Obesity and Systemic Oxidative Stress

Clinical Correlates of Oxidative Stress in The Framingham Study

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Objective—To determine the clinical conditions associated with systemic oxidative stress in a community-based cohort. Information regarding cardiovascular risk factors associated with systemic oxidative stress has largely been derived from highly selected samples with advanced stages of vascular disease. Thus, it has been difficult to evaluate the relative contribution of each cardiovascular risk factor to systemic oxidative stress and to determine whether such risk factors act independently and are applicable to the general population.

Methods and Results—We examined 2828 subjects from the Framingham Heart Study and measured urinary creatinine–indexed levels of 8-epi-$PGF_{2\alpha}$ as a marker of systemic oxidative stress. Age- and sex-adjusted multivariable regression models were used to assess clinical correlates of oxidative stress. In age- and sex-adjusted models, increased urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels were positively associated with female sex, hypertension treatment, smoking, diabetes, blood glucose, body mass index, and a history of cardiovascular disease. In contrast, age and total cholesterol were negatively correlated with urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels. After adjustment for several covariates, decreasing age and total/HDL cholesterol ratio, sex, smoking, body mass index, blood glucose, and cardiovascular disease remained associated with urinary 8-epi-$PGF_{2\alpha}$ levels.

Conclusions—Smoking, diabetes, and body mass index were highly associated with systemic oxidative stress as determined by creatinine-indexed urinary 8-epi-$PGF_{2\alpha}$ levels. The effect of body mass index was minimally affected by blood glucose, and diabetes and may suggest an important role of oxidative stress in the deleterious impact of obesity on cardiovascular disease. **(*Arterioscler Thromb Vasc Biol*. 2003;23:434-439.)**

Key Words: oxidative stress ■ isoprostanes ■ diabetes ■ obesity ■ cardiovascular disease

Oxidative damage has been implicated in the pathogenesis of many chronic progressive diseases, such as cancer, inflammation, and neurodegenerative disorders. Over the last decade, there has been considerable interest in the role of oxidative stress in vascular disease as well. This interest has been driven by a wealth of data indicating that LDL[1] oxidation is a prominent feature of atherosclerosis (for review, see references[1]). More recently, studies have also suggested that oxidative stress is a feature of many risk factors for premature atherosclerosis, such as diabetes,[2] hypertension,[3] and smoking.[4]

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By and large, clinical studies linking oxidative stress to atherosclerosis and its risk factors have involved small, highly selected groups of patients in the advanced stages of disease. This selectivity is the result, in part, of the limited consensus for reliable markers of oxidative stress. Over the last decade, however, there has been considerable progress in characterizing markers of lipid peroxidation that are derived from arachidonic acid.[5] This class of compounds, known as isoprostanes, have now been established as reliable markers of oxidative stress in a number of experimental and clinical settings (for review, see references[6]) As with many markers of oxidative damage, early studies with isoprostanes were limited in scope by the technical demands of sample processing for gas chromatography-mass spectrometry. However, a reliable ELISA for specific isoprostanes has recently been developed.[7]

The availability of such methodology now makes it feasible to address a number of important questions. For example, the relative magnitude with which individual atherosclerosis risk factors contribute to markers of oxidative stress is unknown. In addition, the extent to which hypertension, diabetes, and smoking contribute independently to systemic oxidative stress is also unknown. Finally, the relation be-

Received November 22, 2002; revision accepted January 16, 2003.

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Arterioscler Thromb Vasc Biol. **is available at http://www.atvbaha.org** **DOI: 10.1161/01.ATV.0000058402.34138.11**

tween oxidative stress and atherosclerosis risk factors has not been tested in relatively healthy populations. The purpose of this study, therefore, was to examine the clinical correlates of systemic oxidative stress in a large community-based cohort.

