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Correlates of Markers of Oxidative Status in the General Population

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Oxidative stress has been implicated in the etiology of many chronic diseases, including cardiovascular disease. However, limited information exists on the factors that may influence oxidative status in the general population. In a random sample of the population of two counties in western New York, levels of several markers of oxidative status (i.e., thiobarbituric acid-reactive substances, erythrocyte glutathione, and glutathione peroxidase) were determined. A total of 894 men and 903 women aged 35–79 years were included in the study (1996–1999). In addition, a number of sociodemographic and lifestyle characteristics and cardiovascular disease risk factors were measured. Age, markers of glucose metabolism (e.g., plasma glucose level) and insulin resistance (e.g., serum triglycerides, high density lipoprotein cholesterol, body mass index), and postmenopausal status in women were associated with increased oxidative stress and reduced antioxidant potentials. Oxidative status and antioxidant potentials appear to be significantly associated with a number of major cardiovascular disease risk factors; most of them are linked to abnormalities in glucose and insulin metabolism. *Am J Epidemiol* 2001;154:348–56.

atherosclerosis; body mass index; cardiovascular diseases; glucose; oxidative stress

The potential role of free radicals, reactive oxygen species, and antioxidants in the etiology of chronic diseases, including cardiovascular disease, lung disease, cancer, and others, has stimulated extensive research in recent years (1–3). Oxidative stress resulting from the increased production of free radicals and reactive oxygen species and/or a decrease in antioxidant defense leads to damage of biologic macromolecules and dysregulation of normal metabolism and physiology (1, 2). These processes have been extensively studied in the etiology of atherosclerosis and coronary vascular disease and have led to the idea that the oxidative modification of low density lipoprotein (LDL) and LDL lipid is central to the pathogenesis of this disease (4).

Despite strong biologic plausibility for this hypothesis, establishing a direct link between free radicals and specific disease has been difficult. A number of factors may be responsible for such difficulty. They include: the complex biology underlying these mechanisms; the lack of detailed and careful evaluation of the validity and reliability of laboratory methods used to measure free radicals and their effects; and the limited amount of available information on factors that influence markers of oxidative status in the general population. Most studies to date have concentrated on selected clinical samples of people with disease rather than the general population.

In the present study, we analyzed the relation between a series of cardiovascular disease risk factors and several markers of oxidative status (i.e., plasma thiobarbituric acid-reactive substances (TBARS), erythrocyte glutathione, and plasma glutathione peroxidase) in a group of men and women randomly selected from the general population. These markers of oxidative status were chosen because they have all been implicated in the potential pathways linking oxidation to pathologic processes. In particular, plasma TBARS were chosen because they have been hypothesized to represent a composite number of oxidative damage products, including malondialdehyde (5). Furthermore, LDL TBARS have been found to be longitudinally associated with the progression of carotid atherosclerosis (6). Erythrocyte glutathione was chosen because it has direct radical-scavenging ability and because levels of erythrocyte glutathione may reflect the glutathione activity in other tissues (7). It also represents a substrate for glutathione peroxidase (8). Finally, glutathione peroxidase was chosen because it appears to be sensitive to body selenium stores (9). A thorough review of the function of both glutathione and glutathione peroxidase can be found in a recent review by Brigelius-Flohe (8).

MATERIALS AND METHODS

The data used here pertained to randomly selected healthy White men ($n = 1,065$) and women ($n = 1,078$) aged 35–79 years from the general population of Erie and Niagara coun-

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Abbreviations: EDTA, ethylenediaminetetraacetic acid; HDL, high density lipoprotein; LDL, low density lipoprotein; TBARS, thiobarbituric acid-reactive substances.

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ties in western New York. In 1996–1998, these men and women were selected as healthy controls for a series of population-based case-control studies (unpublished data). Participants under age 65 years were selected from driver's license rolls; those aged 65 years or older were selected from the rolls of the Health Care Financing Administration. The participation rate was approximately 40 percent of those eligible. Our sample for analyses (894 men and 903 women) included only observations with nonmissing data on all variables included in the models.

Data on demographic factors, smoking, alcohol consumption, and other study variables were collected by trained interviewers through in-person interviews conducted primarily at our clinical research center; approximately 4.2 percent of participants were interviewed in their homes. During each interview, height and weight were measured according to a standardized protocol. Body mass index was calculated by dividing weight (in kilograms) by height (in meters) squared.

