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Factors Associated with Oxidative Stress in Human Populations

Gladys Block[1], Marion Dietrich[1], Edward P. Norkus[2], Jason D. Morrow[3], Mark Hudes[4], Bette Caan[5], and Lester Packer[6]

[1] Division of Health Policy and Management, School of Public Health, University of California, Berkeley, CA.
[2] Department of Biomedical Research, Our Lady of Mercy Medical Center, Bronx, NY.
[3] Departments of Medicine and Pharmacology, School of Medicine, Vanderbilt University, Nashville, TN.
[4] Department of Nutritional Sciences and Toxicology, University of California, Berkeley, CA.
[5] Division of Research, Kaiser Permanente, Oakland, CA.
[6] Department of Molecular Pharmacology and Toxicology, School of Pharmacy, University of Southern California, Los Angeles, CA.

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Oxidation of biomolecules may play a role in susceptibility to a number of diseases. However, there are few large-scale survey data describing oxidative damage that occurs in humans and the demographic, physical, or nutritional factors that may be associated with it. Such information is essential for the design and analysis of studies investigating the role of oxidative stress in health and disease. This paper presents data on levels of two biomarkers of lipid peroxidation, malondialdehyde and F_2-isoprostanes, in 298 healthy adults aged 19–78 years. The study was conducted in Berkeley and Oakland, California, in 1998–1999. Sex was the strongest predictor of lipid peroxidation as measured by both biomarkers ($p < 0.0001$); it was stronger than smoking. C-reactive protein was positively associated with lipid peroxidation ($p = 0.004$), as was plasma cholesterol. Plasma ascorbic acid had a strong inverse relation ($p < 0.001$) with both biomarkers. Plasma β-carotene was also associated with F_2-isoprostanes. Other plasma antioxidants were not associated with lipid peroxidation biomarkers, once ascorbic acid was included in the multivariate model. Future surveys and epidemiologic studies should measure at least one marker of oxidative damage, as well as plasma ascorbic acid. These data would permit a better understanding of the role that oxidants and antioxidants play in the health of human populations. *Am J Epidemiol* 2002;156:274–85.

ascorbic acid; biological markers; C-reactive protein; epidemiology, molecular; lipid peroxidation; obesity; oxidative stress; sex characteristics

Abbreviations: Iso-P, F_2-isoprostane(s); MDA, malondialdehyde; TBARS, thiobarbituric acid reactive substances.

Oxidative stress is caused by the presence of free radicals or radical-generating agents in concentrations that overwhelm natural radical-blocking or -scavenging mechanisms. Sources of oxidative stress include exogenous factors, such as cigarette smoke, and endogenous factors, such as the oxidative burst from activated macrophages. Antioxidant mechanisms include antioxidant enzymes and plasma antioxidants, many of which are determined by dietary antioxidant intake. Oxidative stress, in turn, can cause oxidative damage to DNA, proteins, and lipids, and many clinical conditions are associated with increased indices of oxidant stress; this suggests that overwhelming the antioxidant defense system initiates and propagates processes involved in the pathogenesis of many diseases (1).

Confirmation of a role for oxidative damage in disease requires information about the oxidative stress status of human populations and the relation of oxidative damage to diseases and their risk factors. Large representative surveys have measured dietary intakes of antioxidant nutrients (2–4) and blood levels of antioxidant nutrients (2, 4, 5). In contrast to this information on *antioxidant* intake or status, very few

Reprint requests to Dr. Gladys Block, 426 Warren Hall, School of Public Health, University of California, Berkeley, CA 94720 (e-mail: gblock@uclink4.berkeley.edu).

epidemiologic data exist on the extent and distribution of *oxidative damage* in human populations (6).

Many biomarkers of oxidative damage are labor- and time-intensive and may not be appropriate for use in epidemiologic studies or clinical trials involving hundreds or thousands of subjects. For the present study, we selected two biomarkers that are widely used, sensitive, and appropriate for use in large studies: malondialdehyde (MDA) and F_2-isoprostanes (Iso-P). MDA is a decomposition product of peroxidized polyunsaturated fatty acids (7). Iso-P are eicosanoids produced by the random oxidation of arachidonyl-containing lipids by oxygen radicals (8). Increased Iso-P levels in urine or plasma have been found in patients with a variety of disease conditions (9–15), as well as in smokers (16, 17). Increased plasma MDA levels have also been found in persons with many conditions (18–25) and in smokers (25).

The absence of epidemiologic data on oxidative damage in normal human populations represents a serious gap in our knowledge about the distribution, correlates, and causative factors of oxidative damage. In this paper, we describe data on two different measures of lipid peroxidation in 298 healthy persons and a number of physiologic and behavioral factors associated with these measures.