Materials and Methods

Study Sample

The design and selection criteria of the Framingham Study have been extensively described elsewhere.[8] The current study sample was derived from the Offspring cohort that began in 1971 with recruitment of 5124 men and women who were offspring or spouses of the offspring of the original Framingham cohort. The 3537 participants who attended the seventh offspring examination cycle (1998 to 2001) were eligible for the present investigation. All attendees underwent routine medical history and physical examination, laboratory assessment of cardiovascular risk factors, and obesity measurements, including height, weight, waist, and hip circumference. Clinical definition for diabetes included fasting glucose greater than or equal to 126 mg/dL, oral hypoglycemic use, or insulin use. Information about cardiovascular disease (CVD; eg, coronary heart disease, cerebrovascular disease, intermittent claudication, congestive heart failure) events was obtained with the aid of medical histories, physical examinations at the Heart Study (every 4 years), hospitalization records, and by communication with personal physicians. All suspected cardiovascular events were reviewed by a panel of three experienced investigators who evaluated all pertinent medical records. Criteria for these end points have been described previously.[9] Subjects were excluded from this study for the following reasons: attended examination before routine collection of urine (n=378); unavailable urine (n=329); other (n=2). After exclusions, a total of 2828 subjects remained eligible for the current investigation.

Determination of 8-Epi-PGF$_{2\alpha}$

As an index of systemic oxidative stress, we used the urinary content of 8-epi-PGF$_{2\alpha}$, one of many stable products of arachidonic acid formed on nonenzymatic oxidation.[6] Urine samples were used because they contain very little arachidonic acid, thereby limiting artifactual oxidation and isoprostane production after sample collection. Urine samples (3 mL) were collected and temporarily stored at −4°C for up to 4 hours and then maintained at −20°C (for creatinine) or −80°C (for isoprostanes) until analysis. Preliminary experiments demonstrated that collection of samples in this manner had no material impact on the content of 8-epi-PGF$_{2\alpha}$. For analysis, samples were thawed at room temperature, vortexed vigorously, and the content of 8-epi-PGF$_{2\alpha}$ determined using a commercially available ELISA (Cayman, Ann Arbor, MI). Samples analyzed in this manner correlate well with those analyzed using electrospray-negative ionization gas chromatography-mass spectroscopy (GC/MS).[7] Samples were analyzed in duplicate with an average intra-assay coefficient of variation of 9.7%. Any samples with a CV greater than two standard deviations from this mean (21%) were repeated. Urinary creatinine was run on an Abbott Spectrum CCX using a method based on the reaction of creatinine and alkaline picrate according to the manufacturer's instructions with average intra- and interassay coefficients of variation of 2% and 4%, respectively. A sample was repeated using the following criteria: 1) if the mean creatinine level was below 50 mg/100 mL and the difference between the two repeats was greater than 4.0 mg/100 mL 2) or if the mean creatinine level was above or equal to 50 mg/100 mL and the percent difference between the two repeats was greater than 6.5%. Urinary content of 8-epi-PGF$_{2\alpha}$ was indexed to creatinine and expressed as ng/mmol creatinine.

Statistical Analysis

The distribution of urinary creatinine–indexed 8-epi-PGF$_{2\alpha}$ levels was highly skewed; therefore, further analyses were performed with natural logarithmic transformation of the data. The urinary isoprostanes were measured over 7 months using two different lots from Cayman Chemicals. Using internal controls that were run on every plate, a difference between lots was detected ($P<0.001$); subsequently, all analyses were adjusted for the effect of the lot. Ninety-four phantom samples were analyzed along with the rest of the 8-epi-PGF$_{2\alpha}$ samples. Kappa analysis was performed on quartiles of these phantoms versus their known counterparts. The weighted Kappa coefficient was 0.80 showing good reproducibility.

Age- and sex-adjusted linear regression models were constructed to assess association of each of the following possible clinical correlates individually: total cholesterol, systolic blood pressure, diastolic blood pressure, body mass index (BMI), fasting glucose level, history of diabetes, smoking, hypertension treatment, and history of myocardial infarction or CVD. Sex interactions were also tested for each variable listed above. Stepwise multivariable models were performed with log creatinine–indexed 8-epi-PGF$_{2\alpha}$ against the above variables plus total cholesterol to HDL cholesterol ratio. Correlations among siblings were also studied in age, sex, and multivariable models. Analyses were run using the statistical package SAS version 8.1, (PROC REG and PROC MIXED).[10] Any two-sided probability value less than 0.05 was considered to be statistically significant.