Systolic and diastolic blood pressures were measured three times in each participant with a standard mercury sphygmomanometer on the right arm, to the nearest 2-mm scale indicator. The mean of the three measurements was used in the analyses. A total of 40 ml of blood was drawn by a trained phlebotomist between 7:00 a.m. and 9:00 a.m., after at least 12 hours of fasting. Participants were asked to refrain from smoking and physical activity for at least 30 minutes prior to the blood drawing. In premenopausal women, blood drawing took place in the same phase of the menstrual cycle (luteal phase, days 20–24 of the cycle). The blood tubes used were immediately protected from light and kept at 4°C until processing, which occurred within 30 minutes of phlebotomy. Whole blood was added to meta-phosphoric acid/ethylenediaminetetraacetic acid (EDTA)/sodium chloride stabilizing solution for glutathione determination (10). The samples were then frozen at –80°C until batch determinations were made. Batch determinations were carried out within 30 days of the blood drawing.

Information on alcohol consumption was elicited with the use of a computer-assisted interview schedule. The questions focused on alcohol intake during the past 30 days, 12–24 months prior to the date of interview, and during the participant's lifetime. In this paper, we restricted our analyses to alcohol consumption during the past 30 days. Each respondent was asked which of four broad types of alcoholic beverages (beer, wine, wine coolers, and liquor) he or she had consumed during each of the periods mentioned above. The usual drink size for each type of beverage was defined using models and pictures. Respondents who drank alcohol less often than once per week were asked global questions about quantity and frequency. Persons who reported having consumed alcohol once per week or more often in the previous 30 days and drank more than one type of alcoholic beverage were asked about the frequency and number of drinks of each type they consumed on a usual Friday, Saturday, and Sunday and on weekdays. Additionally, they were asked how often they drank alcohol with meals, with snacks, and without food.

For the quantity measure of drinking, we used total ounces of ethanol consumed during the past 30 days. Total ounces of absolute ethanol were estimated by calculating ounces of beverage-specific alcohol intake (number of drinks × drink size × frequency of drinking on usual drinking occasions) and multiplying this by the following conversion factors: 0.045 for beer, 0.121 for wine, and 0.409 for liquor.

Current smoking status was ascertained by means of a questionnaire that elucidated detailed decade-specific information on smoking habits. For the purpose of this study, participants reporting current use of cigarettes were classified as current smokers; participants reporting not having smoked at least 100 cigarettes in their lifetime were classified as never smokers. Other participants were classified as former smokers.

Analytical methods

TBARS were measured by the method of Armstrong and Browne (5) and were expressed in nanomoles per milliliter of malondialdehyde equivalents.

Red blood cell glutathione level was determined according to the method of Browne and Armstrong (11). Automated analysis of plasma glutathione peroxidase was performed on a Cobas Mira automated chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, New Jersey) according to the procedure described by Pippenger et al. (12).

All cholesterol determinations (total and high density lipoprotein (HDL) cholesterol) were performed in EDTA plasma according to the method of Gidez et al. (13) before and after precipitation of LDL and very low density lipoprotein with 1.06 M manganese chloride and 40,000 USP units of sodium heparin. Serum triglyceride levels were determined using a commercial kit and calibrators purchased from Sigma Chemical Company (St. Louis, Missouri) for the Cobas Mira automated chemistry analyzer (14). Glucose determinations were performed with a Paramax automated chemistry analyzer (Dade International, Inc., Aguada, Puerto Rico) employing glucose oxidase methodology (15).

Quality control

To maintain assay precision and accuracy over long periods, we performed several quality control procedures. External standards were included in all assays on every run, and the range of concentrations in the calibration curves encompassed the range of expected sample values. Recovery experiments were performed on all assays by standard addition methodology (16) for assessment of the ability of each particular method to accurately quantify the analyte present in the sample. The most important aspect of quality control was the inclusion of control samples in every assay. Since quality control material was not commercially available for many of the assays, "in-house" controls were generated. These specimens were used to estimate method performance characteristics and to assess variability. Intraassay reproducibility was calculated from 20 determinations in the same run. Long-term interassay reproducibil-

