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Dietary patterns are associated with plasma F₂-isoprostanes in an observational cohort study of adults

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Abstract

Associations between individual foods or nutrients and oxidative markers have been reported. Comprehensive measures of food intake may be uniquely informative, given the complexity of oxidative systems and the possibility for antioxidant synergies. We quantified associations over a 20 y history between 3 food-based dietary patterns, summary measures of whole diet, and a plasma biomarker of lipid peroxidation, F₂-isoprostanes in a cohort of Americans, aged 18–30 at year 0 (1985–86). We assessed diet at years 0, 7, and 20 through a detailed history of past-month food consumption and supplement use, and measured plasma F₂-isoprostanes at years 15 and 20. We created 3 different dietary patterns: 1) *a priori* (“*a priori* diet quality score”) based on hypothesized healthy foods, 2) an empirical pattern reflecting high fruit and vegetable intake (“fruit-veg”), and 3) an empirical pattern reflecting high meat intake (“meat”). We used linear regression to estimate associations between each dietary pattern and plasma F₂-isoprostanes cross-sectionally (at year 20, n=2,736) and prospectively (year 0/7 average diet and year 15/20 average F₂-isoprostanes, n=2,718), adjusting for age, sex, race, total energy intake, education, smoking, body mass index (BMI), waist circumference, physical activity, and supplement use. In multivariable-adjusted cross-sectional analysis, the *a priori* diet quality score and the fruit-veg diet pattern were negatively, and the meat pattern positively, associated with F₂-isoprostanes (all p-values<0.001). These associations remained statistically significant in prospective analysis. Our findings suggest that a long-term adherence to a diet rich in fruits and vegetables and low in red meat may decrease lipid peroxidation.

Keywords

cohort; epidemiology; diet; oxidative stress

INTRODUCTION

Oxidative stress is hypothesized to play a role in the pathogenesis of cardiovascular and other chronic diseases^{1,2}. Oxidative stress is a dynamic and complex biologic process, making its direct assessment challenging. Various markers of oxidative damage have been proposed, of which F₂-isoprostanes, a measure of lipid peroxidation, are considered the most reliable^{3–5}. Findings from observational and intervention studies have been mixed with respect to the association between diet and F₂-isoprostanes^{6–9}. Studies have generally

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focused on individual foods or nutrients, however, and very little is known about the effects of diet as a whole on F₂-isoprostanes^{10,11}.

We considered that a whole diet approach might be particularly well-suited to the study of oxidative processes, which are possibly impacted by a range of foods. The notion that studying diet comprehensively, rather than through isolated foods and nutrients, may yield unique information is not new¹², but has gained traction in the past decade among nutritional epidemiologists^{13,14}. Dietary patterns, summary measures of whole diet, may better reflect the complexity of diet, may better capture synergistic aspects of dietary components, and may be more powerful and reliable measures in observational epidemiology where dietary exposures are prone to larger measurement errors^{12–14}.

In the present study, we quantified the associations of 3 dietary patterns and plasma F₂-isoprostanes in the Coronary Artery Risk Development in Young Adults (CARDIA) study, a large, longitudinal cohort study of black and white Americans. We hypothesized that dietary patterns reflective of a high diet quality (rich in plant foods, low in red meat) would be inversely associated with plasma F₂-isoprostanes, and that these associations would be observable over long-term follow-up. We further hypothesized that whole-diet associations would not be completely explained by consumption of dietary antioxidant nutrients or supplement use.

MATERIALS AND METHODS

Study sample

CARDIA is a multicenter, longitudinal study of the evolution of coronary heart disease risk starting in young adulthood¹⁵. CARDIA began in 1985–1986 with a group of 5,115 black and white adults aged 18–30 years from four metropolitan areas (Birmingham, AL; Chicago, IL; Minneapolis, MN; and Oakland, CA). Study participants were sampled to obtain roughly equal numbers of blacks (51.5%) and whites (48.5%), men (45.5%) and women (54.5%), ages 18–24 y (44.9%) and 25–30 y (55.1%), and with a high school education or less (39.7%) or more than a high school education (60.3%). Follow-up examinations were conducted during 1987–1988 (Year 2), 1990–1991 (Year 5), 1992–1993 (Year 7), 1995–1996 (Year 10), 2000–2001 (Year 15), and 2005–2006 (Year 20). A majority of the surviving cohort membership has been examined at each of the follow-up examinations (91%, 86%, 81%, 79%, 74%, and 72%, respectively). The Young Adult Longitudinal Trends in Antioxidants (YALTA) ancillary study assayed F₂-isoprostanes in year 15 and 20 CARDIA plasma samples. CARDIA and YALTA were approved by institutional review boards of each field centers; each study participant provided informed written consent.

Clinical measures

Participants were asked to fast 12 hours and to avoid heavy physical activity and smoking for the 2 hours before each CARDIA exam. Blood was collected from participants in EDTA-containing and serum Vacutainer tubes and stored at 4°C. Within 90 minutes of collection, blood samples were separated through centrifugation, aliquoted into airtight vials, flash-frozen, and stored at –70°C until shipped. Samples were delivered overnight on dry ice to a central laboratory and stored at –70°C until analysis.

