*. 1998; 22:14–22. [PubMed: 9481595]
9. Rock CL, Thomson C, Caan BJ, Flatt SW, Newman V, Ritenbaugh C, Marshall JR, Hollenbach KA, Stefanick ML, Pierce JP. Reduction in fat intake is not associated with weight loss in most women after breast cancer diagnosis: evidence from a randomized controlled trial. *Cancer*. 2001; 91:25–34. [PubMed: 11148556]
10. Brehm BJ, Spang SE, Lattin BL, Seeley RJ, Daniels SR, D'Alessio DA. The role of energy expenditure in the differential weight loss in obese women on low-fat and low-carbohydrate diets. *J Clin Endocrinol Metab*. 2005; 90:1475–1482. [PubMed: 15598683]
11. Troiano RP, Briefel RR, Carroll MD, Bialostosky K. Energy and fat intakes of children and adolescents in the united states: data from the national health and nutrition examination surveys. *The American journal of clinical nutrition*. 2000; 72:1343S–1353S. [PubMed: 11063476]
12. Swan G. Findings from the latest National Diet and Nutrition Survey. *The Proceedings of the Nutrition Society*. 2004; 63:505–512. [PubMed: 15831121]
13. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed Pharmacother*. 2002; 56:365–379. [PubMed: 12442909]
14. Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR. Changes in consumption of omega-3 and omega-6 fatty acids in the United States during the 20th century. *The American journal of clinical nutrition*. 2011; 93:950–962. doi 103945/ajcn.110.006643. [PubMed: 21367944]
15. Ailhaud G, Massiera F, Weill P, Légrand P, Alessandri JM, Guesnet P. Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Progress in lipid research*. 2006; 45:203–236. doi 101016/j.plipres.2006.01.003. [PubMed: 16516300]
16. Simopoulos AP. Essential fatty acids in health and chronic disease. *The American journal of clinical nutrition*. 1999; 70:560S–569S. [PubMed: 10479232]
17. Mozaffarian D, Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. *Journal of the American College of Cardiology*. 2011; 58:2047–2067. doi 10.1016/j.jacc.2011.06.063. [PubMed: 22051327]
18. Ramsden CE, Zamora D, Leelarthaepin B, Majchrzak-Hong SF, Faurot KR, Suchindran CM, Ringel A, Davis JM, Hibbeln JR. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. *Bmj*. 346:e8707.
19. Amri EZ, Ailhaud G, Grimaldi PA. Fatty acids as signal transducing molecules: involvement in the differentiation of preadipose to adipose cells. *J Lipid Res*. 1994; 35:930–937. [PubMed: 8071615]
20. Jump DB, Clarke SD, Thelen A, Liimatta M. Coordinate regulation of glycolytic and lipogenic gene expression by polyunsaturated fatty acids. *J Lipid Res*. 1994; 35:1076–1084. [PubMed: 8077846]
21. Clarke SD, Jump D. Polyunsaturated fatty acids regulate lipogenic and peroxisomal gene expression by independent mechanisms. *Prostaglandins Leukot Essent Fatty Acids*. 1997; 57:65–69. [PubMed: 9250610]
22. Schwinkendorf DR, Tsatsos NG, Gosnell BA, Mashek DG. Effects of central administration of distinct fatty acids on hypothalamic neuropeptide expression and energy metabolism. *Int J Obes (Lond)*. 2011; 35:336–344. doi 10.1038/ijo.2010.159. [PubMed: 20714328]
23. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. *The American journal of clinical nutrition*. 2000; 71:343S–348S. [PubMed: 10617994]
24. Field AE, Willett WC, Lissner L, Colditz GA. Dietary fat and weight gain among women in the Nurses' Health Study. *Obesity (Silver Spring)*. 2007; 15:967–976. [PubMed: 17426332]
25. Koh-Banerjee P, Chu NF, Spiegelman D, Rosner B, Colditz G, Willett W, Rimm E. Prospective study of the association of changes in dietary intake, physical activity, alcohol consumption, and smoking with 9-y gain in waist circumference among 16 587 US men. *The American journal of clinical nutrition*. 2003; 78:719–727. [PubMed: 14522729]