MATERIALS AND METHODS

Subjects (138 cigarette smokers, 68 persons exposed to secondhand smoke, and 92 nonsmokers) were enrolled in an intervention study on the effect of antioxidant supplements on oxidative damage in active and passive smokers (26). The study was conducted in Berkeley and Oakland, California, between 1998 and 1999, and was approved by the institutional review boards of the University of California, Berkeley, and Kaiser Permanente. The inclusion criteria for smoking status were as follows. Smokers were eligible if they smoked 15 or more cigarettes per day. Passive smokers were eligible if they had not smoked cigarettes for at least 1 year and were exposed indoors to the smoke of at least one cigarette per day on at least 5 days per week. Nonsmokers were eligible if they had not smoked cigarettes for at least 1 year and were not passively exposed to cigarette smoke at all. Exclusion criteria included reported consumption of four or more servings of fruits and vegetables per day (based on response to a single screening question); intake of more than two alcoholic drinks per day; history of alcohol problems less than 1 year previously; pregnancy; use of blood-thinning drugs; hemochromatosis; history of kidney stones, other kidney problems, cancer, stroke, heart attack, hepatitis, or diabetes mellitus; human immunodeficiency virus infection; and consumption of iron supplements or vitamin E supplements at levels over 800 IU/day. Persons who had consumed other vitamin supplements were required to undergo a "washout period" of at least 5 weeks without supplement use preceding the collection of the data described here. Results reported here were based on cross-sectional data obtained from the cohort at baseline, after the washout period and before any intervention.

Dietary information was obtained using the Block98 food frequency questionnaire (Block Dietary Data Systems, Berkeley, California (http://www.nutritionquest.com/faq.html#B98differences)), covering usual dietary intake over the previous year. Venous blood was collected after an overnight fast, drawn into Vacutainers (Becton Dickinson, Rutherford, New Jersey) containing ethylenediaminetetraacetic acid, centrifuged at 5°C for 10 minutes at 1,200 × *g*, protected from light, and stored at –70°C. Plasma aliquots for ascorbic acid measurement were added (1:1) to 10 percent weight/volume meta-phosphoric acid to stabilize the ascorbic acid. Plasma samples were assayed for C-reactive protein, cotinine, ascorbic acid, α- and γ-tocopherol, five carotenoids, cholesterol, triglycerides, transferrin saturation, and two biomarkers of lipid peroxidation, MDA and Iso-P. All plasma samples from active and passive smokers were assayed for MDA and Iso-P. All 92 nonsmoker samples were assayed for MDA, but because of budgetary constraints, only 38 nonsmoker samples were assayed for Iso-P.

MDA in plasma was determined using lipid peroxidation analysis kits (Oxis International, Inc., Portland, Oregon). Plasma MDA concentrations were derived after calculating the third derivative spectra from each sample's absorption spectra (530–610 nm) to enhance the sensitivity and accuracy of the MDA assay. Third derivative spectroscopy mathematically eliminates interference from other biologic compounds (alkanals, 4-hydroxyalkenals, and other biologic compounds) that has been associated with many earlier studies that reported controversially high plasma MDA levels. With this technique, we obtained plasma MDA estimates that were specific for total MDA and were similar to levels obtained using high-performance liquid chromatography (27–30). The within-run coefficient of variation ranged from 1.2 percent to 3.4 percent depending on the concentration of MDA.

Free Iso-P levels in plasma were quantitated, after purification and derivatization, by selected ion monitoring gas chromatography/negative ion chemical ionization-mass spectrometry employing [$^2H_4$]8-iso-prostaglandin $F_2\alpha$ as an internal standard. Compounds were analyzed as pentafluorobenzyl ester and trimethylsilyl ether derivates by monitoring the M-181 ions (m/z 569 Da for endogenous Iso-P and m/z 573 Da for [$^2H_4$]8-iso-prostaglandin $F_2\alpha$). Iso-P data are expressed in ng/ml. This assay has a precision of ±6 percent and accuracy of 96 percent (31).

Concentrations of cotinine were determined by gas chromatography with nitrogen-phosphorus detection (for smokers) and by liquid chromatography atmospheric pressure ionization tandem mass spectrometry (for passive smokers and nonsmokers) (32, 33). Tocopherols and carotenoids were measured by reverse-phase high-performance liquid chromatography (34). Ascorbic acid was determined spectrophotometrically using 2,4-dinitrophenylhydrazine as a chromogen (35). C-reactive protein concentrations were measured by radial immunodiffusion assay (The Binding Site Ltd., San Diego, California). Total cholesterol and triglyceride levels were measured by endpoint spectroscopy (Sigma-Aldrich, St. Louis, Missouri). Transferrin saturation was analyzed by a commercial clinical laboratory (SmithKline Beecham Clinical Laboratories, Norristown, Pennsylvania).

Smoking status was initially categorized according to the criteria stated above. However, for the present analysis, a few subjects were recategorized on the basis of their cotinine levels. Four passive smokers were recategorized as active

TABLE 1. Characteristics of the sample in a study of two biomarkers of lipid peroxidation (malondialdehyde and F_2-isoprostanes) in healthy adults aged 19–78 years (n = 298), Berkeley and Oakland, California, 1998–1999