In years 15 and 20, plasma free F₂-isoprostanes were measured with a gas chromatography–mass spectrometry-based method¹⁶ by the Molecular Epidemiology and Biomarker Research Laboratory (MEBRL) at the University of Minnesota (Minneapolis, MN), as previously described¹⁷. All samples were analyzed within 1 year of collection. Substudies demonstrated stability of F₂-isoprostanes (no ex vivo loss or formation) during blood collection and processing procedures. Analytical variation of the method was 10% for each

of three control pools assembled in 2000 and assayed repeatedly between October, 2000 and August, 2007; the values of these control pools were stable over time, implying that both assay and stored samples were stable. Thus year 15 and 20 plasma F₂-isoprostane concentrations are directly comparable (Figure 1). A separate quality control approach was 368 blind duplicate pairs (which included non-assay variance attributable to phlebotomy, sample handling, and labeling, as well as to chemical analysis). In the blind duplicate analysis, the overall technical error was 20.1% and the test-retest correlation was 0.84.

Other baseline measurements

Standard questionnaires were used to obtain self-reported demographic and behavioral information across CARDIA examinations. Sex, race, date of birth, education, cigarette smoking, weekly alcohol consumption, and vitamin supplement use were ascertained by structured interview or self-administered questionnaire at each examination. Educational status was quantified as the maximum (at any visit) reported number of years of schooling completed. Self-reported smoking status was classified as never, former, or current smoker. Alcohol consumption in drinks per week was computed from self-reported frequency of beer, wine, and liquor consumption. Participants reported their use of dietary supplements, including brand, dose, and frequency of use. Physical activity was assessed with the validated interview-administered CARDIA Physical Activity History, a simplified version of the Minnesota Leisure Time Physical Activity Questionnaire¹⁸, from which a score was calculated by multiplying participants' self-reported activity frequency by the activity intensity and summing over activities. Height and weight were measured by study staff at each examination and recorded to the nearest 0.5 cm and 0.2 kg, respectively. Body mass index (BMI) was defined as weight (kg) divided by height squared (m²).

Dietary assessment and food group creation

Diet was assessed at years 0, 7, and 20 by participant self-report to an interviewer-administered validated diet history. Interviewers asked study participants open-ended questions about dietary consumption in the past month within 100 food categories, referencing 1,609 separate food items in years 0 and 7 and several thousand separate food items in year 20. For example, an affirmative response to the question: "Do you usually eat bread?" was followed with open-ended questions about the type of bread (e.g., white, whole wheat/mixed grains, rye) and, for each bread type, the frequency of consumption (as servings per day, week, or month). The complete CARDIA diet history can be viewed online at: <http://www.cardia.dopm.uab.edu/doc/d10006.pdf>.

Detailed diet history data were coded by the University of Minnesota Nutrition Coordinating Center (NCC). Recipes were decomposed into constituent foods and assigned a number of servings per day. Foods were placed into 166 food groups using the food grouping system devised by the NCC. Food group intake was calculated as the total number of servings per day each food within the food group was consumed. From the 166 food groups within the NCC system, we excluded 6 infant products (not relevant), unsweetened water, and non-grain flour (rarely consumed). We further collapsed the remaining 158 food groups into 46 food groups, as shown in Supplemental Table 1, to approximate those previously established by study co-authors^{19,20}. Food groups were created based on considerations of similar nutrient characteristics, hypothesized biologic effects, and comparability to food groups defined in previous studies.^{19,20}

Description of dietary patterns

We created a dietary pattern, *a priori*, by classifying foods groups as beneficial (n=20), adverse (n=13), or neutral (n=13) in terms of hypothesized health effects (Supplemental Table 2)^{19,20}. We categorized the 33 non-neutral food groups into quintiles of consumption,

scored the food group consumption as 0 to 4 for beneficial or 4 to 0 (the reverse) for adverse food depending on study participants' quintile level of consumption, and summed across all 33 food groups. For food groups with a large proportion of non-consumers, we coded non-consumers as 0 and consumers were categorized into quartiles. The theoretical maximum score was 132, with a baseline mean of 64 (SD: 13, range: 25–110). Higher scores indicate higher hypothesized diet quality (1 point corresponds to a shift in a single food group to the next higher quintile), and we thus refer to this summary measure as the “*a priori* diet quality score.”

We additionally derived two dietary patterns empirically, based on the self-reported dietary consumption of study participants. We used principal components analysis (PCA) with orthogonal rotation to derive uncorrelated dietary patterns from the 46 food groups. This is an established approach to studying dietary patterns^{13,21}. We refer to these dietary patterns as the “fruit-veg” and “meat” diet patterns to reflect dietary consumption patterns that were relatively high in fruits and vegetables or meat, respectively. PCA results, presented as factor loadings, were generally consistent across years 0 and 7 (Supplemental Table 2).

The two methods for constructing dietary patterns differ conceptually. The *a priori* diet quality score is based on our beliefs, guided by the scientific literature, about the health effects of foods, while the two empirically-derived dietary patterns are based on a statistical correlation structure of food consumption, based on CARDIA participants' reported consumption. We note that the PCA still incorporates *a priori* considerations about diet and nutrition reflected in decisions about diet assessment and food group creation. All 3 dietary patterns are completely food-based, and in this way differ from approaches to dietary pattern construction that incorporate both foods and nutrients^{12,13}.

