Effect of Dietary Patterns on Measures of Lipid Peroxidation
Results From a Randomized Clinical Trial

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Background—Free radical–mediated oxidative damage to lipids is thought to be an important process in the pathogenesis of atherosclerosis. Although previous studies have demonstrated a beneficial impact of antioxidant vitamin supplements on lipid peroxidation, the effect of dietary patterns on lipid peroxidation is unknown.

Methods and Results—During the 3-week run-in period of a randomized trial, 123 healthy individuals were fed a control diet, low in fruits, vegetables, and dairy products, with 37% of calories from fat. Participants were then randomized to consume for 8 weeks: (1) the control diet, (2) a diet rich in fruits and vegetables but otherwise similar to the control diet, and (3) a combination diet rich in fruits, vegetables, and low-fat dairy products and reduced in fat. Serum oxygen radical–absorbing capacity, malondialdehyde (an in vitro measure of lipid peroxidation), and breath ethane (an in vivo measure of lipid peroxidation) were measured at the end of run-in and intervention periods. Between run-in and intervention, mean (95% CI) change in oxygen radical–absorbing capacity (U/mL) was −35 (−93, 13) in the control diet, 26 (−15, 67) in the fruits and vegetables diet (P=0.06 compared with control), and 19 (−22, 54) in the combination diet (P=0.10 compared with control). Median (interquartile range) change in ethane was 0.84 (0.10, 1.59) in the control diet, 0.02 (−0.61, 0.83) in the fruits and vegetables diet (P=0.04 compared with control), and −1.00 (−1.97, 0.25) in the combination diet (P=0.005 compared with control). Change in malondialdehyde did not differ between diets.

Conclusions—This study demonstrates that modification of diet can favorably affect serum antioxidant capacity and protect against lipid peroxidation. (Circulation. 1998;98:2390-2395.)

Key Words: diet • lipids • antioxidants • atherosclerosis

The oxidative modification of lipids, particularly oxidation of LDL cholesterol, has been proposed to be an important if not obligatory intermediate step in the formation of atherosclerotic lesions. Evidence to support this hypothesis is based in part on observational studies that show associations between oxidized LDL cholesterol and both the presence of atherosclerotic lesions and the progression of carotid artery atherosclerosis. The extent of lipid peroxidation depends on at least 3 factors: the generation of oxygen free radicals, the presence of lipid substrates, and the activity of antioxidants.

Increased consumption of fruits and vegetables may protect against free radical–mediated lipid peroxidation by providing dietary sources of antioxidants such as carotenoids and flavonoids. Several longitudinal observational studies have demonstrated an inverse association between the dietary intake of fruits and vegetables and the risk of atherosclerotic cardiovascular diseases (ASCVD). Although many nutrients from fruits and vegetables (fiber, potassium, and antioxidants) may be responsible for this effect, some studies have shown inverse relations between ASCVD and serum levels or dietary intake of antioxidants. For instance, ecological studies have shown an inverse relation between serum concentrations of vitamin E, vitamin C, and carotenoids and risk for ASCVD.

An increasing but inconclusive body of evidence suggests that diet supplementation with antioxidant vitamins may prevent atherosclerotic cardiovascular disease. In prospective clinical trials of patients with angiographically-proven coronary artery disease, there was a lower incidence of nonfatal myocardial infarction and lower rates of progression of coronary artery lesions among patients supplemented with vitamin E. Several clinical trials have also tested the effects of vitamin supplements on various measurements of lipid peroxidation. In these trials, supplementation of diet with vitamin E, beta carotene, vitamin C, or combination supplements reduced the extent of in vitro lipid peroxidation and inhibited in vitro oxidative modification of LDL cholesterol. In the aggregate, these trials suggest that supplementation of diet with high doses of these nutrients...
reduce lipid peroxidation and may have important antiatherogenic properties.