ity and control ranges were generated by running five samples per day over a period of 20 days. The mean value ± two standard deviations was then calculated as the acceptable range for all control materials, which were regularly evaluated by means of decision limit cusum analysis of Levy-Jennings control plots (17) and Westgard multirule charts (18). Controls were analyzed in duplicate with all patient determinations. The coefficients of variation of the mean values were as follows: intraassay coefficients—cholesterol, 2.7 percent; HDL cholesterol, 4.8 percent; triglycerides, 3.6 percent; glucose, 2.0 percent; TBARS, 7.6 percent; glutathione, 3.3 percent; and glutathione peroxidase, 4.4 percent; interassay coefficients—cholesterol, 5.1 percent; HDL cholesterol, 8.7 percent; triglycerides, 7.0 percent; glucose, 3.1 percent; TBARS, 9.2 percent; glutathione, 4.0 percent; and glutathione peroxidase, 8.6 percent.

Total and HDL cholesterol, triglyceride, and TBARS control specimens were established from a pool of EDTA plasma collected from "normal" fasting volunteers. Approximately 20 ml of plasma from 15 donors was pooled and then filtered through Whatman type 1 filter paper (Whatman International Ltd., Maidstone, England). Two hundred 1.25-ml aliquots were made in 1.5-ml cryovials (Corning Costar Corporation, Cambridge, Massachusetts) and were frozen at –76°C. The remaining plasma pool was diluted 1:2 with isotonic phosphate-buffered saline containing 5 percent bovine serum albumin and was then portioned and stored as above. These quality control samples showed no change in measured parameters for at least 1 year.

Erythrocyte glutathione control specimens were prepared from pooled whole blood that had been processed with meta-phosphoric acid. The meta-phosphoric acid extract was aliquoted into 1.0-ml cryovials and frozen at –76°C. These extracts were stable for at least 1 year.

Glutathione peroxidase control specimens were prepared from pooled heparinized plasma aliquoted into 1.0-ml cryovials and were stored at –76°C. These quality control samples showed no change in enzyme activity for at least 4 years.

Statistical methods

All statistical analyses were performed by sex and menopausal status using the statistical package SAS for Windows, version 6.12 (19). The characteristics of the study population are presented in terms of mean values and standard deviations. The correlations between oxidation markers and selected variables were calculated as simple product moment (Pearson) correlation coefficients. Comparisons of mean oxidation markers by sex and menopausal status were performed by analysis of variance. Partial correlations were calculated using multivariate analysis of variance. Finally, the correlates of oxidation markers were estimated by multiple linear regression analyses. TBARS and glutathione proved to be nonnormally distributed. However, when appropriate transformation was implemented and analyses were performed, the results were similar to those obtained with nontransformed variables; therefore, we decided to display results from nontransformed variables because of the better clarity and interpretation of the study findings. The inclusion of quadratic terms did not improve the model estimates, which suggests that a linear model may best represent the observed association.

RESULTS

Table 1 gives the characteristics of the study participants. Men in this sample were, on average, significantly older than women and had lower total and HDL cholesterol levels and higher systolic and diastolic blood pressures and glucose levels than women. In addition, they reported using more alcohol than women and had a significantly lower prevalence of nonsmokers and past smokers in comparison with women. Regarding markers of oxidative status, glutathione level was lower in men than in women; no significant difference was detected between the two sexes for TBARS or glutathione peroxidase. When women were analyzed according to menopausal status, postmenopausal women had, on average (and as expected), significantly older age, higher total cholesterol and triglyceride levels, and a higher blood pressure and body mass index than premenopausal women. With regard to markers of oxidative status, postmenopausal women had higher TBARS and glutathione and lower glutathione peroxidase levels than premenopausal women; however, statistical significance was reached only for TBARS and glutathione peroxidase.

When the mean levels of markers of oxidative status were analyzed according to smoking status, no significant or consistent difference was detected between never smokers, current smokers, and former smokers among either men or pre- or postmenopausal women.

No consistent relation between alcohol drinking status and either TBARS and glutathione was detected (data not shown).

In table 2, we summarize the results of correlation analyses for the markers of oxidative status and the other variables considered. In both men and women, TBARS were inversely related to glutathione and positively related to glutathione peroxidase. Age was inversely related to glutathione peroxidase and positively related to glutathione. TBARS were more strongly related to age in females than in males. No association between current total alcohol consumption and any of the oxidative status variables was detectable. Body mass index was positively associated with TBARS and inversely associated with glutathione and glutathione peroxidase. Systolic blood pressure was inversely associated with glutathione peroxidase. Glutathione level in men was significantly associated with diastolic blood pressure but not systolic blood pressure. With regard to serum lipids, triglycerides appeared to be positively related to TBARS and inversely associated with glutathione in both sexes and to be inversely associated with glutathione peroxidase in women. Plasma glucose had a strong positive association with plasma level of TBARS and was negatively associated with glutathione in women. Among women, total cholesterol was positively associated with TBARS, positively related to glutathione peroxidase, and inversely related to glutathione. HDL cholesterol was inversely related to glutathione and positively associated with glutathione peroxidase in both men and women.