Reliability of dietary data

A baseline reliability study of nutrient consumption comparing two CARDIA diet histories administered 1 month apart found substantial sex- and energy-adjusted test-retest correlations, ranging from 0.27 (polyunsaturated fat) to 0.82 (saturated fat)²¹. We lack reliability data for foods and dietary patterns in the CARDIA diet validation study. However, in another large prospective cohort study, short term reliability coefficients for dietary patterns²³ were generally in the same range as for foods²⁴ and nutrients²⁵. In our data, participants' dietary patterns were characterized by long-term stability exceeding that observed for food or nutrient consumption. Correlation coefficients for dietary patterns at years 0 and 7 were 0.66 for the *a priori* diet quality score, 0.50 for the fruit-veg diet pattern, and 0.59 for the meat diet pattern, while correlation coefficients for nutrients or foods were generally lower than 0.40. For example, the correlation coefficient for years 0 and 7 dietary alpha-tocopherol was 0.24 and the coefficient for years 0 and 7 fruit consumption was 0.33.

Statistical Analysis

We used linear regression to quantify cross-sectional and prospective associations between dietary patterns and plasma F₂-isoprostanes. In the prospective analyses, we present averages of each of the 3 dietary patterns assessed at years 0 and 7 and of F₂-isoprostanes measured at years 15 and 20. We averaged diet and biomarker data because there was not biologic reason for choosing specific years for prospective study, and because averaging across examinations increased sample size (in those in whom only one of the measures was available) and may improve the stability of dietary²⁶ and biomarker²⁷ data obtained in epidemiologic studies. However, we note that findings for prediction of year 20 F₂-isoprostanes from year 0 diet patterns did not yield substantially different findings from those presented (data not shown).

Separate regression analyses were conducted for each of the 3 dietary patterns, as independent variables, and plasma F₂-isoprostanes. Dietary patterns were modeled as quintiles of consumption and continuously, using the p-value from the continuous model to assess linear trend. All regression models adjusted for participant sex (male, female), study center (Birmingham, Chicago, Minneapolis, Oakland), race (white, black), age (continuous), and total energy (continuous). A fuller model included educational attainment (high school, some college, college), current cigarette smoking (current, never/former), supplement use (yes/no), physical activity (continuous exercise units), BMI, and waist circumference. In subsequent analysis, we additionally adjusted for dietary consumption of several antioxidants, including alpha-tocopherol, ascorbic acid, vitamin A, and alpha- and beta-carotene. We included the nutrient-adjusted analysis to examine the extent to which antioxidant nutrients appeared to account for associations between dietary patterns and F₂-isoprostanes. We also quantified associations between dietary patterns and F₂-isoprostanes separately among supplement users and non-users. For cross-sectional analysis, we adjusted for year 20 covariate values; for prospective analysis, we adjusted for covariate values averaged across years 0 and 7. If dietary, covariate, or F₂-isoprostanes data were available for only one of the 2 years (year 0 or 7), we used the single year's value in the analysis. Potential intermediates were handled the same. To address possible non-normality in F₂-isoprostanes, we included linear regression analysis of natural logarithm-transformed F₂-isoprostanes. We also considered the possibility that associations may differ for high values of F₂-isoprostanes, and thus included logistic regression analysis of those at or above vs. below the 75th percentile of F₂-isoprostanes. All analyses were completed with the PC version (9.2) of the Statistical Analysis System (SAS, Cary, NC).

We excluded from analysis clinic attendees who had missing dietary data (n=4 at year 0, 143 at year 7, and 406 at year 20) or who reported implausibly high or low energy intake (<800 or >8000 kcal/day for men, <600 or >6000 kcal/day for women) (n=128 at year 0, 94 at year 7, and 54 at year 20). Data on plasma F₂-isoprostanes were missing on 670 participants at year 15 and 486 at year 20. After exclusions and accounting for missing data, we had 2,736 participants available for year 20 cross-sectional analysis, and 2,718 available for prospective analysis. Other exclusions were analysis-specific, based on missing data for covariates.

RESULTS

Dietary patterns

The *a priori* diet quality score, created from hypothesized health effects of foods, reflected a diet consistent with dietary guidelines, with low consumption of red meat and refined grains and high consumption of fruits and vegetables and whole grains. For example, among participants in the 1st, 3rd, and 5th quintiles of the *a priori* diet quality score, the respective mean daily servings of red meat were 2.1, 1.4, and 0.8; whole grains were 0.9, 1.6, 2.2; and green vegetables were 0.2, 0.6, and 1.6. The *a priori* diet quality score was not low fat, but had fat consumption consistent with a Mediterranean diet, with higher consumption of oil (2.1, 2.1, and 3.8 servings/day within 1st, 3rd, and 5th quintiles), and nuts and seeds (0.6, 1.0, and 1.9), and lower consumption of fried potatoes (0.3, 0.2, and 0.1) and butter (2.5, 2.0, 1.4).

Principal components analysis revealed 2 major patterns of dietary consumption among CARDIA participants. One pattern, the “fruit-veg diet” was characterized by higher consumption of fruit, green vegetables, yellow vegetables, whole grains, and nuts and seeds (Table 1). The “meat diet,” was characterized by higher consumption of red meat, processed meat, butter, fried potatoes, and refined grains (Table 1). Food-specific loading scores for each pattern are shown in Supplemental table 2. The fruit-veg diet positively correlated and

the meat diet was negatively correlated with the *a priori* diet quality score (respectively, at year 20: $r = 0.69$ and $r = -0.42$). The meat and fruit-veg dietary patterns were the first 2 principal components and together explained 7.5 percent of the total variance in the 46 food groups at year 0, 8.1 percent at year 7, and 7.3 at year 20. The meat diet explained slightly more of the variance than the fruit-veg diet (4.4 vs. 3.2, 4.6 vs. 3.5, and 3.9 vs. 3.4 percent from orthogonal rotated analysis of year 0, 7, and 20 data, respectively). Other factors explained less than 2 percent each of the total variance. In addition to explaining the most variance, the first two factors were conceptually appealing: the patterns distinguished participants on consumption of major foods groups and are broadly consistent with other studies reporting results for “Western” and “prudent” dietary patterns derived from PCA.