Few trials have studied the effects of diets rather than vitamin supplements on lipid peroxidation. To our knowledge, just 2 dietary intervention trials have examined the impact of modifying single nutrients. In a trial of 12 healthy women studied in a metabolic ward, consumption of a diet depleted of dietary carotenoids for 2 months resulted in increased rates of lipid peroxidation.

In the other trial, increased consumption of monounsaturated fatty acids was associated with a lower susceptibility of LDL cholesterol to in vitro peroxidation. In a clinical trial of fruit and vegetable consumption, a diet high in fruits and vegetables increased serum antioxidants, including carotenoids and flavonoids; however, the effects on lipid peroxidation were not examined. To our knowledge, no trial has examined the impact of healthy dietary patterns with several desirable factors (rich in fruits and vegetables and reduced in saturated fat, total fat, and cholesterol) on lipid peroxidation. Such a dietary pattern (termed the combination diet in this study) should reduce the extent of lipid peroxidation through 2 mechanisms: by decreasing lipid substrate available for peroxidation (ie, serum lipids) and increasing the concentration of antioxidants derived from diet.

In the setting of a randomized, controlled feeding study, we tested the hypothesis that diets rich in fruits and vegetables, particularly one that is also reduced in fat, will have beneficial effects on lipid peroxidation.

Methods

This research was conducted as an ancillary study within the Dietary Approaches to Stop Hypertension (DASH) trial, a National Heart, Lung, and Blood Institute–sponsored clinical trial designed to assess the effect of dietary patterns on blood pressure. This ancillary study was conducted at the Johns Hopkins clinical center and was designed and analyzed only by the coauthors. Details of the DASH protocol and of its main results have been published elsewhere. The protocol was approved by a local institutional review board. All participants provided written informed consent.

Participants

Study participants consisted of 123 healthy adults (age ≥22 years) who were not receiving antihypertensive medication and who had a diastolic blood pressure of 80 to 95 mm Hg and a systolic blood pressure <160 mm Hg. Persons with a total serum cholesterol of ≥260 mg/dL were excluded. Other major exclusion criteria for entry into the trial were poorly controlled diabetes, a cardiovascular event within 6 months, chronic disease that might interfere with trial participation, pregnancy or lactation, body mass index >35 kg/m², medications that affect blood pressure, unwillingness to stop all vitamin and mineral supplements, unwillingness to stop antacids containing calcium or magnesium, and consumption of >14 alcoholic drinks per week.

Diets

During a 3-week run-in, all participants ate the control diet. This diet was relatively low in fruits and vegetables (total of 4 servings per day), and its macronutrient profile corresponded to average US dietary consumption. At the end of run-in, participants were randomized to 1 of 3 diets consumed during an 8-week intervention period: the control diet, a fruit and vegetable diet, or a combination diet. The fruits and vegetables diet provided approximately 9 servings of fruits and vegetables per day and was rich in potassium, magnesium, and fiber. Otherwise it was similar to the control diet. The combination diet emphasized fruits and vegetables (total of ~10 servings per day), low-fat dairy products, and other reduced-fat foods; this diet was rich in potassium, magnesium, fiber, calcium, and protein and reduced in saturated fat, total fat, and cholesterol. A nutrient analysis of the diets is published elsewhere.

Database estimates of N-3 polyunsaturated fatty acid (PUFA) content of the control, fruits and vegetables, and combination diets were 2.4%, 3.0%, and 5.5% of total daily caloric intake, respectively. Meals were prepared in a metabolic kitchen and served in an outpatient dining facility. Throughout the 11 weeks of feeding, participants agreed to eat only the food provided to them and nothing else. Caloric intake was adjusted to maintain a stable weight.