TABLE 1. Characteristics of a random sample of the population of two counties, by gender and menopausal status, western New York, 1996–1998

	Men (n = 894)		All women (n = 903)		Premenopausal women (n = 237)		Postmenopausal women (n = 666)	
	Mean or %	SD†	Mean or %	SD	Mean or %	SD	Mean or %	SD
Age (years)	58.6	11.0*	57.3	10.4	45.5	6.3***	61.6	8.0
Cholesterol (mg/dl)	230.7	50.3***	250.3	55.8	235.7	53.1***	255.4	55.7
HDL† cholesterol (mg/dl)	55.2	14.2***	66.3	16.7	67.2	17.1	66.0	16.5
Triglycerides (mg/dl)	145.8	81.7	147.0	84.7	116.4	67.9***	157.7	87.6
Glucose (mg/dl)	110.1	31.0***	103.2	33.5	100.7	35.2	104.1	32.9
Blood pressure (mmHg)								
Diastolic	74.1	10.0***	71.6	9.3	70.3	8.6**	72.0	9.5
Systolic	124.0	15.3***	119.4	16.9	111.9	14.1***	122.1	17.1
Body mass index‡	28.2	4.5	27.9	5.8	27.1	6.5*	28.2	5.6
Ounces of ethanol consumed in past 30 days	13.3	21.3***	5.6	10.8	6.4	12.3	5.3	10.2
Smoking (%)								
Nonsmoker	34.0		49.0		50.2		48.5	
Current smoker	14.0		14.9		14.3		15.0	
Past smoker	51.2		36.2		35.4		36.5	
TBARS† (nmol/ml)	1.4	0.4	1.3	0.5	1.3	0.4**	1.4	0.5
GSH† (mg/dl packed red blood cells)	49.5	15.5***	55.0	15.7	54.3	13.8	55.4	16.3
GSH-Px† (IU/liter)	648.6	93.4	641.9	92.5	678.3	81.3***	629.0	92.8

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$ (p value for comparison between men and women or between premenopausal women and postmenopausal women).

† SD, standard deviation; HDL, high density lipoprotein; TBARS, thiobarbituric acid-reactive substances; GSH, erythrocyte glutathione; GSH-Px, glutathione peroxidase.

‡ Weight (kg)/height (m)^2.

When these associations were analyzed separately in pre- and postmenopausal women (data not shown), it appeared that the interrelations among the various markers of oxidative status were stronger in postmenopausal women while their association with age, body mass index, and cardiovascular disease risk factors was stronger in premenopausal women.

Assay of TBARS measures malondialdehyde present in the sample as well as malondialdehyde that is generated from lipid hydroperoxides by the hydrolytic conditions of the reaction. Since the primary substrate for lipid peroxidation is polyunsaturated fatty acids, it has been suggested that the amount of substrate available in the sample (i.e., levels of

TABLE 2. Simple Pearson correlation coefficients for oxidation markers and covariates, western New York, 1996–1998

	Men (n = 894)			Women (n = 903)		
	TBARS†	GSH†	GSH-Px†	TBARS	GSH	GSH-Px
TBARS (nmol/ml)	1.000			1.000		
GSH (mg/dl packed red blood cells)	–0.066*	1.000		–0.104**	1.000	
GSH-Px (IU/liter)	0.102**	–0.219***	1.000	0.133***	–0.222***	1.000
Age (years)	0.015	0.197***	–0.298***	0.120***	0.093**	–0.253***
Cholesterol (mg/dl)	0.062	–0.108***	0.224***	0.149***	–0.075*	0.126***
HDL† cholesterol (mg/dl)	–0.020	–0.177***	0.373***	0.060	–0.136***	0.295***
Triglycerides (mg/dl)	0.234***	–0.132***	–0.031	0.157***	–0.072*	–0.075*
Glucose (mg/dl)	0.470***	–0.063	–0.038	0.454***	–0.136***	0.015
Blood pressure (mmHg)						
Diastolic	0.038	–0.137***	0.011	0.065*	–0.057	–0.030
Systolic	0.082**	–0.017	–0.114***	0.143***	–0.007	–0.116***
Body mass index‡	0.175***	–0.129***	–0.077*	0.088**	–0.050	–0.162*
Ounces of ethanol consumed in past 30 days	0.044	–0.019	0.031	0.016	–0.008	0.051

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

† TBARS, thiobarbituric acid-reactive substances; GSH, erythrocyte glutathione; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein.