Year 20 characteristics of the study sample according to dietary patterns

Dietary patterns were associated with participant characteristics (Table 1). People who ranked high on the *a priori* diet quality score or the fruit-veg diet pattern were more likely to be white, have a college degree, and use supplements. They were less likely to smoke. In contrast, participants who ranked high on the meat diet were more likely to be black or to smoke, and were less likely to have a college degree or use supplements. BMI and waist circumference decreased across quintiles of the *a priori* diet quality score and fruit-veg pattern, and increased across quintiles of the meat dietary pattern. Physical activity increased across quintiles of the *a priori* diet quality score and the fruit-veg pattern, but was stable across quintiles of the meat pattern. In energy-adjusted models, exercise decreased across quintiles of the meat dietary pattern.

Year 20 characteristics of the study sample according to plasma F₂-isoprostanes

Plasma F₂-isoprostanes were negatively associated with physical activity, supplement use, and educational attainment, and were positively associated with cigarette smoking, BMI, and waist circumference (Table 2). Across quintiles of F₂-isoprostanes, dietary intake of alpha-tocopherol, ascorbic acid, vitamin A, and alpha- and beta-carotene decreased (Table 2). Year 15 and 20 F₂-isoprostanes were correlated at 0.62. The distribution of F₂-isoprostanes was left-skewed, as evidenced by the large standard deviation for the 5th quintile of F₂-isoprostanes.

Cross-sectional associations between dietary patterns and plasma F₂-isoprostanes

At year 20, plasma F₂-isoprostanes concentrations were associated with each of the 3 diet measures (Figure 2), using multivariable-adjusted regression models (adjusted for age, gender, race, study center, total energy consumption, smoking, education, supplement use, physical activity, BMI, and waist circumference). F₂-isoprostanes concentrations decreased across quintiles of the *a priori* diet quality score (mean = 61.6, 56.7, 56.6, 50.0, and 44.3 pg/mL, $p_{\text{trend}} = 0.0001$) and the fruit-veg dietary pattern (mean = 62.1, 55.2, 54.6, 51.5, 45.7 pg/mL, $p_{\text{trend}} = 0.0001$), while they increased across quintiles of the meat dietary pattern (mean = 48.0, 53.1, 52.4, 55.3, 60.4 pg/mL, $p_{\text{trend}} = 0.0001$). Findings were materially similar from regression analysis of natural logarithm-transformed F₂-isoprostanes, though the transformation yielded a smaller range of geometric mean values from the 1st to the 5th quintile of dietary pattern (Supplemental Table 3). Logistic regression analysis of those at or above vs below the 75th percentile did not reveal anomalous associations between diet and high F₂-isoprostanes (data not shown).

The associations between dietary pattern and F₂-isoprostanes were similar among supplement users and non-users: F₂-isoprostanes were negatively associated with the *a priori* diet quality score and the fruit-veg pattern, and positively associated with the meat pattern (Table 3). Additional adjustment for dietary antioxidant nutrients, including alpha-tocopherol, ascorbic acid, vitamin A, and alpha- and beta-carotene, did not materially

change estimated mean F₂-isoprostanes across quintiles of consumption of the *a priori* diet quality score or the fruit-veg dietary pattern among supplement users or non-users. The association between the meat dietary pattern and F₂-isoprostanes was attenuated to a greater extent upon adjustment for dietary antioxidants, particularly among supplement users. For example, among supplement users, comparing the 1st and 5th quintiles of meat dietary pattern consumption, the estimated F₂-isoprostanes means were 44.8 (41.6, 48.0) and 58.0 (53.2, 62.7) before adjustment for dietary antioxidant nutrients and 47.0 (43.8, 50.3) and 53.8 (48.8, 58.7) after adjustment (Table 3). Similarly, in analysis of natural logarithm-transformed F₂-isoprostanes, the *a priori* diet quality score and fruit-veg dietary pattern displayed stronger associations with F₂-isoprostanes in supplement-stratified multivariable-adjusted analysis that included adjustment for dietary nutrients than did the meat dietary pattern (Supplemental Table 3). In separate models of possible statistical interaction, we did not find evidence that associations between dietary patterns and F₂-isoprostanes differed according to smoking status (data not shown).

We obtained multivariable-adjusted standardized regression coefficients for individual antioxidant nutrients in the total sample. These findings indicate that the regression coefficient for the association between the *a priori* diet quality score and F₂-isoprostanes was stronger (−6.1; 95% CI: −7.4, −4.8), than those for dietary alpha-tocopherol (−3.8; −5.0, −2.6), ascorbic acid (−0.2; −1.3, 0.9), vitamin A (−2.9; −4.0, −1.7), alpha-carotene (−1.6; −2.6, −0.5), or beta-carotene (−2.9; −4.0, −1.7).

Prospective associations between dietary patterns and plasma F₂-isoprostanes

In multivariable-adjusted analysis, the year 0/7 average *a priori* diet quality score and fruit-veg dietary pattern were both negatively associated, and the year 0/7 average meat dietary pattern was positively associated, with year 15/20 average F₂-isoprostanes (Figure 3a). We next looked at whether year 0/7 diet was associated with the change in F₂-isoprostanes between years 15 and 20. We note that in our data year 20 F₂-isoprostanes were on average lower than year 15 concentrations. In multivariable-adjusted analysis, year 15 to 20 declines in F₂-isoprostanes were positively associated with the year 0/7 *a priori* diet quality score and fruit-veg dietary pattern, and negatively associated with the year 0/7 meat dietary pattern (Figure 3b).