	Percentage or mean value	Percentile cutpoint			Range of values
		25th	50th	75th	
Sex (% male)	40.6				
Smoking					
Current smoking (%)	46.0				
Exposure to secondhand smoke (%)	23.2				
No. of cigarettes smoked per day (active smokers)	22.7 (7.5)*	20	20	25	15–60
No. of cigarettes exposed to per day (passive smokers)	11.7 (13.6)	3.5	6.5	12.5	1–70
Age (years)	46.6 (13.6)	37	48	56	19–78
Weight (pounds†)					
Males	192 (36.6)	168	188	212	120–330
Females	162.3 (38.4)	136	156	185	100–330
Body mass index‡	27.6 (5.5)	23.6	26.5	30.9	17.2–54.8
Mean percentage of dietary calories derived from fat§,¶	38.3 (7.5)	34.0	38.7	42.5	16–64
Daily no. of servings of fruit or juice§,#	1.1 (0.9)	0.5	1.0	1.5	0.1–6.3
Daily no. of servings of vegetables§,#	2.6 (1.6)	1.4	2.2	3.4	0.3–10.8
Plasma total ascorbate level (mg/dl)	0.97 (0.45)	0.60	0.98	1.31	0.12–2.08
Plasma β-carotene level (μg/dl)	16.3 (17.2)	6.1	11.8	20.0	0.10–134.7
Plasma α-tocopherol level (mg/dl)	1.49 (0.63)	1.12	1.34	1.65	0.39–4.99
Plasma malondialdehyde level (μmol/liter)	0.81 (0.55)	0.40	0.73	1.13	0.02–3.15
Plasma F_2-isoprostane level (ng/ml)	0.050 (0.025)	0.034	0.042	0.060	0.006–0.169

* Numbers in parentheses, standard deviation.
† 1 pound = 0.45 kg.
‡ Weight (kg)/height $(m)^2$.
§ As estimated by the Block98 food frequency questionnaire (http://www.nutritionquest.com/faq.html#B98differences).
¶ Comparable data from the Third National Health and Nutrition Examination Survey (1988–1994): 34% of kilocalories were derived from fat (2).
# Comparable data from the California Dietary Practices Survey (1997): 3.8 servings of fruits and vegetables per day (39).

smokers, and four nonsmokers were recategorized as passive smokers. In the resulting categorization, all but one of the nonsmokers had cotinine levels below the limit of quantification (36); passive smokers had cotinine levels less than 4,000 pg/ml (median, 357 pg/ml), and all but one of the active smokers had cotinine levels greater than 18,000 pg/ml (median, 257,000 pg/ml).

Body mass index was calculated as weight (in kilograms) divided by height (in meters) squared and was categorized as normal weight or overweight, using the classification recommended by the National Heart, Lung, and Blood Institute (37). Food servings were calculated by multiplying frequency of consumption by reported portion size for each food, summing the grams consumed in each food group, and dividing by the standard serving size as defined in the US Department of Agriculture Food Guide Pyramid (38).

Univariate statistics are expressed as mean values and standard deviations. For bivariate analyses, MDA and Iso-P levels were examined within quartiles of potential determinants or covariates, and trend tests were performed. For multivariate analyses, MDA was square-root-transformed and Iso-P was log-transformed. Multivariate analyses were conducted after examination of potential effect modifiers, and variables were included if they were statistically significant based on type III sum of squares. Variables examined included sex, age, race, body weight, and body mass index; smoking status; plasma cotinine level; levels of plasma antioxidants, including carotenoids, α- and γ-tocopherol, and total ascorbic acid; levels of plasma lipids, including serum cholesterol and triglycerides; dietary intake of nutrients and food groups; and C-reactive protein and transferrin saturation.

RESULTS

The mean age of the subjects was 46.6 years, and 41 percent were male (table 1). Slightly less than half were current smokers, and approximately one quarter were exposed to secondhand smoke. The mean number of servings of fruits and vegetables as estimated by the food frequency questionnaire was only slightly lower than the California average (3.7 servings vs. 3.8 servings (39)). The percentage of energy obtained from fat was somewhat higher than the US national average (38.3 percent vs. 34 percent (40)).

TABLE 2. Bivariate relations of demographic and behavioral factors with plasma malondialdehyde and F_2-isoprostane levels in healthy adults aged 19–78 years (n = 298), Berkeley and Oakland, California, 1998–1999

	Malondialdehyde (μmol/liter)				F_2-isoprostanes (ng/ml)			
	No.	Mean	SD*	p value†	No.	Mean	SD	p value†
Sex								
Female	177	0.97	0.57		142	0.056	0.027	
Male	121	0.57	0.43	<0.0001	93	0.042	0.018	<0.0001
Race								
White	177	0.76	0.52		131	0.055	0.026	
Black	70	1.05	0.59		64	0.041	0.022	
Other	48	0.67	0.53	<0.0001	37	0.050	0.024	0.001
Age group (years)								
19–42	106	0.76	0.52		89	0.050	0.023	
43–53	96	0.90	0.52		77	0.048	0.023	
54–78	96	0.78	0.62	0.78	69	0.054	0.029	0.34
Body mass index‡								
Normal weight (<25)	99	0.81	0.58		78	0.042	0.014	
Overweight (25–29)	102	0.75	0.50		80	0.049	0.026	
Obese I (30–34)	57	0.82	0.60		50	0.052	0.021	
Obese II (≥35)	34	0.99	0.49	0.17	20	0.075	0.038	<0.0001
Current smoking status								
Nonsmoker	92	0.45	0.42		38	0.045	0.017	
Passive smoker	69	0.88	0.53		66	0.055	0.028	
Smoker of <30 cigarettes/day	110	0.96	0.51		105	0.051	0.025	
Smoker of ≥30 cigarettes/day	27	1.21	0.61	<0.0001	26	0.040	0.021	0.04
Alcohol consumption								
<1 drink/week	113	0.83	0.58		91	0.051	0.030	
1–6 drinks/week	157	0.81	0.56		124	0.050	0.021	
1–2 drinks/day	28	0.72	0.41	0.40	20	0.049	0.020	0.62

* SD, standard deviation.