‡ Weight (kg)/height (m)^2.

triglycerides) may be a major determinant of the amount of TBARS produced (20). Therefore, serum triglyceride levels may explain why TBARS were associated with a number of other cardiovascular disease risk factors and obesity. To avoid this potentially important confounder, we examined partial correlation coefficients while controlling for triglycerides. For TBARS, the partial correlation coefficients were of only slightly lower magnitude than the unadjusted coefficients; however, they maintained the level of statistical significance reached in the unadjusted analysis. In addition, as expected, the relation between the cardiovascular disease risk factors and the other markers of oxidative status was not influenced by adjustment for triglycerides (data not shown).

In table 3, we summarize the results of multivariate regression analyses using each of the markers of oxidation as the dependent variable in a regression model for the whole sample, after adjustment for sex. Glucose and HDL cholesterol were positively associated with TBARS and glutathione peroxidase and negatively associated with glutathione. In addition, age appeared to be negatively associated with glutathione peroxidase and positively associated with glutathione. Triglycerides were positive independent correlates of TBARS and were inversely associated with glutathione; total cholesterol was positively associated with TBARS and glutathione peroxidase. Body mass index was inversely related to glutathione and glutathione peroxidase. The percentage of variance (r^2) explained by the model was highest for TBARS ($r^2 = 0.24$).

Finally, in tables 4 and 5, we present results from the multivariate regression analyses using the markers of oxidation as dependent variables for women and men, respectively. Among women (table 4), glucose, total cholesterol, and HDL cholesterol were independent correlates of TBARS and glutathione peroxidase. In addition, triglycerides were an independent correlate of glutathione, and body mass index was an independent correlate of glutathione peroxidase. When age was replaced with menopausal status in the multivariate model, the results confirmed that menopausal status was a significant correlate of both TBARS ($\beta = 0.066$, standard error 0.031) and glutathione peroxidase ($\beta = -48.290$, standard error 6.780), with postmenopausal women exhibiting higher levels of TBARS and lower levels of glutathione peroxidase. Among men (table 5), plasma glucose and serum triglycerides were independent correlates of TBARS; age, triglycerides, and HDL cholesterol were correlates of glutathione; and age, cholesterol, and HDL cholesterol were correlates of glutathione peroxidase. The association between TBARS, glutathione, glutathione peroxidase, and glucose remained statistically significant even after elimination of individuals with plasma glucose levels ≥140 mg/dl or ≥200 mg/dl from the analysis; this suggests that the associations between markers of oxidative status and glucose observed in the sample as a whole were not driven by the presence of diabetic participants (data not shown).

DISCUSSION

The main findings from this study, which focused on a large population-based sample of adult men and women, are that there are significant independent associations between several cardiovascular disease risk factors and markers of oxidative status.

TABLE 3. Multiple linear regression coefficients for oxidation markers in men and women, western New York, 1996–1998

	TBARS†		GSH†		GSH-Px†	
	Parameter estimate	Scaled parameter‡	Parameter estimate	Scaled parameter‡	Parameter estimate	Scaled parameter‡
Intercept	0.374***		65.075***		678.12***	
Age (years)	–0.0003	–0.003	0.251***	2.695	–2.196***	–23.620
Glucose (mg/dl)	0.006***	0.201	–0.049***	–1.572	0.238***	7.644
Triglycerides (mg/dl)	0.001***	0.056	–0.022***	–1.849	–0.036	–3.063
Cholesterol (mg/dl)	0.0003	0.016	–0.003	0.156	0.197***	10.75
HDL† cholesterol (mg/dl)	0.003***	0.051	–0.205***	–3.357	1.501***	24.574
Body mass index§	–0.001	–0.008	–0.224**	–1.173	–1.031**	–5.400
Systolic blood pressure (mmHg)	0.0004	0.007	–0.032	–0.523	–0.138	–2.260
Ounces of ethanol consumed in past 30 days	0.0002	0.004	0.018	0.350	–0.039	–0.735
Current smoker¶	0.013	0.005	–0.357	–0.127	–5.992	–2.125
Past smoker¶	0.021	0.010	–1.142	–0.566	0.694	0.344
Gender¶	–0.067***	–0.003	6.476***	3.239	–24.74***	–12.375
Adjusted R^2	0.244***		0.106***		0.170***	
No. of observations	1,797		1,797		1,797	

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

† TBARS, thiobarbituric acid-reactive substances; GSH, erythrocyte glutathione; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein.