DISCUSSION

In this cohort of young and middle-aged black and white Americans, we found significant associations among three food-based dietary patterns and plasma F₂-isoprostanes. Our findings suggest that diets high in plant foods and low in red meat may decrease systemic oxidative stress. These associations could not be explained by differences in demographic variables, smoking, physical activity, BMI, waist circumference, or supplement use. This study demonstrates the utility of summary measures of diet—dietary patterns—to assess associations between diet and F₂-isoprostanes.

In this analysis, we studied summary measures of whole diet (dietary patterns), rather than individual nutrients or foods. The use of dietary patterns has grown over the past decade, and our findings contribute to a literature showing that dietary patterns provide a powerful method for studying diet and health. Dietary patterns may better allow for the complexity of food and food synergy^{13,14}, and may be especially well-suited to the study of oxidative stress—a complex biologic process that is likely influenced by a variety of dietary components⁶. The benefit of studying diet comprehensively is supported the strength of dietary patterns associations after accounting for vitamin supplement use and dietary antioxidant nutrient consumption. Although there are few studies of oxidative stress and

dietary patterns, our data are consistent with data from observational⁹ and intervention^{7,11} studies showing an inverse association between plant-based diets and F₂-isoprostanes.

In our data, the apparent benefit conferred by plant-based diets on F₂-isoprostanes concentrations could not be explained by vitamin supplement use or by the consumption of dietary antioxidants, including alpha-tocopherol, ascorbic acid, vitamin A, and alpha- and beta-carotene. These findings may indicate that plant-based diets affect oxidative stress through dietary components that have not been identified or measured or through complex pathways involving dietary synergy. In contrast, dietary antioxidants seemed to account for more of the association between the meat dietary pattern and F₂-isoprostanes among supplement users, and, to a lesser extent, supplement non-users. These findings may reflect the greater variability in phytochemical-rich plant foods in the *a priori* diet quality score and the fruit-veg dietary pattern, as compared to the meat dietary pattern, seen, for example, in the consumption of whole grains, nuts and seeds, and green and yellow vegetables across quintiles of dietary pattern in Table 1.

Our analysis included the two primary methods for dietary pattern construction: 1) a pattern based on *a priori* considerations of the health effects of foods, and 2) patterns empirically-derived from dietary consumption of the study sample. Each approach provides an overall summary of dietary consumption, and, in the present analysis, all three summaries are purely foods-based. Dietary patterns derived from principal components analysis generally do not incorporate nutrients, but, to our knowledge, our *a priori* diet quality score is the first *a priori* pattern that does not incorporate nutrients or supplement use, in contrast to other published *a priori* dietary patterns, such as the Mediterranean diet²⁸ and the Alternative Health Eating Index²⁹. Our focus on foods is consistent with our support for a greater emphasis on foods in dietary research^{14,30}, as well as with calls for a foods-based approach to dietary recommendations³¹.

Dietary patterns research has been critiqued for the variability in published approaches to defining patterns. In addition to a focus on foods-based diet summaries, our selection of dietary patterns was motivated by an interest in testing the stability of diet-isoprostanes associations with several patterns and to restrict analysis to approaches that have been established in the literature. Our two PCA-derived patterns can be considered conceptually comparable to “Western” and “prudent” patterns in publications from other cohort studies^{32,33}, although we recognize that the exact weighting of foods will vary among study samples and depending on foods included in the dietary assessment. The approach used to create the *a priori* diet quality score has also been previously published, and has been shown to predict a range of cardiometabolic outcomes in other samples^{19,20}. From our reading of the dietary patterns literature, we believe that specific approaches to variable construction may be less relevant than the finding that studying whole diets is a powerful method to verify the importance of food in physiologic processes. Still, the field of dietary patterns is new and future research will benefit from work focused on elucidating important distinctions among patterns.

Our study has several strengths and limitations. The large CARDIA study sample and extensive covariate data enable us to provide effect estimates that are stable and adjusted for important confounding variables. Applying both *a priori* and principal component analysis approaches allowed us to test the robustness of findings related to the construction of dietary pattern. The repeated measurement of diet and biologic markers allowed us to average diet and biomarker data, which increases the reliability of our prospective analysis. Our longitudinal design also enabled us to include both cross-sectional and prospective analysis. Cross-sectional results do not temporally distinguish the measurement of diet and F₂-isoprostanes, both of which were assessed at year 20, while prospective analysis included

assessment of diet and covariables that preceded the measurement of F₂-isoprostanes. The prospective associations mean that the conditions for mid-life oxidative stress exist many years before mid-life. The timing of data collection was not planned with the current study in mind, and although the analysis was prospective in nature, we were unable to examine concurrent change in diet and F₂-isoprostanes, which weakens our ability to make causal statements. Our study sample was relatively young (aged 38–50 in year 20) and limited to black and white race groups, and it is possible that our findings may not represent older or more diverse populations.

Our analysis was limited to a single measure of oxidative damage, lipid peroxidation. Among available markers, F₂-isoprostanes are considered the gold standard for assessing lipid peroxidation^{3–5}. A study of experimentally-induced oxidative damage supports the use of either urinary or plasma F₂-isoprostanes as reliable time- and dose-dependent markers of general oxidative status *in vivo*³⁴. Evidence from many, but not all, epidemiologic studies supports higher F₂-isoprostanes in physiologic states consistent with the oxidative stress hypothesis³. In the CARDIA sample, F₂-isoprostanes have been inversely associated with circulating carotenoids³⁵, and positively associated with smoking, BMI, coronary artery calcification (CAC)¹⁷, and insulin resistance³⁶.