† For two-level variables, the p value represents the results of a t test. For three-level or four-level ordered variables, the p value represents the results of a trend test, performed by including the ordinal variable in a linear regression analysis.

‡ Weight (kg)/height $(m)^2$.

MDA and Iso-P were only weakly correlated with each other (r = 0.13, p = 0.05) (data not shown). In addition, the two biomarkers had different relations with cotinine, a biomarker of nicotine exposure. Cotinine was positively correlated with MDA (r = 0.31, $p < 0.0001$) but inversely associated with Iso-P (r = –0.16, p = 0.02) (data not shown).

Bivariate relations between the two lipid peroxidation markers and demographic characteristics are shown in table 2. Both plasma MDA and plasma Iso-P were strongly associated with sex, with women having significantly higher levels of both markers than men ($p < 0.0001$). Body mass index was significantly and strongly associated with Iso-P but was not associated with MDA. Smoking status was significantly associated with MDA but not with Iso-P. African Americans had significantly higher MDA levels but significantly lower Iso-P levels. Age and alcohol intake were not associated with either biomarker, either overall (table 2) or after stratification by sex (data not shown). Among dietary factors (table 3), only fruit intake was significantly associated with lipid peroxidation status (inversely). Neither total fat intake nor fat subtype (data not shown) was associated with lipid peroxidation.

C-reactive protein had a positive relation with both biomarkers of lipid peroxidation, and transferrin saturation had an inverse relation (table 4). Plasma ascorbic acid and several plasma carotenoids were significantly and inversely related to both biomarkers. In these bivariate analyses, plasma α-tocopherol was not associated with either MDA or Iso-P, while plasma γ-tocopherol had a significant and positive association with both peroxidation markers. Plasma cholesterol was significantly positively associated with MDA but not with Iso-P.

In multiple regression analyses (table 5 for MDA and table 6 for Iso-P), sex, age, race, smoking, and body mass index

TABLE 3. Bivariate relations of dietary factors with plasma malondialdehyde and F_2-isoprostane levels in healthy adults aged 19–78 years (n = 298), Berkeley and Oakland, California, 1998–1999

Dietary factor and quartile	Malondialdehyde (μmol/liter)				F_2-isoprostanes (ng/ml)			
	No.	Mean	SD*	p value†	No.	Mean	SD	p value†
Fruits								
Q1* (low)	72	1.04	0.64		64	0.055	0.025	
Q2	74	0.82	0.49		47	0.052	0.030	
Q3	83	0.77	0.54		62	0.049	0.026	
Q4 (high)	69	0.61	0.45	<0.0001	62	0.045	0.018	0.015
Vegetables								
Q1 (low)	74	0.89	0.59		60	0.051	0.025	
Q2	75	0.81	0.55		61	0.052	0.024	
Q3	72	0.79	0.55		54	0.045	0.025	
Q4 (high)	76	0.74	0.51	0.10	59	0.053	0.025	0.96
Dairy foods								
Q1 (low)	77	0.93	0.64		51	0.053	0.030	
Q2	71	0.77	0.52		60	0.046	0.020	
Q3	75	0.77	0.53		66	0.048	0.022	
Q4 (high)	75	0.76	0.50	0.07	58	0.055	0.027	0.51
Grains								
Q1 (low)	74	0.84	0.60		59	0.052	0.027	
Q2	76	0.84	0.52		58	0.051	0.021	
Q3	75	0.79	0.54		58	0.046	0.021	
Q4 (high)	73	0.77	0.56	0.34	60	0.052	0.030	0.80
Meats								
Q1 (low)	80	0.77	0.59		61	0.052	0.024	
Q2	71	0.73	0.52		56	0.051	0.024	
Q3	74	0.87	0.52		58	0.046	0.021	
Q4 (high)	73	0.87	0.58	0.16	60	0.053	0.030	0.97
Total fats								
Q1 (low)	74	0.77	0.60		58	0.052	0.028	
Q2	75	0.83	0.52		59	0.049	0.020	
Q3	75	0.83	0.57		59	0.047	0.021	
Q4 (high)	74	0.81	0.53	0.68	59	0.053	0.029	0.99

* SD, standard deviation; Q, quartile.

† For two-level variables, the p value represents the results of a t test. For three-level or four-level ordered variables, the p value represents the results of a trend test, performed by including the ordinal variable in a linear regression analysis.

were included in both models for comparability. As in the bivariate analyses, sex was by far the strongest predictor of both MDA and Iso-P. Women had substantially higher plasma levels of both biomarkers, even after adjustment for body mass index and the other variables. The only other factor strongly associated with both biomarkers was plasma ascorbic acid (inversely). With ascorbic acid included in the model, no other plasma antioxidants remained significantly associated with MDA, and only β-carotene was retained in the Iso-P model.