Blood Collection and Analyses

Blood samples were collected after overnight fasts, once at the end of run-in (baseline), and again at the end of the 8-week intervention. Blood was drawn from the antebrachial vein into a Vacutainer tube. Serum was allowed to clot for 15 minutes and then centrifuged at 2000g for 15 minutes at 4°C. Serum samples were then pipetted into 2 mL polyethylene storage containers, topped with nitrogen gas, and stored for 5 months at −70°C for a period of up to 4 months that should have no substantial effect on measures of malondialdehyde (MDA) or oxygen radical–absorbing capacity (ORAC); separate analyses of serum samples stored for 5 months demonstrated that differences between replicate measurements (before and after storage) were similar to reported run-to-run coefficient of variations (CV) for these assays.

Breath Collection and Ethane Analysis

Breath ethane is an end product of N-3 PUFA oxidation and reflects in vivo lipid peroxidation. Breath collection occurred at the end of the run-in and intervention periods. Breath was collected in a well-ventilated room from seated participants before they ate their noon or evening meal and after they rested for at least 1 minute. Thirty to 60 seconds of breath (≈10 L) were collected from each participant by means of a 1-way, nonbreathing Rudolf valve connected by respiratory tubing to a 22-L gas-tight collection bag. A sample of room air was also collected at each sampling period. The concentration of ethane was determined by capillary gas chromatography.

Carbon dioxide concentration of the participant’s breath was analyzed by a Beckman LB-3 CO₂ monitor (Sensor Medics) for the purpose of standardizing the ethane values to an alveolar CO₂ concentration of 40 mm Hg. Breath ethane concentration is corrected for background ethane and the dead space in the breath collection system (as determined by CO₂) and calculated as follows: (sample ethane − background ethane)×(40/measured CO₂). All analyses were performed within 24 hours of collection, well within the 72-hour period of sample stability. Ethane values are reported as picomoles per liter, with a reported run-to-run CV of 3.0%.

Malondialdehyde Assay

The MDA assay provides in vitro estimates of PUFA peroxidation. Determinations of MDA were made on freshly thawed serum by a modification of the Yagi method at the Genox Corporation. The serum sample was incubated for 1 hour at 95°C with thiobarbituric acid–MDA adduct was measured by absorption at 530 nm. A standard curve for absorption and MDA concentration was generated from which lipid peroxidation was reported as micromoles of MDA equivalents. The run-to-run CV for the MDA assay at Genox was 6.5%.

ORAC Assay

The ORAC assay estimates the ability of serum to resist oxidative damage, reflecting the combined effects of all antioxidants in the serum rather than any individual antioxidant. An indicator protein sensitive to oxidative damage (β-phycocerythrin) was added to serum and allowed to undergo oxidation after the addition of a water soluble peroxyl radical generator 2,2’-azo-bis-(2-aminopropane) dihydrochloride (AAPH) at 37°C. The oxidation of the fluorescent protein was monitored spectrofluorometrically at 560 nm emission
TABLE 1. Characteristics of Participants at End of Run-in Feeding Period by Diet Assignments

<table>
<thead>
<tr>
<th>Diet</th>
<th>Control</th>
<th>Fruits and Vegetables</th>
<th>Combination</th>
<th>All (n=123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y*</td>
<td>49.6 (11.9)</td>
<td>49.1 (10.1)</td>
<td>46.9 (11.2)</td>
<td>48.5 (11.0)</td>
</tr>
<tr>
<td>% Women</td>
<td>52</td>
<td>43</td>
<td>46</td>
<td>47</td>
</tr>
<tr>
<td>% Black</td>
<td>65</td>
<td>64</td>
<td>73</td>
<td>67</td>
</tr>
<tr>
<td>% Current smokers</td>
<td>13</td>
<td>10</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>28.1 (3.7)</td>
<td>27.6 (3.8)</td>
<td>27.0 (3.4)</td>
<td>27.5 (3.6)</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
<td>200 (30)</td>
<td>194 (31)</td>
<td>198 (29)</td>
<td>197 (29)</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>131 (24)</td>
<td>124 (29)</td>
<td>126 (31)</td>
<td>127 (34)</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>46.8 (11.8)</td>
<td>49.6 (12.2)</td>
<td>50.6 (20.3)</td>
<td>49.0 (15.2)</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>115 (51)</td>
<td>105 (48)</td>
<td>107 (58)</td>
<td>108 (52)</td>
</tr>
<tr>
<td>Albumin, g/dL</td>
<td>4.92 (0.37)</td>
<td>4.85 (0.35)</td>
<td>4.93 (0.38)</td>
<td>4.90 (0.36)</td>
</tr>
</tbody>
</table>

*Continuous data reported as mean and SD.