In conclusion, our data support cross-sectional and prospective associations between foods-based dietary patterns and plasma concentrations of F₂-isoprostanes, a biomarker of lipid peroxidation. Associations were robust to the method of dietary pattern creation, with both *a priori* and principal components analysis-derived patterns showing relations to plasma F₂-isoprostanes, and to multivariable-adjustment for BMI, waist circumference, smoking, and physical activity. These findings could not be explained by vitamin supplement use or by dietary consumption of antioxidant nutrients. These data support protective effects of a diet high in fruits, vegetables, nuts, seeds, and whole grains, and low in red and processed meats and refined grains on oxidative damage.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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	Control Pool B	Control Pool C	Control Pool A
N	194	142	226
Mean	36.8	56.8	74.0
CV	11.1%	10.0%	9.4%

Figure 1.
F₂-isoprostanes concentrations 2000–2007 in repeatedly measured control pools stored at –70°C since 2000

a.

b.

c.

Figure 2.

Multivariable-adjusted mean year 20 F₂-isoprostanes (pg/mL) according to quintiles of year 20 *a priori* diet quality score² (a), fruit-veg dietary pattern (b), and meat dietary pattern (c)³.

1. Estimated mean F₂-isoprostanes are plotted on dietary pattern quintile medians.
2. For graphical purposes, the *a priori* diet quality score was centered at its mean.
3. All p-values for trend in F₂-isoprostanes across quintiles of dietary patterns < 0.0001.

a.

b.

Figure 3.

Multivariable-adjusted¹ mean year 15/20 F₂-isoprostanes (pg/mL) (a) and mean change in F₂-isoprostanes (pg/mL) from year 15 to 20 (b) according to quintiles² of year 0/7 average dietary patterns.

- Adjusted for age, gender, race, study center, total energy consumption, education, smoking, physical activity, supplement use, BMI, and waist circumference. Year 15 to 20 change analysis was also adjusted for year 15 F₂-isoprostanes.
- F₂-isoprostanes are plotted at dietary pattern quintile medians. The *a priori* diet quality score was centered for graphical purposes. All p-values for trend < 0.01

Table 1

Characteristics of study sample at year 20 according to quintiles of year 20 dietary pattern scores, CARDIA 2005–06, n=2,736¹

	<i>A priori</i> diet quality score ^{2,4}				Fruit-veg dietary pattern ^{3,5}				Meat dietary pattern ^{3,6}			
	Q1	Q3	Q5		Q1	Q3	Q5		Q1	Q3	Q5	
n	540	614	571		547	548	547		547	548	547	
Dietary pattern score (mean, SD)	3.4 (0.4)	4.7 (0.2)	6.2 (0.4)		-1.1 (0.2)	-0.2 (0.1)	1.5 (0.9)		-1.0 (0.2)	-0.2 (0.1)	1.6 (1.0)	
F ₂ -isoprostanes (pg/mL)	63.0 (35.4)	56.2 (31.9)	43.5 (17.6)		64.0 (35.5)	53.7 (28.5)	44.6 (19.7)		51.2 (28.6)	53.2 (28.6)	56.5 (29.6)	
Age, years	24.2 (3.8)	25.2 (3.5)	26.0 (3.3)		24.1 (3.9)	25.3 (3.5)	25.6 (3.4)		25.8 (3.4)	25.2 (3.7)	24.8 (3.6)	
Female (%)	49.3	54.4	66.0		56.1	55.1	54.3		82.1	60.0	28.0	
White (%)	31.3	54.9	81.3		27.6	62.2	70.6		62.9	56.6	41.3	
College degree (%)	32.8	50.7	77.1		28.9	58	68.7		64.4	54.7	35.8	
Current smoker (%)	28.4	20.9	9.2		27.4	15.1	12.7		9.4	15.2	32.1	
Supplement users (%)	32.6	52.9	67.6		30.9	55.3	62.9		62.2	53.5	36.9	
Body Mass Index, kg/m ²	30.6 (7.3)	29.8 (6.8)	27.0 (5.7)		30.6 (7.3)	29.4 (6.9)	28.3 (6.3)		27.8 (6.3)	29.6 (6.4)	30.4 (7.6)	
Waist circumference, cm	95.1 (15.7)	92.9 (15)	85.9 (13.6)		93.9 (15.3)	92.0 (15.4)	89.6 (15.2)		85.3 (13.9)	92 (13.9)	97.2 (16.6)	
Physical activity, exercise units	240 (233)	332 (275)	452 (297)		221 (217)	340 (264)	459 (308)		351 (272)	305 (255)	353 (305)	
Mean total energy, kcal/day	2,495 (1,185)	2,251 (1,039)	2,292 (806)		2,035 (1,019)	2,252 (1,001)	2,927 (1,104)		1,539 (574)	2,121 (542)	3,718 (1,111)	
Dietary alpha-tocopherol, mg/day	8.4 (6.5)	9.9 (8.5)	15.4 (10.6)		6.2 (3.5)	10.4 (10.7)	18.7 (11.3)		10.1 (10.4)	10.3 (9.2)	14.3 (8.6)	
Dietary ascorbic acid, mg/day	114 (124)	163 (251)	204 (177)		95.3 (182.5)	165 (232)	261 (220)		139 (134)	161 (217)	207 (234)	
Dietary vitamin A, IU/day	5,320 (3,653)	8,636 (6,326)	15,168 (10,784)		4228 (2683)	8,107 (4,061)	19,122 (14,153)		11,242 (13,012)	8,774 (6,850)	10,688 (8,012)	
Dietary alpha-carotene, mcg/day	282 (433)	564 (997)	1,113 (1,472)		207 (306)	507 (621)	1,498 (2,292)		845 (189)	553 (757)	673 (995)	
Dietary beta-carotene, mcg/day	1,953 (1,759)	3,679 (3,094)	7,439 (5,850)		1,541 (1,300)	3,406 (2,020)	9,347 (7,533)		5,462 (6,934)	3,900 (3,620)	4,395 (4,158)	
Fruit, sv/day	0.8 (1)	1.6 (1.9)	2.5 (2.0)		0.6 (0.7)	1.4 (1.1)	3.0 (2.6)		2.1 (2.4)	1.6 (1.7)	1.5 (1.6)	
Green vegetables, sv/day	0.2 (0.3)	0.6 (0.6)	1.6 (1.3)		0.2 (0.2)	0.6 (0.5)	1.8 (1.4)		1.1 (1.3)	0.7 (0.8)	0.6 (0.7)	
Yellow vegetables, sv/day	0.1 (0.2)	0.2 (0.3)	0.5 (0.6)		0.1 (0.1)	0.2 (0.2)	0.6 (0.7)		0.4 (0.6)	0.2 (0.3)	0.2 (0.3)	
Oil, sv/day	2.1 (4.6)	2.1 (4.1)	3.8 (6.0)		1.6 (3.2)	2.0 (4.0)	4.2 (6.8)		1.7 (3.1)	2.4 (4.5)	4.4 (8.7)	
Refined grains, sv/day	4.6 (3)	3.5 (2.5)	2.5 (1.7)		3.8 (2.6)	3.5 (2.5)	3.3 (2.5)		1.5 (0.9)	3.0 (1.3)	6.4 (3.1)	
Red meat, sv/day	2.1 (1.9)	1.4 (1.3)	0.8 (0.9)		1.7 (1.7)	1.4 (1.4)	1.3 (1.4)		0.5 (0.4)	1.1 (0.7)	3.0 (2.1)	
Whole grains (sv/day)	0.9 (1.1)	1.6 (1.4)	2.2 (1.5)		0.7 (0.9)	1.6 (1.2)	2.5 (2.0)		1.6 (1.4)	1.6 (1.5)	1.5 (1.5)	