TABLE 1. Baseline Characteristics of the Study Sample

	Men N=1286	Women N=1542
Age, y (range)	61±10 (37–88)	61±9 (33–87)
Total cholesterol, mg/dL	193±35	207±36
Total/HDL cholesterol	4.5±1.4	3.6±1.2
Systolic blood pressure, mm Hg	129±18	126±20
Diastolic blood pressure, mm Hg	76±10	73±10
Fasting glucose, mg/dL	109±30	101±25
BMI, kg/m^2	28.8±4.6	27.6±5.7
Diabetes	17%	11%
Smoking	13%	14%
History of cardiovascular disease	18%	9%
History of myocardial infarction	8%	2%
Hypertension treatment	37%	30%
Taking lipid lowering medication	24%	17%
Urinary 8-epi-PGF$_{2\alpha}$, pg/mL	1644±1393	1429±1414
Urinary creatinine, mg/dL	145±76	102±69
8-epi-PGF$_{2\alpha}$/creatinine, ng/mmol (range)	145±101 (15–1845)	173±119 (10–1601)
Log 8-epi-PGF$_{2\alpha}$/creatinine, ng/mmol (range)	4.8±0.6 (2.7–7.5)	5.0±0.6 (2.3–7.4)

All numbers are mean±SD or percent. Log denotes natural logarithm.

Results

Subject Characteristics and Individual Predictors of Urinary 8-Epi-PGF$_{2\alpha}$ Levels

The characteristics of subjects in this study are displayed in Table 1. The mean age of the participants was 61 years, 55% of whom were women. As expected, a greater proportion of men had prevalent CVD than did women. On average, women exhibited urinary creatinine–indexed 8-epi-PGF$_{2\alpha}$ levels that were 16% higher than men ($P<0.001$; Table 1). This finding was not simply a consequence of higher urinary creatinine levels in men because women had higher urinary 8-epi-PGF$_{2\alpha}$ levels at each decile of urinary creatinine (data not shown).

TABLE 2. Age- and Sex-Adjusted Correlates of Log Urinary 8-epi-$PGF_{2\alpha}$ Indexed to Urinary Creatinine

	Regression Coefficients	*P* Value
Age, 10 y	−0.037	0.001
Sex, female vs male	0.157	<0.0001
Total cholesterol, 35 mg/dL	−0.024	0.03
Total/HDL cholesterol, 2 units	−0.008	0.63
Systolic blood pressure, 20 mm Hg	0.029	0.02
Diastolic blood pressure, 10 mm Hg	0.006	0.60
BMI, 5 kg/m^2	0.087	<0.0001
Glucose, 25 mg/dL	0.065	<0.0001
Diabetes, present	0.172	<0.0001
Smoking, present	0.490	<0.0001
Hypertension treatment, present	0.053	0.03
History of myocardial infarction, present	0.117	0.03
History of CVD, present	0.149	<0.0001

All regression coefficients represent the estimated change in log creatinine-indexed urinary 8-epi-$PGF_{2\alpha}$ (ng/mmol creatinine) per specified number of units (approximately 1 SD) of the variable. Each covariate was analyzed separately, adjusting for sex, age, and lot. Two-tailed *P* values were obtained for T=coefficient/(standard error of coefficient) using the t distribution.

TABLE 3. Multivariable Correlates of Log Urinary 8-epi-$PGF_{2\alpha}$ Indexed to Urinary Creatinine

Variable, unit	Regression Coefficient, (95% CI)	*P* Value
Age, 10 y	−0.023	<0.05
	(−0.045,−0.0004)	
Sex, female vs male	0.163	<0.0001
	(0.121,0.206)	
Smoking, present	0.503	<0.0001
	(0.443,0.563)	
BMI, 5 kg/m^2	0.094	<0.0001
	(0.074,0.114)	
Glucose, 25 mg/dL	0.042	<0.0001
	(0.022,0.062)	
Total cholesterol/HDL, 2 units	−0.077	<0.0001
	(−0.110,−0.044)	
History of CVD, present	0.101	0.002
	(0.037,0.164)	

All regression coefficients represent the estimated change in log creatinine-indexed urinary 8-epi-$PGF_{2\alpha}$ (ng/mmol creatinine) per specified number of units (approximately 1 SD) of the variable. All covariates were analyzed simultaneously, adjusting for lot, selected by a stepwise method with *P* value <0.10 to enter and stay in the model (see Statistical Analysis). Two-tailed *P* values were obtained for T=coefficient/(standard error of coefficient) using the t distribution; 95% confidence intervals [CI] were obtained using coefficient±1.96×(standard error of coefficient).