(540 nm excitation) every 5 minutes until extinction. The presence of antioxidants in the serum reduces the rate of decline of the fluorescence of the protein. A water-soluble vitamin E analog, Trolox, was used to establish a standard curve. One ORAC unit is equivalent to the protection provided by one micromole of Trolox. This assay was performed at the Genox Corporation, which reported a run-to-run CV of 4.4%.

**Other Assays**

Serum albumin was determined spectrophotometrically by Sigma Diagnostics albumin assay with a reported run-to-run CV of 1.3%. Serum lipids were measured in a Hitachi 704 chemistry analyzer. HDL cholesterol was measured on the chemistry analyzer with the magnetic HDL method (Polymedico). LDL cholesterol concentration was calculated by the equation LDL cholesterol = total cholesterol – HDL cholesterol – triglyceride/5. All triglyceride levels were <400 mg/dL.

Serum carotenoids and retinol concentrations were determined by high-pressure liquid chromatography in a subgroup of participants, the 34 individuals enrolled in the fourth feeding group. The reported run-to-run CV was between 0.6% and 5.1% for all analytes (retinol, lutein, zeaxanthin, cryptoxanthin, lycopene, β-carotene, and α-tocopherol).

**Data Analyses and Statistical Considerations**

In this study, the sample size was fixed at the number of participants enrolled at the Hopkins clinical center of the DASH trial. Power calculation suggested that a target sample size of 114 would have 80% power to detect a 35% change in breath ethane and 4% change in ORAC.

For continuous variables with a normal distribution, means±SD values are presented. The breath ethane values did not have a normal distribution; hence, medians with interquartile ranges are presented for baseline levels and medians with 95% CI for the changes between run-in and intervention. Characteristics of study participants at baseline were compared with the use of χ² tests for categorical variables (sex and ethnicity), ANOVA for continuous variables, and Wilcoxon for continuous variables with nongaussian distribution.

To test our hypothesis, we performed pairwise comparisons in which changes in the outcome variables (ORAC, MDA, and ethane) in the fruits and vegetables diet were compared with those in the control diet, and changes from the combination diet were compared with those from the control diet. For ORAC and MDA, we used ANCOVA, adjusting for baseline values. For ethane, we used median regression analysis, adjusting for baseline ethane. All analyses were performed with STATA and SAS software.

**Results**

From a total of 2247 persons who were screened, 135 (6.0%) started run-in. Of these, 124 (92%) were randomized. A total of 123 of 124 randomized participants completed the feeding study. Paired specimens of serum and breath were available in 121 (98%) of the participants. Self-report of perfect adherence to diet assignment (no nonstudy foods consumed and all study foods eaten) occurred in 96%, 96%, and 94% of person-days in those assigned to the control, fruits and vegetables, and combination diets.

Baseline characteristics of participants at the end of run-in are presented in Table 1. Participants tended to be middle-aged (mean age, 48.5 years). Most were black (66%). Both sexes were well represented. There were no significant or substantial differences at baseline between diet groups in any of the variables listed in Table 1. Baseline levels of ORAC and MDA were similar in the 3 diets (P>0.20) (Table 2). Breath ethane was not normally distributed; hence medians are presented. There was an imbalance in the distribution of baseline ethane levels across groups, with the highest level occurring in the combination group (P=0.02).