	<i>A priori</i> diet quality score ^{2,4}			Fruit-veg dietary pattern ^{3,5}			Meat dietary pattern ^{3,6}		
	Q1	Q3	Q5	Q1	Q3	Q5	Q1	Q3	Q5
Seeds and nuts (sv/d)	0.6 (1.4)	1.0 (1.6)	1.9 (2.3)	0.4 (0.8)	1.0 (1.5)	2.3 (3.0)	1.2 (2.0)	1.0 (1.6)	1.0 (1.9)
Processed meat (sv/day)	1.3 (1.1)	0.9 (0.9)	0.6 (0.8)	1.0 (1.0)	0.8 (0.8)	0.9 (1.1)	0.3 (0.3)	0.8 (0.7)	1.8 (1.5)
Fried potatoes (sv/day)	0.3 (0.4)	0.2 (0.2)	0.1 (0.1)	0.3 (0.4)	0.2 (0.2)	0.1 (0.2)	0.1 (0.1)	0.1 (0.1)	0.4 (0.5)
Butter (sv/day)	2.5 (2.6)	2.0 (2.2)	1.4 (1.9)	1.7 (2.0)	2.1 (2.4)	2.3 (2.7)	0.9 (0.9)	1.8 (1.8)	3.7 (3.3)

¹ Tests for trend of continuous variables were based on general linear regression with dietary pattern as continuous independent variable. Chi-square tests were used for categorical variables across all 5 levels of the dietary pattern.

² A priori score is expressed in standard deviation (SD) units to facilitate comparison with the PCA scores. The overall mean (SD) of the *a priori* diet quality score at year 20 was 70.3 (12.8).

³ Meat diet and fruit-vegetable diet score are principal components, centered on zero with a standard deviation of 1.0.

⁴ All variables significant at p<0.01

⁵ All variables significant at p<0.01 except gender (0.39), processed meat (0.021)

⁶ All variables significant at p<0.01 except physical activity (p=0.22), dietary vitamin A (0.21), dietary alpha-carotene (0.25), dietary beta-carotene (0.036), whole grains (0.34), seeds and nuts (0.33)

Table 2

Characteristics of study sample at year 20 according to quintiles of year 20 plasma F₂-isoprostanes, CARDIA 2005–06