Factors significantly associated with MDA but not with Iso-P in the multivariate models included plasma cholesterol and C-reactive protein, both of which were positively associated with MDA. Factors significantly associated with Iso-P but not with MDA included body mass index and race. Omitting plasma cholesterol from the MDA model did not cause the body mass index variable to approach significance (data not shown). Race again had significant but inconsistent associations, African Americans having higher MDA levels but lower Iso-P levels. γ-Tocopherol was moderately significant in the MDA model, inversely after adjustment for other covariates, in contrast to the finding in the bivariate analyses. Age was not associated with either biomarker of lipid peroxidation; neither was alcohol consumption, dietary energy intake, polyunsaturated fat intake, transferrin saturation, or other carotenoids.

Current smoking was strongly positively associated with MDA (table 5), with nonsmokers having half the MDA levels of smokers even after data were controlled for sex, plasma cholesterol, and the other factors shown. Iso-P was not consistently associated with smoking, although results were statistically significant (table 6); indeed, persons in the highest smoking category appeared to have lower Iso-P levels.

DISCUSSION

In this study, we examined a number of demographic, physical, plasma, and dietary factors for their contribution to lipid peroxidation, as measured by the biomarkers plasma MDA and Iso-P, in 298 healthy adults. Our results should be useful to researchers in both the design and the analysis of observational or intervention studies that explore the relation between lipid peroxidation and disease.

Sex. The significantly *higher* lipid peroxidation among women in this study was unexpected, and we could uncover no explanatory factors. We hypothesize that the higher percentage of body fat in women may be a factor, but controlling the data for body mass index did not reduce the sex effect, and we were unable to measure body fat more accurately. This finding is of particular interest in view of the fact that women have been found to be at greater risk of lung cancer than men exposed to similar levels of cigarette smoke (41). Our results are consistent with those of Coudray et al. (42), who found higher levels of lipid peroxidation in women than in men. They also found a significant positive correlation of lipid peroxidation with plasma cholesterol and body mass index, as we did. They conducted no multivariate analyses. Mooney et al. (43) found that autoantibodies to 5-hydroxymethyl–2′-deoxyuridine, oxidized DNA, were 50 percent higher in women than in men, after adjustment for number of cigarettes smoked per day. Loft et al. (44) did not find higher levels of body-weight-adjusted urinary 8-hydroxydeoxyguanosine in women than in men, nor did Tagesson et al. (45) find higher 8-hydroxydeoxyguanosine levels in female Swedish glass workers.

Smoking. To our knowledge, this study is the only study on oxidative damage markers in smokers and passive smokers in which smoking status was categorized not just on the basis of self-report but also on the basis of plasma cotinine level, and in which current use of vitamin supplements was strictly controlled. The two biomarkers of lipid peroxidation had different associations with smoking status—plasma Iso-P showing no positive relation with smoking, in contrast to MDA. Several studies have found elevated Iso-P levels in persons with a variety of disease conditions (46, 47), and a few have reported elevated *urinary excretion* of Iso-P in smokers (16, 48). However, few studies have examined *plasma* Iso-P in both smokers and nonsmokers (17). Our results differ from those of Morrow et al. (17), who found elevated plasma Iso-P levels in smokers. Since the Iso-P analyses were conducted by the same researcher using the same assay techniques, it is unlikely that methodological differences explain the lack of agreement between the findings of Morrow et al. and the present report.

One major difference between this study and that of Morrow et al. (17) was the intensity of smoking in the two study groups. Only 20 percent of smokers in our study smoked 30 or more cigarettes per day, whereas the *mean* number of cigarettes smoked per day in the study by Morrow et al. was 37 (1.85 packs per day). In addition, the heavy smokers in the Morrow et al. study may have smoked cigarettes immediately before their blood was drawn, whereas our study subjects were not permitted to smoke during the 1-hour period immediately prior to each blood drawing. Thus, the explanation for the differing results may be that heavy smoking, such as smoking of two packs per day, is associated with elevated plasma Iso-P levels but more moderate smoking is not; that smoking immediately prior to having blood drawn may provoke a short-term increase in plasma Iso-P levels that resolves within an hour; or that Iso-P have too short a half-life in plasma to be detected after an overnight fast but would have been detectable in urine.

Finally, it is possible that the heavy smokers in the study by Morrow et al. were also heavy alcohol consumers and that the observed smoking association with Iso-P was actually an association with heavy alcohol intake. This would be consistent with our observation of no association or even an inverse association between Iso-P and cotinine. Although we found no relation with alcohol for either biomarker, our sample was restricted to persons who consumed two or fewer alcoholic drinks per day; 39 percent consumed no alcohol, the median alcohol intake was one drink per week, and only 1 percent consumed two alcoholic drinks per day.

We found MDA to be a strong marker of smoke-induced lipid peroxidation, as have other investigators (45), and our earlier work found MDA to be a useful marker of current smoking, provided that there was control for or stratification on plasma ascorbic acid level (49). Some studies did not find elevated MDA levels in smokers (50, 51). Researchers in the latter study (51) used a method (thiobarbituric acid reactive substances (TBARS)) that may be nonspecific and may provide erroneously high estimates for plasma MDA.