Results of the dietary interventions on lipid peroxidation measures are also reported in Table 2. There was a significant increase in breath ethane in the control group between the run-in and intervention periods (median=0.91, [95% CI, 0.14, 1.57]), no change in the fruits and vegetables diet (median=0.02, [95% CI −0.61, 0.82]), and a decrease in the combination diet (median=−0.80 [95% CI, −1.92, 0.28]) (Table 2). In median regression analyses, adjusting for baseline ethane measured at run-in, breath ethane at the end of intervention was higher in the control diet compared with the fruits and vegetables diet (P=0.04) and the combination diet (P=0.005) (Figure 1). In a subgroup analysis restricted to the nonsmokers, the effects of the diets on breath ethane persisted (P=0.05 and P=0.006 for comparisons of the fruits and vegetables diet and combination diet with the control diet, respectively).

MDA increased between run-in and intervention in all diets. However, neither within-diet changes from baseline nor
between-diet differences achieved statistical significance. In the fruits and vegetables diet and the combination diet, serum ORAC increased, whereas it fell in the control diet (Figure 2, $P = 0.10$ comparing control diet and fruits and vegetables diet, $P = 0.06$ comparing control and combination diets).

Serum carotenoids, retinol, and $\alpha$-tocopherol were measured in the 34 participants enrolled in the fourth feeding group (control diet, $n = 12$; fruits and vegetables diet, $n = 10$; combination diet, $n = 12$). Baseline levels ($n = 34$) were as follows: $\beta$-carotene (0.279±0.152 µg/mL), cryptoxanthin (0.110±0.061 µg/mL), lutein (0.237±0.120 µg/mL), lycopene (0.571±0.214 µg/mL), zeaxanthin (0.064±0.026 µg/mL), retinol (0.688±0.167 µg/mL), and $\alpha$-tocopherol (10.09±2.51 µg/mL). Percent change from baseline in these serum antioxidants by diet are displayed in Figure 3. Consumption of the fruits and vegetables diet and the combination diet resulted in significantly higher serum, cryptoxanthin, zeaxanthin, and $\beta$-carotene compared with changes in the control diet ($P < 0.05$). Compared with control, serum lutein significantly increased in the combination diet. Changes in serum levels of lycopene, retinol, and $\alpha$-tocopherol were small in each of the diets and were not statistically significant across diets.

### Discussion

This study demonstrated that modification of diet without use of vitamin supplements can favorably affect serum antioxidant capacity and protect against in vivo lipid peroxidation. In the control diet, breath ethane significantly increased from the end of run-in to the end of intervention, 8 weeks later. Hence, the control diet, with its low levels of antioxidants, may

| Table 2: Levels of ORAC, Ethane, and MDA Measured at End of Run-In (Pre) and Intervention (Post) Periods and Change From Run-In ($\Delta$) |
|---|---|---|---|---|---|
| Diet | Control (A) | Fruits and Vegetables (B) | Combination (C) | $P$ for Comparison of Diets |
| Ethane Pre* | 1.44 (0.78, 2.38) | 1.70 (1.44, 4.10) | 2.78 (1.76, 4.23) | 0.84 (0.10, 1.59) | 0.04 (0.10, 1.59) |
| Ethane Post* | 2.26 (1.76, 3.00) | 1.76 (1.25, 3.13) | 1.94 (1.29, 3.11) | 0.02 (0.61, 0.83) | 0.005 (0.005, 0.005) |
| MDA Pre† | 21.31 (2.58) | 20.94 (3.17) | 22.14 (3.31) | 0.14 (0.68, 0.95) | 0.64 (0.64, 0.64) |
| MDA Post† | 21.45 (2.79) | 21.53 (3.56) | 22.54 (3.98) | 0.59 (0.29, 1.46) | 0.48 (0.48, 0.48) |
| ORAC Pre‡ | 1532 (112) | 1531 (124) | 1501 (126) | 0.14 (0.68, 0.95) | 0.06 (0.06, 0.06) |
| ORAC Post‡ | 1498 (144) | 1557 (135) | 1516 (136) | 0.59 (0.29, 1.46) | 0.10 (0.10, 0.10) |