	Quintile of F ₂ -isoprostanes					p-value ¹
	1	2	3	4	5	
n	547	547	550	545	547	
F ₂ -Isoprostanes, pg/mL	28.1 (3.9)	37.5 (2.3)	46.2 (3.0)	58.4 (4.3)	98.9 (36.0)	
Age at baseline, years	25.6 (3.4)	24.9 (3.5)	25.1 (3.5)	24.9 (3.7)	25.1 (3.7)	0.34
Female (%)	48.1	45.2	51.5	61.5	75.9	<0.0001
White (%)	60.9	58.3	57.1	49.2	51.0	0.0010
College degree (%)	65.5	60.5	52.9	47.7	41.1	<0.0001
Current smoker (%)	11.2	16.0	18.7	22.6	25.3	<0.0001
Supplement users (%)	65.3	52.8	52.0	40.9	44.2	<0.0001
Body Mass Index, kg/m ²	26.7 (4.6)	27.7 (5.6)	28.7 (6.4)	30.7 (7.1)	32.9 (8.2)	<0.0001
Waist circumference, cm	86.9 (12.5)	89.1 (13.5)	90.7 (15.2)	94.6 (16.4)	97.3 (16.5)	<0.0001
Physical activity, exercise units	396 (284)	386 (287)	375 (290)	285 (248)	242 (229)	<0.0001
Dietary alpha-tocopherol, mg/day	13.7 (11.0)	11.8 (10.0)	10.8 (7.1)	9.9 (6.2)	9.3 (9.6)	<0.0001
Dietary ascorbic acid, mg/day	184 (206)	175 (231)	166 (153)	142 (122)	138 (203)	0.0066
Dietary vitamin A, IU/day	11,458 (9,911)	10,040 (9,030)	9,839 (8,272)	9,033 (10,308)	7,780 (6,231)	<0.0001
Dietary alpha-carotene, mcg/day	838 (1,418)	678 (1,202)	681 (1,083)	608 (1,599)	462 (716)	<0.0001
Dietary beta-carotene, mcg/day	5,264 (5,294)	4,575 (4,783)	4,405 (4,312)	3,896 (5,388)	3,345 (3,229)	<0.0001

¹ Tests for trend of continuous variables were based on general linear regression with F₂-isoprostanes as continuous independent variable. Chi-square tests were used for categorical variables across all 5 levels of F₂-isoprostanes.

Table 3

Year 20 multivariable (MV)-adjusted mean F₂-isoprostanes (95% confidence interval) according to year 20 quintile of dietary patterns, among supplement non-users and users, CARDIA 2005/2006.

		Quintile of Dietary Pattern					
		1	2	3	4	5	p-value, trend
Supplement non-users							
<i>A priori</i> diet quality score							
n		364	296	289	205	185	
MV-adjusted ¹		63.5 (60.2, 66.8)	60.0 (56.5, 63.4)	60.7 (57.2, 64.1)	51.1 (46.9, 55.3)	44.6 (40.0, 49.3)	<0.0001
+dietary nutrients ²		62.6 (59.2, 66.0)	59.5 (56.1, 63.0)	60.8 (57.4, 64.3)	52.0 (47.7, 56.3)	45.9 (41.0, 50.7)	<0.0001
Fruit-veg dietary pattern							
n		378	289	245	224	203	
MV-adjusted		65.5 (62.2, 68.9)	56.4 (52.9, 59.9)	56.7 (52.9, 60.5)	56.5 (52.4, 60.5)	46.8 (42.3, 51.3)	<0.0001
+dietary nutrients		64.5 (61.0, 68.1)	55.9 (52.4, 59.5)	56.9 (53.1, 60.7)	57.0 (52.9, 61.0)	48.6 (43.5, 53.8)	<0.0001
Meat dietary pattern							
n		207	236	255	296	345	
MV-adjusted		51.7 (46.9, 56.5)	55.0 (50.7, 59.2)	54.9 (51.1, 58.7)	59.6 (56.1, 63.1)	63.3 (58.8, 67.8)	<0.0001
+dietary nutrients		53.7 (48.8, 58.6)	55.6 (51.3, 59.9)	55.4 (51.6, 59.2)	59.2 (55.7, 62.6)	61.7 (57.1, 66.3)	0.0057
Supplement users							
<i>A priori</i> diet quality score							
n		176	214	325	296	386	
MV-adjusted		60.4 (56.5, 64.4)	53.3 (49.8, 56.7)	52.4 (49.6, 55.1)	48.4 (45.6, 51.3)	43.3 (40.6, 46.0)	<0.0001
+dietary nutrients		59.0 (54.9, 63.1)	52.5 (49.0, 56.0)	51.7 (48.9, 54.5)	48.6 (45.7, 51.4)	44.9 (41.9, 47.8)	<0.0001
Fruit-veg dietary pattern							
n		169	258	303	323	344	
MV-adjusted		58.8 (54.7, 63.0)	53.6 (50.4, 56.8)	52.2 (49.4, 55.1)	47.3 (44.5, 50.0)	44.4 (41.4, 47.3)	<0.0001
+dietary nutrients		56.8 (52.5, 61.2)	52.6 (49.3, 55.8)	51.5 (48.6, 54.3)	47.3 (44.5, 50.1)	46.8 (43.4, 50.2)	0.0001
Meat dietary pattern							
n		340	311	293	251	202	

	Quintile of Dietary Pattern					p-value, trend
	1	2	3	4	5	
MV-adjusted	44.8 (41.6, 48.0)	50.9 (47.9, 53.8)	49.6 (46.7, 52.5)	51.0 (47.8, 54.3)	58.0 (53.2, 62.7)	<0.0001
+dietary nutrients	47.0 (43.8, 50.3)	52.0 (49.0, 54.9)	49.3 (46.4, 52.3)	50.4 (47.2, 53.7)	53.8 (48.8, 58.7)	0.032

1 . Adjusted for age, sex, race, study center, total energy consumption, education, smoking, physical activity, BMI, and waist circumference.

2 . Additionally adjusted for dietary alpha-tocopherol, ascorbic acid, vitamin A, and alpha- and beta-carotene.