26. Cook NR, Lee IM, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, Buring JE. Low-dose aspirin in the primary prevention of cancer: the Women's Health Study: a randomized controlled trial. *Jama*. 2005; 294:47–55. [PubMed: 15998890]
27. Lee IM, Cook NR, Gaziano JM, Gordon D, Ridker PM, Manson JE, Hennekens CH, Buring JE. Vitamin E in the primary prevention of cardiovascular disease and cancer: the Women's Health Study: a randomized controlled trial. *Jama*. 2005; 294:56–65. [PubMed: 15998891]
28. Lee IM, Cook NR, Manson JE, Buring JE, Hennekens CH. Beta-carotene supplementation and incidence of cancer and cardiovascular disease: the Women's Health Study. *J Natl Cancer Inst*. 1999; 91:2102–2106. [PubMed: 10601381]
29. Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clin Chem*. 2006; 52:2265–2272. [PubMed: 17053155]
30. Matthan NR, Ip B, Resteghini N, Ausman LM, Lichtenstein AH. Long-term fatty acid stability in human serum cholesteryl ester, triglyceride, and phospholipid fractions. *Journal of lipid research*. 2010; 51:2826–2832. doi 10.1194/jlr.D007534. [PubMed: 20448292]
31. Zeleniuch-Jacquotte A, Chajes V, Van Kappel AL, Riboli E, Toniolo P. Reliability of fatty acid composition in human serum phospholipids. *European journal of clinical nutrition*. 2000; 54:367–372. [PubMed: 10822282]
32. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. *Epidemiology*. 1990; 1:466–473. [PubMed: 2090285]
33. Nyholm M, Gullberg B, Merlo J, Lundqvist-Persson C, Rastam L, Lindblad U. The validity of obesity based on self-reported weight and height: Implications for population studies. *Obesity (Silver Spring)*. 2007; 15:197–208. doi 10.1038/oby.2007.536. [PubMed: 17228048]
34. Gillum RF, Sempos CT. Ethnic variation in validity of classification of overweight and obesity using self-reported weight and height in American women and men: the Third National Health and Nutrition Examination Survey. *Nutr J*. 2005; 4:27. [PubMed: 16209706]
35. Willett WC, Sampson L, Stampfer MJ, Rosner B, Bain C, Witschi J, Hennekens CH, Speizer FE. Reproducibility and validity of a semiquantitative food frequency questionnaire. *American journal of epidemiology*. 1985; 122:51–65. [PubMed: 4014201]
36. Guenther PM, Reedy J, Krebs-Smith SM. Development of the Healthy Eating Index-2005. *J Am Diet Assoc*. 2008; 108:1896–1901. doi 10.1016/j.jada.2008.08.016. [PubMed: 18954580]
37. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative dietary indices both strongly predict risk of chronic disease. *J Nutr*. 2012; 142:1009–1018. doi 10.3945/jn.111.157222. [PubMed: 22513989]
38. Glymour MM, Weuve J, Berkman LF, Kawachi I, Robins JM. When is baseline adjustment useful in analyses of change? An example with education and cognitive change. *American journal of epidemiology*. 2005; 162:267–278. doi 10.1093/aje/kwi187. [PubMed: 15987729]
39. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res*. 1997; 38:2012–2022. [PubMed: 9374124]
40. Subbaiah PV, Kaufman D, Bagdade JD. Incorporation of dietary n-3 fatty acids into molecular species of phosphatidyl choline and cholesteryl ester in normal human plasma. *The American journal of clinical nutrition*. 1993; 58:360–368. [PubMed: 8237847]
41. Skeaff CM, Hodson L, McKenzie JE. Dietary-induced changes in fatty acid composition of human plasma, platelet, and erythrocyte lipids follow a similar time course. *J Nutr*. 2006; 136:565–569. [PubMed: 16484525]
42. Simopoulos AP, Leaf A, Salem N Jr. Essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. *Ann Nutr Metab*. 1999; 43:127–130. doi 12777. [PubMed: 10436312]
43. Hamazaki T, Okuyama H. The Japan Society for Lipid Nutrition recommends to reduce the intake of linoleic acid. A review and critique of the scientific evidence. *World review of nutrition and dietetics*. 2003; 92:109–132. [PubMed: 14579687]
44. Harris WS, Mozaffarian D, Rimm E, Kris-Etherton P, Rudel LL, Appel LJ, Engler MM, Engler MB, Sacks F. Omega-6 fatty acids and risk for cardiovascular disease: a science advisory from the