*Median (interquartile range).
†Median (95% CI).
‡Mean (SD).
§Mean (95% CI).
$P$ values are calculated from ANOVA for MDA and ORAC and Wilcoxon (rank sums) for ethane. Complete data on ORAC and MDA were available on 121 participants ($n = 39$, control diet; $n = 141$, fruits and vegetables diet; and $n = 41$, combination diet).
reflect a deficiency state resulting in higher rates of lipid peroxidation. In contrast, the combination diet reduced breath ethane. Participants randomized to the fruits and vegetables diet had an intermediate response. Although breath ethane levels did not significantly change between run-in and intervention in the fruits and vegetables diet, the change in breath ethane was significantly different from that of the control diet ($P=0.04$). These trends in ethane production across diets support the hypothesis that diets rich in fruits and vegetables provide nutrients that protect against in vivo lipid peroxidation.

Both the combination diet and the fruits and vegetables diet increased the antioxidant capacity of the serum (as measured by the ORAC assay). These results indicate that there are components of the serum that delayed oxidation of the target protein, $eta$-phycocerythrin, and resulted in higher ORAC. Previous studies have demonstrated that flavenoids and tocopherols protect the $eta$-phycocerythrin against oxidative damage in vitro. In our study, the serum levels of several carotenoids including $eta$-carotene, cryptoxanthin, and zeaxanthin increased in the fruits and vegetables diet and in the combination diet. Lutein increased only in the combination diet (Figure 3). This pattern of change in serum levels of antioxidants is similar to that reported in a clinical trial of nutritional advice to increase the consumption of fruits and vegetables reported in our trial ($P<0.05$) are indicated (*) and were calculated by t tests.

measured at the end of intervention was higher in participants who ate the control diet in comparison to participants in the fruits and vegetables diet ($P=0.10$) and combination diet ($P=0.001$). A second issue relates to cigarette smoking, which is associated with higher levels of breath ethane. Ethane is a component of cigarette smoke. As well, cigarette smokers have higher in vivo lipid peroxidation and ethane production. However, smokers made up only 11% of participants and were equally distributed between the 3 diets. Furthermore, in subgroup analyses excluding smokers, the between-diet differences in breath ethane persisted.

In this trial, there were no between-diet differences in MDA, a measure of in vitro lipid peroxidation that is associated with conditions that predispose to the development of atherosclerosis (cigarette smoking, diabetes, hyperlipidemia, hypertension, and obesity). However, it is well recognized that the assay lacks specificity and repeatability. Less well recognized is the dependence of this assay on the proportion of fats derived from linoleic PUFAs. As the ratio of polyunsaturated to saturated fatty acids (P/S) in serum lipids increases, formation of lipid peroxidation products will also increase even though total fat and saturated fat decrease. In this study, the P/S ratio was 0.5 in the control diet, 0.6 in the fruits and vegetables diet, and 1.3 in the combination diet. This represents a more than doubling of the P/S ratio in the combination diet over the control diet. Such a difference in the P/S ratio may have a major influence on the MDA assay, potentially overwhelming the effects of increased dietary intake of antioxidants. Hence, in this diet intervention study that simultaneously modified fat and antioxidant intake, this in vitro assay is not an appropriate outcome variable.

Results of this study support the hypothesis that diets rich in fruits and vegetables can increase the antioxidant capacity of serum (ORAC) and protect against in vivo lipid peroxidation (breath ethane). Such links between healthy dietary patterns and reduced rates of lipid peroxidation contribute to a better understanding of the role of diet in the pathogenesis of atherosclerosis. As well, these results provide additional scientific rationale for recommendations to increase the consumption of fruits and vegetables and reduce dietary fat intake as a means to prevent atherosclerotic cardiovascular disease.

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References


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