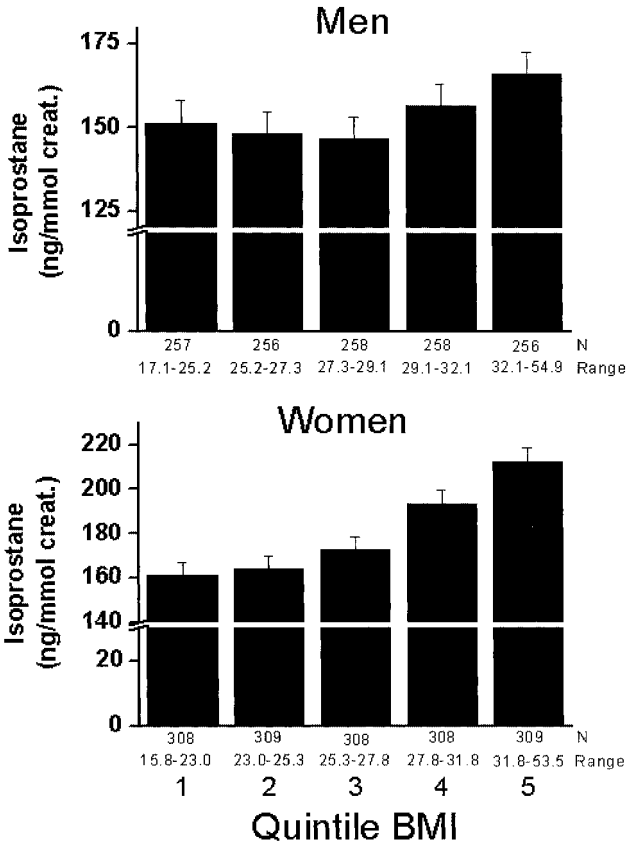
Both age and sex were important clinical correlates of urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels, with age demonstrating an inverse relation to this estimate of systemic oxidative stress. We performed age- and sex-adjusted regression using clinical and laboratory variables from Table 1 as correlates of urinary creatinine-indexed 8-epi-$PGF_{2\alpha}$, and these results are contained in Table 2. Data for men and women are combined because we found no significant interaction on the basis of sex. Both smoking and diabetes were strongly and positively associated with an increase in urinary 8-epi-$PGF_{2\alpha}$, which is consistent with previous reports.[2,4] We also observed a strong association between BMI and urinary 8-epi-$PGF_{2\alpha}$ levels (Table 2). Although animal studies suggest that hypertension[11] and hypercholesterolemia[12] may be associated with systemic oxidative stress, we found only weak relations between these two clinical conditions and urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels.

Multivariable Correlates of Creatinine-Indexed Urinary 8-Epi-$PGF_{2\alpha}$ Levels

The results of multivariable regression models using clinical and laboratory variables are contained in Table 3. Age was marginally significant in the multivariable model. The strongest positive predictor of urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ was smoking, with smokers demonstrating a 65% higher mean level than nonsmokers (raw mean levels of 240±145, and 148±100 ng/mmol creatinine in smokers and nonsmokers, respectively). Fasting glucose was also associated with our estimate of systemic oxidative stress. Each 25 mg/dL increase in fasting glucose was associated with a 4.3% increase in urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels. Diabetes and serum glucose were correlated; when diabetes was substituted for serum glucose in the multivariable model, individuals with a diagnosis of diabetes demonstrated urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels that were 10.8% higher than nondiabetics, with raw means being 181±128 ng/mmol creatinine versus 157±108 ng/mmol creatinine. In the multivariable model, total cholesterol to HDL cholesterol ratio also emerged as a negative correlate of 8-epi-$PGF_{2\alpha}$ levels ($P<0.0001$).