American Heart Association Nutrition Subcommittee of the Council on Nutrition, Physical Activity, and Metabolism; Council on Cardiovascular Nursing; and Council on Epidemiology and Prevention. *Circulation*. 2009; 119:902–907. doi 10.1161/CIRCULATIONAHA.108.191627. [PubMed: 19171857]

45. Gaillard D, Negrel R, Lagarde M, Ailhaud G. Requirement and role of arachidonic acid in the differentiation of pre-adipose cells. *Biochem J*. 1989; 257:389–397. [PubMed: 2539084]
46. Corey EJ, Shih C, Cashman JR. Docosahexaenoic acid is a strong inhibitor of prostaglandin but not leukotriene biosynthesis. *Proc Natl Acad Sci U S A*. 1983; 80:3581–3584. [PubMed: 6304720]
47. Massaro M, Habib A, Lubrano L, Del Turco S, Lazzerini G, Bourcier T, Weksler BB, De Caterina R. The omega-3 fatty acid docosahexaenoate attenuates endothelial cyclooxygenase-2 induction through both NADP(H) oxidase and PKC epsilon inhibition. *Proc Natl Acad Sci U S A*. 2006; 103:15184–15189. [PubMed: 17018645]
48. Ringbom T, Huss U, Stenholm A, Flock S, Skattebol L, Perera P, Bohlin L. Cox-2 inhibitory effects of naturally occurring and modified fatty acids. *J Nat Prod*. 2001; 64:745–749. [PubMed: 11421736]
49. Mirnikjoo B, Brown SE, Kim HF, Marangell LB, Sweatt JD, Weeber EJ. Protein kinase inhibition by omega-3 fatty acids. *J Biol Chem*. 2001; 276:10888–10896. [PubMed: 11152679]
50. Hennig B, Watkins BA. Linoleic acid and linolenic acid: effect on permeability properties of cultured endothelial cell monolayers. *Am J Clin Nutr*. 1989; 49:301–305. [PubMed: 2563626]
51. Ukropec J, Reseland JE, Gasperikova D, Demcakova E, Madsen L, Berge RK, Rustan AC, Klimes I, Drevon CA, Sebkova E. The hypotriglyceridemic effect of dietary n-3 FA is associated with increased beta-oxidation and reduced leptin expression. *Lipids*. 2003; 38:1023–1029. [PubMed: 14669966]
52. Lepperdinger G. Inflammation and mesenchymal stem cell aging. *Current opinion in immunology*. 2011; 23:518–524. doi 10.1016/j.coi.2011.05.007. [PubMed: 21703839]
53. Couet C, Delarue J, Ritz P, Antoine JM, Lamiere F. Effect of dietary fish oil on body fat mass and basal fat oxidation in healthy adults. *Int J Obes Relat Metab Disord*. 1997; 21:637–643. [PubMed: 15481762]
54. Krebs JD, Browning LM, McLean NK, Rothwell JL, Mishra GD, Moore CS, Jebb SA. Additive benefits of long-chain n-3 polyunsaturated fatty acids and weight-loss in the management of cardiovascular disease risk in overweight hyperinsulinaemic women. *Int J Obes (Lond)*. 2006; 30:1535–1544. [PubMed: 16552404]
55. Thorsdottir I, Tomasson H, Gunnarsdottir I, Gisladdottir E, Kiely M, Parra MD, Bandarra NM, Schaafsma G, Martinez JA. Randomized trial of weight-loss-diets for young adults varying in fish and fish oil content. *Int J Obes (Lond)*. 2007; 31:1560–1566. doi 10.1038/sj.ijo.0803643. [PubMed: 17502874]
56. Kunesova M, Braunerova R, Hlavaty P, Tvrzicka E, Stankova B, Skrha J, Hilgertova J, Hill M, Kopecky J, Wagenknecht M, Hainer V, Matoulek M, Parizkova J, Zak A, Svacina S. The influence of n-3 polyunsaturated fatty acids and very low calorie diet during a short-term weight reducing regimen on weight loss and serum fatty acid composition in severely obese women. *Physiol Res*. 2006; 55:63–72. [PubMed: 15857162]
57. Guebre-Egziabher F, Rabasa-Lhoret R, Bonnet F, Bastard JP, Desage M, Skilton MR, Vidal H, Laville M. Nutritional intervention to reduce the n-6/n-3 fatty acid ratio increases adiponectin concentration and fatty acid oxidation in healthy subjects. *European journal of clinical nutrition*. 2008; 62:1287–1293. doi 10.1038/sj.ejcn.1602857. [PubMed: 17700650]
58. Teegala SM, Willett WC, Mozaffarian D. Consumption and health effects of trans fatty acids: a review. *J AOAC Int*. 2009; 92:1250–1257. [PubMed: 19916363]
59. Axen KV, Dikeakos A, Sclafani A. High dietary fat promotes syndrome X in nonobese rats. *J Nutr*. 2003; 133:2244–2249. [PubMed: 12840187]
60. Kavanagh K, Jones KL, Sawyer J, Kelley K, Carr JJ, Wagner JD, Rudel LL. Trans fat diet induces abdominal obesity and changes in insulin sensitivity in monkeys. *Obesity (Silver Spring)*. 2007; 15:1675–1684. [PubMed: 17636085]