‡ These values represent the amount of change in the dependent variable associated with a one-standard-deviation change in the independent variable.

§ Weight (kg)/height (m)^2.

¶ Nonsmokers and males were the reference categories.

TABLE 4. Multiple linear regression coefficients for oxidation markers in women, western New York, 1996–1998

	TBARS†		GSH†		GSH-Px†	
	Parameter estimate	Scaled parameter‡	Parameter estimate	Scaled parameter‡	Parameter estimate	Scaled parameter‡
Intercept	0.309*		70.709***		655.19***	
Age (years)	–0.0004	–0.004	0.128	1.328	–1.123**	–11.679
Glucose (mg/dl)	0.006***	0.198	–0.070***	–2.282	0.341***	11.132
Triglycerides (mg/dl)	0.0004*	0.031	–0.014*	–1.163	–0.024	–2.070
Cholesterol (mg/dl)	0.001*	0.032	0.002	0.128	0.173***	9.770
HDL† cholesterol (mg/dl)	0.004***	0.063	–0.164***	–2.665	1.148**	18.656
Body mass index§	–0.004	–0.022	–0.164	–0.967	–1.397**	–8.240
Systolic blood pressure (mmHg)	0.0001	0.001	0.003	0.057	–0.233	–3.950
Ounces of ethanol consumed in past 30 days	–0.0003	–0.003	0.042	0.441	0.096	0.993
Current smoker¶	0.021	0.007	–1.694	–0.597	–8.680	–3.059
Past smoker¶	0.024	0.011	–1.206	–0.581	–8.285	–3.994
Menopausal status¶	0.071	0.031	0.420	0.185	–28.765***	–12.659
Adjusted R^2	0.230***		0.053***		0.144***	
No. of observations	903		903		903	

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

† TBARS, thiobarbituric acid-reactive substances; GSH, erythrocyte glutathione; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein.

‡ These values represent the amount of change in the dependent variable associated with a one-standard-deviation change in the independent variable.

§ Weight (kg)/height (m)^2.

¶ Nonsmokers and premenopausal women were the reference categories.

The most common approach to measurement of free radicals and oxidative stress has been to measure the products of free radical reactions with biologic macromolecules. Measurement of lipid peroxidation and polyunsaturated fatty acid oxidation has been most commonly employed, because of the sensitivity of 1,4-pentadienes to free radical attack and proton abstraction. The resulting lipid-centered free radicals rearrange and react with molecular oxygen to form lipid hydroperoxides and, following reductive reactions, hydroxy derivatives. Hydrolysis of lipid hydroperoxides leads to a

TABLE 5. Multiple linear regression coefficients for oxidation markers in men, western New York, 1996–1998

	TBARS†		GSH†		GSH-Px†	
	Parameter estimate	Scaled parameter‡	Parameter estimate	Scaled parameter‡	Parameter estimate	Scaled parameter‡
Intercept	0.378*		67.091***		643.81***	
Age (years)	–0.002	–0.025	0.331***	3.657	–2.329***	–25.748
Glucose (mg/dl)	0.007***	0.207	–0.031	–0.965	0.112	3.500
Triglycerides (mg/dl)	0.001***	0.073	–0.028***	–2.310	–0.046	–3.776
Cholesterol (mg/dl)	–0.00002	–0.001	0.007	0.349	0.222***	11.237
HDL† cholesterol (mg/dl)	0.002	0.025	–0.256***	–3.618	1.941***	27.425
Body mass index§	0.003	0.012	–0.307**	–1.367	–0.392	–1.750
Systolic blood pressure (mmHg)	0.001	0.014	–0.062	–0.951	–0.100	–1.530
Ounces of ethanol consumed in past 30 days	0.0004	0.011	0.017	0.405	–0.097	–2.345
Current smoker¶	0.005	0.002	1.028	0.367	–1.511	–0.540
Past smoker¶	0.023	0.011	–1.243	–0.622	9.452	4.728
Adjusted R^2	0.263***		0.116***		0.213***	
No. of observations	894		894		894	

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

† TBARS, thiobarbituric acid-reactive substances; GSH, erythrocyte glutathione; GSH-Px, glutathione peroxidase; HDL, high density lipoprotein.