Ascorbic acid. Plasma ascorbic acid level was the only factor that had a significant inverse association with both MDA and Iso-P in our multivariate analyses (table 5 and 6). Numerous other investigators have reported that plasma ascorbic acid is inversely associated with smoking (51–54), but there has been little cross-sectional population research on ascorbic acid and oxidative damage (55).

C-reactive protein. C-reactive protein, a marker of inflammation, was associated with significant elevations in both biomarkers of lipid peroxidation in bivariate analyses and with MDA in the multivariate model. Cigarette smoke stimulates the recruitment and activation of phagocytes, which in turn contribute to the free-radical burden in smokers (56). C-reactive protein has been shown to be a powerful predictor of cardiovascular disease risk (57, 58). We believe that ours is the only study to have simultaneously examined both smoking status and the associated inflammatory/immune response to smoking in relation to markers of oxidative damage.

Alcohol. We excluded from this study persons who reported regularly consuming more than two alcoholic drinks per day in order to remove high alcohol consumption

TABLE 4. Bivariate relations of plasma factors with plasma malondialdehyde and F_2-isoprostane levels in healthy adults aged 19–78 years (n = 298), Berkeley and Oakland, California, 1998–1999

Plasma factor and quartile	Malondialdehyde (μmol/liter)				F_2-isoprostanes (ng/ml)			
	No.	Mean	SD*	p value†	No.	Mean	SD	p value†
Transferrin saturation								
Q1* (low)	74	0.94	0.51		60	0.056	0.029	
Q2	71	0.80	0.54		58	0.051	0.022	
Q3	77	0.79	0.56		58	0.047	0.023	
Q4 (high)	75	0.70	0.58	0.008	58	0.045	0.024	0.012
C-reactive protein								
Q1 (low)	72	0.66	0.50		57	0.043	0.017	
Q2	78	0.72	0.52		58	0.047	0.023	
Q3	74	0.81	0.53		62	0.055	0.028	
Q4 (high)	74	1.05	0.59	<0.0001	58	0.053	0.028	0.018
Plasma cholesterol								
Q1 (low)	72	0.61	0.44		58	0.047	0.021	
Q2	77	0.83	0.56		59	0.050	0.024	
Q3	75	0.84	0.53		59	0.046	0.022	
Q4 (high)	74	0.95	0.62	<0.0001	59	0.055	0.030	0.38
Plasma ascorbic acid								
Q1 (low)	74	1.14	0.57		58	0.058	0.030	
Q2	75	0.86	0.53		59	0.055	0.025	
Q3	75	0.64	0.48		59	0.045	0.022	
Q4 (high)	74	0.60	0.48	<0.0001	59	0.041	0.018	0.0004
Plasma α-carotene								
Q1 (low)	78	0.98	0.56		62	0.056	0.028	
Q2	69	0.83	0.54		53	0.049	0.029	
Q3	77	0.79	0.57		60	0.051	0.021	
Q4 (high)	74	0.63	0.48	0.0004	60	0.043	0.019	0.013
Plasma β-carotene								
Q1 (low)	74	0.90	0.56		58	0.056	0.029	
Q2	75	0.87	0.47		59	0.053	0.022	
Q3	74	0.85	0.58		59	0.046	0.022	
Q4 (high)	75	0.61	0.56	0.005	59	0.044	0.023	0.0006

Table continues

as a source of lipid peroxidation, and we observed no peroxidative effects linked to alcohol consumption within the low range of intake permitted. One study found that consumption of two alcoholic drinks per day produced a nonsignificant rise in urinary Iso-P levels (59), while substantially higher doses were required to produce a statistically significant elevation in urinary F_2-isoprostane levels. Alcohol intake has been found to be higher among smokers (50, 51, 60). It appears that after adjustment for smoking and other factors, alcohol intake (in this low range) may not have long-term effects on lipid peroxidation. Nevertheless, acute effects due to alcohol cannot be ruled out.

Age. Subjects in this study ranged in age from 19 years to 78 years, and no association between lipid peroxidation and age was noted. Coudray et al. (42) also found no association with age in persons aged 59–71 years, although the mean lipid peroxidation level in that older cohort was higher than the mean in a younger cohort previously studied by the same group. Age was significantly associated with MDA in subjects undergoing hip replacement (n = 66) (61). However, none of these studies controlled for the effects of cholesterol level, body mass index, or other factors. Our observation of the lack of an age effect is consistent with a hypothesis that while oxidative damage to DNA may accumulate with age (62–64), oxidative damage to *lipids* is related not to age but to behavioral and physiologic conditions concurrent with aging—such as increasing levels of body fat and cholesterol, smoking (when present), and inflammation (reflected in C-reactive protein) that may increase with arthritis and other conditions of aging. Even in relation to oxidative DNA