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

TABLE 1

	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, y	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, %			
<i>cis</i> polyunsaturated fatty acid	33.2 ± 3.6	33.2 ± 3.6	0.90
<i>cis</i> ω6 polyunsaturated fatty acid	26.9 ± 2.9	27.1 ± 2.9	0.45
<i>cis</i> 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
<i>trans</i> fatty acid	1.98 ± 0.61	2.03 ± 0.58	0.34

BMI: body mass index.

^aValues were mean±standard deviation (SD) for continuous variables and % for categorical variables.

P for t -tests for continuous variable and chi-square test for categorical variable

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Table 2

Body weight change (kg)^a over a mean of 10.4 years follow-up according to erythrocyte polyunsaturated fatty acids

	Quantiles				P, trend ^b
<i>cis</i> ω6 fatty acid	1 st	2 nd	3 rd	4 th	
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
18:2ω 6 (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
18:3ω 6 (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
20:3ω 6 (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
20:4ω 6 (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
<i>cis</i> 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

	Quartiles				P, trend ^b
	1 st	2 nd	3 rd	4 th	
18:3ω 3 (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
20:5ω 3 (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
22:6ω 3 (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
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

	Quartiles				P, trend ^b
	1 st	2 nd	3 rd	4 th	
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

^aValues were mean±Standard Error.

^bLinear trends were tested using the median value in each quartile of erythrocyte fatty acids as an ordinal variable.

^cModel adjusted for age, race (white, non-white), randomized treatment (vitamin E, aspirin, β-carotene, or placebo), hypertension case/control status (case, control).

^dModel additionally adjusted for total energy intake (continuous), physical activity (continuous), smoking (never, former, current), alcohol use (0, >0-<5, 5-<15, 15 g/d), post-menopausal status (yes, no, uncertain), post-menopausal 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

Table 3

Hazard ratios^a of becoming overweight or obese according to quartiles of erythrocyte polyunsaturated fatty acid

	Quartiles				P, trend ^b
	1 st	2 nd	3 rd	4 th	
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
18:2ω 6 (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
18:3ω 6cc (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
20:3ω 6 (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
20:4ω 6 (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
18:3ω 3 (alpha-linolenic acid)					
N of becoming overweight	50	43	46	47	

	Quartiles				P, trend ^b
	1 st	2 nd	3 rd	4 th	
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
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

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^aValues were hazard ratio (95% confidence intervals).

^bLinear trends were tested using the median value in each quartile of erythrocyte fatty acids as an ordinal variable.

^cModel adjusted for age, race (white, non-white), randomized treatment (vitamin E, aspirin, β -carotene, or placebo), hypertension case/control status (case, control).

^dModel additionally adjusted for total energy intake (continuous), physical activity (continuous), smoking (never, former, current), alcohol use (0, >0-<5, 5-<15, 15 g/d), post-menopausal status (yes, no, uncertain), post-menopausal 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).