‡ These values represent the amount of change in the dependent variable associated with a one-standard-deviation change in the independent variable.

§ Weight (kg)/height (m)^2.

¶ Nonsmokers were the reference category.

complex mixture of small acyl compounds, aldehydes, alcohols, and hydrocarbons (5). One of these compounds, malondialdehyde, reacts with 2-thiobarbituric acid to form a fluorescent adduct; this is the most commonly employed method for assessing lipid peroxidation. However, the 2-thiobarbituric acid reaction is not exclusively specific for malondialdehyde, as other related compounds have been shown to react—hence the name thiobarbituric acid-reactive substances (TBARS). Furthermore, malondialdehyde can arise from oxidative mechanisms other than lipid peroxidation. Therefore, the test is considered to be a general indicator of oxidative stress rather than a specific marker of lipid peroxidation (5).

Lipid peroxides are quickly metabolized to a less toxic hydroxy derivative by glutathione peroxidase, a selenium metalloenzyme which uses glutathione as a proton donor to reduce hydrogen peroxide to water and lipid hydroperoxides to corresponding lipid hydroxy derivatives with the production of glutathione disulfide (21). Glutathione disulfide is recycled by glutathione reductase using nicotinamide adenine dinucleotide phosphate from the hexose monophosphate shunt. Glutathione is also a nonspecific free radical scavenger able to donate protons to unpaired electrons, thereby "quenching" the free radical (8).

Both glutathione and glutathione peroxidase are present in many cellular systems, and they are intimately involved in the processes controlling oxidative stress. It is not clear whether erythrocyte glutathione and plasma glutathione peroxidase reflect the intracellular level of systems involved in atherosclerosis and its clinical manifestation. However, intracellular glutathione has been shown to reverse endothelial dysfunction and improve nitric oxide activity (22). In several studies, erythrocyte glutathione was found to be lower in coronary artery disease patients than in controls (23); macrophage glutathione was inversely related to cell-mediated oxidation of LDL cholesterol (21); and glutathione in vitro was able to reduce blood viscosity in blood samples from individuals with atherosclerosis (24).

Finally, cellular glutathione peroxidase has been found to be inversely related to cell-mediated oxidation of LDL cholesterol (21), absent or reduced in carotid atherosclerosis plaques as compared with normal mammary arteries (25), and reduced in patients with coronary artery disease (26), while plasma glutathione peroxidase has been shown to reduce phosphatidylcholine hydroperoxide in rats (27).

In our population sample, we found no evidence of a significant association between smoking and oxidative stress. This lack of association is puzzling, because cigarette smoking is a well-known environmental oxidant. The literature relating cigarette smoking to markers of oxidative stress has provided conflicting results, with the majority of studies finding no association (28–30). The lack of association may be due to the lack of specificity of TBARS as a marker of lipid peroxidation (20). However, even studies that have used more specific markers of lipid peroxidation (i.e., antibodies against oxidized LDL cholesterol) or isoprostanes have found inconsistent results (31–38). The majority of these studies focused on small and selected samples, however. It is not clear why there is a lack of association between markers of oxidation and smoking. Among the many potential reasons is the possibility that passive smoking and/or exposures to other environmental oxidants are important unmeasured confounders.

Another factor known to have important oxidative action for which we found no association with the markers considered is alcohol intake. The information relating alcohol intake to oxidation comes from studies focusing on heavy drinkers or alcoholics (39). Very little is known about the relation between alcohol and oxidation in the general population and the interaction between alcohol and dietary antioxidants. In addition, it has been hypothesized that different alcoholic beverages may have different oxidative actions—particularly that wine may contain important antioxidant compounds (40). This hypothesis has been supported by a number of in vitro studies (41, 42). It is also supported by preliminary analyses of data from a smaller sample of the present study in which we found that alcohol from beer and liquor but not alcohol from wine was significantly and positively associated with TBARS (43).