In addition to smoking and diabetes, our data support a relation between obesity and creatinine-indexed urinary levels of 8-epi-$PGF_{2\alpha}$. With respect to BMI, we found that each 5 kg/m^2 was associated with a 9.9% increase in urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels. To ensure this relation was truly a reflection of obesity, we substituted waist to hip ratio for BMI in the multivariable model and found it was also significantly associated with creatinine-indexed urinary 8-epi-$PGF_{2\alpha}$ levels ($P<0.0001$). To characterize the relation between obesity and oxidative stress, we plotted creatinine-indexed 8-epi-$PGF_{2\alpha}$ as a function of BMI quintile in nonsmokers and these data are contained in the Figure. Overall, the multivariable model was able to explain approximately 15.8% of the variability in urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$. The most important contributors to the multivariable model were smoking, BMI, sex, glucose, total cholesterol to HDL cholesterol ratio, and prevalent CVD that contributed 7.7%, 2.4%, 2.2%, 0.5%, 0.6%, and 0.3%, respectively, to explaining variability of urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels.

We performed several secondary analyses to ensure that our findings were not confounded by the presence of illness or medication treatment. We found that excluding patients



Systemic oxidative stress related to BMI. Nontransformed urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels (isoprostane) are plotted against BMI quintile for nonsmokers: men (top panel) and women (lower panel). The range of BMI (kg/m^2) for each quartile as well as the number of observations are included. Data represent the mean±SEM of each quintile. The relation between 8-epi-$PGF_{2\alpha}$ levels and BMI was significant for both men and women by multivariable linear regression ($P<0.001$).

with CVD or on lipid-lowering medication had no material effect on the results contained in Table 3. If we excluded patients on hypertension treatment, systolic blood pressure gained a marginal positive association with urinary 8-epi-$PGF_{2\alpha}$ levels ($P=0.07$). With respect to heritability, we found a sibling correlation of 0.06, indicating modest heritability (roughly 12%). Taking into account siblings in the multivariable model had no material impact on the analyses listed in Table 3.

Discussion

Considerable evidence links CVD and its risk factors to increased oxidative stress, although such evidence is derived from selected samples of individuals with advanced cardiovascular risk factors or disease. Thus, the extent to which this evidence applies to healthier populations is unclear. The principal finding of this study is that systemic oxidative stress, estimated as creatinine-indexed urinary 8-epi-$PGF_{2\alpha}$, is related to smoking, diabetes, and obesity in a large community-based cohort. In contrast, adjusted analyses did not demonstrate any strong positive association between creatinine-indexed urinary 8-epi-$PGF_{2\alpha}$ levels and other variables, such as total cholesterol, blood pressure, or age. In fact, with respect to the latter, age appeared associated with a decrease in this marker of systemic oxidative stress.

Previous studies have established a link between smoking and oxidative stress. Cigarette smoke is replete with a number of oxidizing species capable of producing lipid peroxidation.[13] Consistent with this notion, smokers typically demonstrate reduced circulating levels of vitamin C, an important antioxidant in human plasma.[14] Morrow and colleagues[4] demonstrated that both plasma levels and urinary metabolites of F_2-isoprostanes (of which 8-epi-$PGF_{2\alpha}$ is a member) are increased in smokers compared with age- and sex-matched controls. In that study, plasma-free isoprostanes in smokers were ≈1.4-fold greater than in nonsmokers. In this study, we found a 65% increase in isoprostanes as a function of smoking, in good agreement with the published data.[4] This finding represents an important internal control for our study as the relation between smoking and isoprostanes as a marker of oxidative stress is well established.[4,15–17]