TABLE 4. Continued

Plasma factor and quartile	Malondialdehyde (μmol/liter)				F_2-isoprostanes (ng/ml)			
	No.	Mean	SD	*p* value	No.	Mean	SD	*p* value
Plasma β-cryptoxanthin								
Q1 (low)	72	0.92	0.57		58	0.060	0.032	
Q2	76	0.98	0.54		59	0.052	0.025	
Q3	77	0.67	0.54		59	0.047	0.020	
Q4 (high)	73	0.66	0.50	0.0003	59	0.041	0.016	<0.0001
Plasma lutein/zeaxanthin								
Q1 (low)	72	0.80	0.49		59	0.061	0.033	
Q2	78	0.94	0.58		57	0.047	0.016	
Q3	74	0.79	0.61		60	0.046	0.021	
Q4 (high)	74	0.69	0.50	0.10	59	0.045	0.022	0.0018
Plasma lycopene								
Q1 (low)	74	0.93	0.49		58	0.060	0.030	
Q2	74	0.73	0.55		59	0.048	0.026	
Q3	75	0.72	0.56		59	0.045	0.017	
Q4 (high)	75	0.86	0.59	0.61	59	0.046	0.022	0.0050
Plasma α-tocopherol								
Q1 (low)	72	0.81	0.49		61	0.053	0.025	
Q2	74	0.82	0.59		55	0.048	0.024	
Q3	80	0.88	0.60		61	0.045	0.018	
Q4 (high)	72	0.72	0.53	0.54	58	0.053	0.030	0.59
Plasma γ-tocopherol								
Q1 (low)	77	0.63	0.51		56	0.042	0.017	
Q2	69	0.82	0.59		60	0.045	0.019	
Q3	76	0.91	0.49		59	0.053	0.025	
Q4 (high)	76	0.87	0.60	0.002	60	0.059	0.032	<0.0001

* SD, standard deviation; Q, quartile.

† For two-level variables, the *p* value represents the results of a *t* test. For three-level or four-level ordered variables, the *p* value represents the results of a trend test, performed by including the ordinal variable in a linear regression analysis.

damage, it may be useful to examine all apparent age-related oxidative damage after controlling for other factors that are not inevitable concomitants of age, such as cholesterol level and inflammation, as well as ascorbic acid level. Given the importance of ascorbic acid in our data, it is possible that even DNA damage may not increase substantially among persons who have maintained a high ascorbic acid intake.

Race. Results for race in this study were inconsistent. We know of only one other study that has examined lipid peroxidation levels by race: Ito et al. (65) found higher TBARS (a marker of lipid peroxidation) among Japanese in the United States than among Caucasians in the United States. In our multivariate model (table 6), African Americans had significantly *lower* Iso-P levels ($p < 0.0001$) than either Whites or persons in the "Other" category. This was true for both men and women (data not shown). In contrast, race was not significant in the MDA model (table 5), and indeed African Americans had slightly *higher* MDA levels than Whites. Further exploration of this observation is needed.

A number of study features may be relevant to our current findings. We had a relatively large sample overall and in each of the three smoking categories (45.1 percent active smokers, 23.2 percent passive smokers, 31.7 percent not actively or passively exposed) and considerable diversity in terms of age, body mass index, and ethnic group. The extensive washout period among previous users of vitamin supplements made it possible to examine the relation between various demographic and biologic factors without the complicating factor of antioxidant supplementation.

A large study by Trevisan et al. (6) analyzed erythrocyte glutathione, plasma glutathione peroxidase, and plasma TBARS as indicators of oxidative status. The TBARS method measures levels of MDA and other reactive substances; it has been widely used but has been criticized as being unspecific. The findings of Trevisan et al. (6) agreed with our data with regard to age but not with regard to smoking. Two biomarkers used by Trevisan et al. also indicated higher levels of oxidative stress in women, although TBARS did not. These differences could be due to the nature of the study populations (ours was multiethnic, while Trevisan et al.'s contained only Whites) or to the variables measured or omitted in each study. Trevisan et al. measured glucose levels and found a highly

TABLE 5. Determinants of plasma malondialdehyde level (multivariable model*) in healthy adults aged 19–78 years, Berkeley and Oakland, California, 1998–1999

Factor	Adjusted† mean malondialdehyde level (μmol/liter)	*F* statistic‡	*p* value
Sex		69.38	<0.0001
Female	0.96		
Male	0.55		
Age	Direct§	0.35	0.5551
Race		2.64	0.0734
White	0.71		
African American	0.83		
Other	0.67		
Current smoking status		19.22	<0.0001
Nonsmoker	0.41		
Passive smoker	0.74		
Smoker of <30 cigarettes/day	0.86		
Smoker of ≥30 cigarettes/day	1.02		
Body mass index¶		0.53	0.6608
Normal weight (<25)	0.74		
Overweight (25–29)	0.69		
Obese I (30–34)	0.74		
Obese II (≥35)	0.77		
Plasma ascorbic acid	Inverse#	10.36	0.0014
Plasma γ-tocopherol	Inverse	4.43	0.0363
Plasma C-reactive protein	Direct	8.45	0.0039
Plasma cholesterol	Direct	15.76	<0.0001

* With no missing data, $n = 289$. Model $R^2 = 0.50$. No other variables contributed to the model at $p < 0.05$. Variables examined included body weight; dietary energy intake; intakes of polyunsaturated, saturated, and total fat; levels of α-tocopherol, α-carotene, β-carotene, β-cryptoxanthin, lutein/zeaxanthin, lycopene, and triglycerides; and transferrin saturation.

† Adjusted for the other variables shown.

‡ The *F* statistic measures the contribution of each variable to the prediction of plasma malondialdehyde level.

§ Positive association of the continuous variable with the outcome.

¶ Weight (kg)/height $(m)^2$.