The positive association between age and glutathione is intriguing and unexpected, because on the basis of the available data we would expect a decline of glutathione with age (44). The negative association found with glutathione peroxidase may be interpreted as an indication that either the intrinsic activity or the absolute amount of this enzyme declines with age.

The most striking association found in our study was the positive association between TBARS and glucose and the inverse association between glutathione and glutathione peroxidase and glucose. These data indicate that disturbances in glucose and insulin metabolism may be linked to oxidative status. Support for these hypotheses has already been presented in a number of clinical studies focusing on diabetic patients (45–47). Our findings confirm that this association is strong in the population as a whole throughout the distribution of plasma glucose levels. The importance of insulin and glucose metabolism is further supported by the significant associations between markers of oxidative status and both body mass index and serum triglycerides—variables that have been associated with elevated levels of insulin and insulin resistance.

Finally, our findings indicate that in women, menopause is associated with a significant increase in oxidative stress; however, in both sexes, age is associated with higher levels of glutathione and lower levels of glutathione peroxidase. Previous studies in experimental animals and humans have suggested a potentially important antioxidant role for both endogenous and exogenous estrogens (48, 49). To our knowledge, ours is the first study to report that in a large sample of the general population, postmenopausal women appear to have a higher level of oxidative stress. These results confirm previous findings from Asada et al. (50), who compared 18 premenopausal women with 10 postmenopausal women. Important information needed to confirm the potential antioxidant action of estrogen is whether postmenopausal women using hormone replacement therapy have lower levels of oxidative stress than postmenopausal women who are not taking hormones. Unfortunately, at this time, we do not have these data. The

positive association of HDL cholesterol with both TBARS and glutathione peroxidase and the inverse association with glutathione in the adjusted analyses are at first somewhat puzzling, because of the well-known inverse association between HDL cholesterol and insulin resistance (51). However, recently it has been suggested that in addition to reverse cholesterol transport, HDL cholesterol may play an important role in extracting oxidized lipid from LDL cholesterol, particularly through enzymes present in its protein core (i.e., paraoxonase) (52, 53). Our data support this hypothesis in part but indicate that the link between HDL cholesterol metabolism and oxidation is a complex one.

Our study has several important strengths: 1) it focused on a large sample of men and women from the general population; 2) blood collection was performed according to highly standardized procedures designed to control for possibly important focuses of biologic and analytical validity; and 3) it used multiple markers of oxidative status. The major weakness of the present study is its cross-sectional nature and our subsequent inability to make conclusive statements about a cause-effect relation between markers of oxidative status and cardiovascular disease risk factors and markers of insulin resistance. While it is generally accepted that it is insulin resistance that generates oxidative stress, it has been suggested that oxidative stress may result in insulin resistance (54). TBARS have been criticized as being a nonspecific marker of lipid peroxidation; however, some experimental studies have shown good correlation between increases in TBARS and increases in levels of isoprostane (a potentially more specific marker of lipid peroxidation) in response to induced oxidative stress (55, 56). In addition, the strong significant association we found between TBARS and markers of insulin and glucose metabolism indicate that despite its lack of specificity for lipid peroxidation, this marker appears to have some value.

In summary, our study indicates that some of the proposed markers of oxidative status may be measured in large population-based studies and that there are important variables representing significant risk factors for cardiovascular disease that are associated with these markers. In particular, disturbances in glucose and insulin metabolism, and menopause for women, may be important determinants of oxidative stress in the body; and oxidation may be an important pathophysiologic link between abnormalities in glucose and insulin metabolism and the clinical manifestation of cardiovascular disease and atherosclerosis. However, the observed associations are not consistent across the different markers, and therefore they cannot be used interchangeably. We do not have conclusive evidence that the glutathione measured in erythrocytes and the glutathione peroxidase measured in plasma adequately reflect levels and activity in other tissues of the body, even though the available evidence seems to suggest that they may represent reasonable surrogates. In addition, the amount of variance in markers of oxidative status explained by the cardiovascular disease risk factors analyzed is limited. This could be a result of methodological limitations of the methods used to measure free radical metabolism and/or an indication that there are other important determinants of oxidative status in the population (i.e., diet, other environmental factors, genetic factors, etc.). Studies that analyzed reliable and valid techniques for measuring oxidative status in large unselected samples of the general population and that aimed to explore whether oxidative status independently predicts clinical events would provide important details on the putative link between oxidative status and cardiovascular disease.

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