In addition to smoking, we found a significant positive association between creatinine-indexed urinary 8-epi-$PGF_{2\alpha}$ and both clinical diabetes and fasting glucose levels (Table 3). These data are in good agreement with previous smaller studies linking hyperglycemia with increased levels of 8-epi-$PGF_{2\alpha}$[2] and lipid hydroperoxides,[18] two markers of lipid peroxidation. Similarly, urinary levels of 8-epi-$PGF_{2\alpha}$ are increased in patients with both types I and II diabetes and decrease significantly with aggressive control of hyperglycemia.[19] Several mechanisms have been proposed to explain this link between hyperglycemia and lipid peroxidation. Glucose may combine directly with LDL phospholipids or apo B lysine groups to form advanced glycosylation end products that facilitate lipid peroxidation.[20] In addition, autoxidation of glucose and nonenzymatic glycation of proteins may generate superoxide,[21] a radical species implicated in vascular cell–mediated LDL oxidation.[22] Hyperglycemia also induces the enzymatic production of superoxide through activation of NAD(P)H oxidase in vascular cells.[23] Our data support this basic research and suggest the effect of glucose on oxidative stress is applicable to a relatively healthy community-based cohort.

Among the more novel findings of the current study was the positive association between indices of obesity, such as BMI and waist/hip ratio and urinary levels of 8-epi-$PGF_{2\alpha}$. These data agree with a smaller studies linking BMI with plasma isoprostanes[24,25] and other markers of oxidative stress such as reduced erythrocyte glutathione and glutathione peroxidase.[26] The notion that obesity is associated with a state of heightened oxidative stress is not without precedent. Obesity is associated with insulin resistance,[27] and emerging evidence links insulin resistance to oxidative stress.[28] For example, hydrogen peroxide impairs insulin signaling[29] and inhibits glucose transport,[30] two cardinal features of insulin resistance. Moreover, tumor necrosis factor-α, a cytokine known to induce insulin resistance, mediates its effect through hydrogen peroxide generation.[31] Insulin itself promotes hydrogen peroxide generation in fat cells,[32] prompting speculation that oxidative stress is a principal mechanism of insulin resistance with chronic hyperinsulinemia. Thus, the

data presented here are in good agreement with basic investigation concerning insulin and oxidative stress.

Total cholesterol was weakly inversely associated with urinary 8-epi-$PGF_{2\alpha}$ levels in both age- and sex-adjusted and multivariable models. This finding may be puzzling to some because previous studies have linked hypercholesterolemia[33–35] to excess oxidative stress. In this regard, such studies typically have involved small numbers of patients (<38) often with extreme hypercholesterolemia typical of FH patients.[33–36] In contrast, our study involved many more subjects and total cholesterol levels more typical for a Western population (≈200 mg/dL).[37] With respect to the former, we were able to construct multivariable models to control for confounding, whereas previous studies did not.[33–36] With respect to differences in cholesterol levels, it is possible the relation between cholesterol and oxidative stress described by other studies is not applicable to "average" cholesterol levels. There is one study by Reilly and colleagues[36] linking moderate hypercholesterolemia with urinary excretion of both 8-epi-$PGF_{2\alpha}$ and class VI isoprostanes in 24 patients. That study sample differed significantly from ours in that the mean age was younger (41 years), smokers were excluded, and the average total cholesterol was higher (290 mg/dL). Moreover, in that study, the control group (serum cholesterol ≈170 mg/dL) did not demonstrate any relation between cholesterol levels and urinary isoprostanes.[36] Considering that cholesterol levels from our sample more closely resemble the control group from that study,[36] one might speculate that study sample differences explain these discrepant results.

In contrast to previous animal studies,[38] we did not find any meaningful positive association between a marker of oxidative stress and hypertension (Table 3). Systolic blood pressure was marginally and positively associated with urinary 8-epi-$PGF_{2\alpha}$ levels in age- and sex-adjusted models but not in the multivariable stepwise model. Animal data suggest the association of hypertension with oxidative stress may only apply to certain hypertensive states, such as those associated with high renin levels[11] or salt sensitivity.[39] Because the determinants of blood pressure in our sample are likely multifactorial, the proportion with oxidative stress–mediated hypertension may be too small to drive an association between hypertension and oxidative stress. Alternatively, because approximately one third of our subjects were receiving antihypertensive therapy, we may have lost the discriminatory value of blood pressure as a correlate of oxidative stress. This concern is mitigated somewhat by the secondary analysis in which we excluded patients on hypertensive treatment and still did not observe a strong association between blood pressure and urinary creatinine–indexed 8-epi-$PGF_{2\alpha}$ levels. Perhaps the development of more sophisticated means of classifying hypertension may help resolve this issue.