Negative association of the continuous variable with the outcome.

TABLE 6. Determinants of plasma F_2-isoprostane level (multivariable model*) in healthy adults aged 19–78 years, Berkeley and Oakland, California, 1998–1999

Factor	Adjusted† mean F_2-isoprostane level (ng/ml)	*F* statistic‡	*p* value
Sex		35.66	<0.0001
Female	0.051		
Male	0.037		
Age	Direct§	1.44	0.2315
Race		18.65	<0.0001
White	0.050		
African American	0.035		
Other	0.047		
Current smoking status		3.65	0.0134
Nonsmoker	0.044		
Passive smoker	0.048		
Smoker of <30 cigarettes/day	0.047		
Smoker of ≥30 cigarettes/day	0.037		
Body mass index¶		11.70	<0.0001
Normal weight (<25)	0.036		
Overweight (25–29)	0.039		
Obese I (30–34)	0.046		
Obese II (≥35)	0.056		
Plasma ascorbic acid	Inverse#	15.07	<0.0001
Plasma β-carotene	Inverse	6.69	0.0103

* With no missing data, $n = 230$. Model $R^2 = 0.42$. No other variables contributed to the model at $p < 0.05$. Variables examined included body weight; dietary energy intake; intakes of polyunsaturated, saturated, and total fat; levels of plasma cholesterol, α-tocopherol, γ-tocopherol, α-carotene, β-cryptoxanthin, lutein/zeaxanthin, lycopene, and triglycerides; and transferrin saturation.

† Adjusted for the other variables shown.

‡ The *F* statistic measures the contribution of each variable to the prediction of F_2-isoprostane levels.

§ Positive association of the continuous variable with the outcome.

¶ Weight (kg)/height $(m)^2$.

Negative association of the continuous variable with the outcome.

significant relation with TBARS, while we did not measure glucose. Conversely, we measured plasma ascorbic acid and found highly significant associations. In addition, a substantial proportion of subjects in the Trevisan study were probably taking vitamin supplements, which would have raised their plasma ascorbic acid levels, whereas none of our subjects were taking vitamin supplements. Finally, it may be that the TBARS assay used by Trevisan et al. was less precise than the MDA assay we used.

It has been argued that plasma MDA may be too nonspecific to be a useful measure of oxidative stress status (66–68). In our data, the MDA assay was very sensitive to level of exposure to cigarette smoke, an exposure known to generate 10^{15} free radicals in every puff (69), and to C-reactive protein. Extra processing in our assay removed interference from alkanals and other endogenous compounds and eliminated baseline drift. We believe that if the predictive factors assessed in this study are controlled for in the design or analysis of other studies and the analytical refinement used here is applied, plasma MDA level is a sensitive marker of lipid peroxidation that is related to smoking status and its associated inflammation. The MDA assay is simpler to use than the Iso-P assay, and large-scale epidemiologic research on the role of oxidative stress is likely to go forward only

when measurement methods that are (relatively) inexpensive and easy to use are available.

Similarly, the superior precision of high-performance liquid chromatography for measuring ascorbic acid is often emphasized, to the detriment of less-expensive methods that can be applied more easily to studies requiring analysis of a large number of samples. However, research has shown good correlation between ascorbic acid estimated by the 2,4-dinitrophenylhydrazine method and by high-performance liquid chromatography (70–73). Our intervention study results (26) show that the 2,4-dinitrophenylhydrazine spectrophotometric assay of ascorbic acid is sensitive to between-group differences and within-group intervention-related changes, and our other research (74) has also shown its feasibility and sensitivity in very large-scale epidemiologic studies.

An interesting finding in this study was the strong and significant association of Iso-P with body mass index. Iso-P may be a sensitive marker of lipid peroxidation derived from oxidation of adipose tissue. The strong relation of both biomarkers to sex seems extremely important and justifies further research.

In summary, the two biomarkers examined here were consistent with each other, in bivariate analyses, in finding 1) significantly increased lipid peroxidation among women and among persons with higher levels of C-reactive protein; 2) significantly decreased lipid peroxidation with higher levels of fruit intake, plasma ascorbic acid, plasma carotenoids, and transferrin saturation; and 3) no association with age, alcohol, other dietary factors, or plasma α-tocopherol level. The biomarkers differed from one another in that only MDA was strongly associated with smoking and plasma cholesterol and only Iso-P was strongly associated with body mass index and plasma lycopene. Race was inconsistent. The differences between MDA and Iso-P in sensitivity to smoking status (MDA performing better than Iso-P) and to body mass index (Iso-P performing better than MDA) may reflect differences in sensitivity to specific oxidant pathways, but further research is needed to explain these differences.

Extensive data suggest that oxidative damage may play a major role in the causation of a number of human diseases. Large-scale epidemiologic studies that have examined oxidative damage are extremely limited in number, partly because of uncertainty as to the best marker to use. We report two markers of lipid peroxidation, plasma MDA and Iso-P, to be useful as markers of oxidative stress, and we suggest that both markers have potential value for future epidemiologic studies. Studies investigating causal mechanisms or the association between oxidative stress and disease should collect data on one or both of these markers, as well as data on plasma vitamin C, to expand our understanding of the role of these factors in disease processes.

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