Another surprising feature of this study was the negative correlation between aging and oxidative stress estimated as creatinine-indexed 8-epi-$PGF_{2\alpha}$ levels. A number of experimental studies in humans and animals have linked aging to a state of heightened oxidative stress (reviewed in the references[40]), in apparent contradiction to the current results. We believe the current investigation still must be viewed favorably in comparison with such previous data as much of that data involved small samples predominantly with case-control designs,[40] whereas this cross-sectional investigation involved a large sample not selected on the basis of disease or other characteristics. Furthermore, we were able to construct multivariable models to control for confounding, whereas smaller studies typically involve too few subjects for such analysis. Nevertheless, aging is associated with changes in total body fat mass, circulating free fatty acids, and renal function, all of which could conceivably alter the value of creatinine-indexed urinary levels of 8-epi-$PGF_{2\alpha}$. Further investigation will be required to determine the precise relation between aging and this marker of lipid peroxidation.

Finally, we found that urinary levels of 8-epi-$PGF_{2\alpha}$ were significantly higher in women than in men. We expected this finding was caused by the lower levels of urinary creatinine in women compared with men (Table 1), however, even at similar levels of urinary creatinine, we found that 8-epi-$PGF_{2\alpha}$ levels in urine were higher in women. Our data agree with a smaller study of 298 healthy subjects aged 19 to 78 years by Block and colleagues[24] in which plasma levels of 8-epi-$PGF_{2\alpha}$ were also higher in women than in men. Thus, both our data and that of Block and colleagues appear to challenge contemporary dogma that men exhibit increased evidence of oxidative stress compared with women.[41]

There are several limitations of the present study that warrant consideration. First and foremost, we used a spot analysis of urinary 8-epi-$PGF_{2\alpha}$ as an index of oxidative stress rather than a 24-hour collection. This choice was necessitated by the constraints inherent in a large epidemiological investigation where there is a need to ensure compliance among participants. Nevertheless, it is possible that other markers of oxidative stress may provide additional information that was not detected in this study. Additionally, because the sample was derived from a single measurement in ambulatory volunteers, 8-epi-$PGF_{2\alpha}$ levels may have been confounded by medication status. Another limitation of this study was our use of an ELISA rather than GC/MS, the gold standard for isoprostane analysis. This compromise was driven by the need to process large numbers of samples in a timely manner. However, previous studies have demonstrated a significant correlation between the two methods ($r=0.63$).[7] It is also worth noting that a less precise ELISA (compared with GC/MS) would result in a random misclassification of levels and this would bias us toward the null hypothesis, that is, tend to weaken associations between variables and urinary 8-epi-$PGF_{2\alpha}$ levels. As such, the associations reported in this study would likely be stronger if an assay with a better signal-to-noise ratio were used. Another limitation of the present study is that our study sample was predominantly white, so our results may not be generalizable to other ethnicities. Finally, one must consider that 8-epi-$PGF_{2\alpha}$ may not be the best choice in future studies as a urinary marker of oxidative stress. For example, Li and colleagues[33] have shown that other isoprostanes are more abundant in urine than 8-epi-$PGF_{2\alpha}$ and that not all clinical conditions display the same pattern of urinary isoprostane excretion.

In summary, the results reported here indicate that smoking, diabetes, and obesity are strong independent predictors of systemic oxidative stress when the latter is measured as creatinine-indexed urinary levels of 8-epi-$PGF_{2\alpha}$. These data suggest that obesity is associated with a state of excess oxidative stress and suggest yet another contributing mechanism for excess CVD with obesity.

Acknowledgments

This study was supported by NIH/NHLBI contracts N01-HC-25195 and the NIH Boston University Cardiovascular Center, N01-HV-28178 and NIH grants HL64753 (E.J.B.), DK55656 (J.F.K.), HL60886 (J.A.V and J.F.K), and HL71039 (R.S.V.). Dr. Keaney is an Established Investigator of the American Heart Association, and Dr. Vasan is the recipient of a research career award (HL04334) from the NIH.

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