Journal of the American College of Cardiology
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Published by Elsevier Inc.

Vol. 47, No. 5, 2006
ISSN 0735-1097/06/$32.00
doi:10.1016/j.jacc.2005.09.063

The Relationship Between Plasma Levels of Oxidized and Reduced Thiols and Early Atherosclerosis in Healthy Adults

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OBJECTIVES The study investigated the relationship between biomarkers of oxidative stress and early atherosclerosis.

BACKGROUND Oxidative stress is an important etiologic factor in the pathogenesis of vascular disease. We hypothesized that oxidative stress would predict early atherosclerosis in a relatively healthy population.

METHODS One hundred fourteen healthy non-smokers, without known clinical atherosclerosis, had carotid intima-media thickness (IMT) measured using ultrasound. Oxidative stress was estimated by measuring plasma levels of: 1) glutathione (GSH), an important intracellular antioxidant thiol, its oxidized disulfide form (GSSG), and their redox state (E_h GSH/GSSG), and 2) cysteine (Cys), an important extracellular antioxidant thiol, its oxidized disulfide form cystine (CySS), and their redox state (E_hCys/CySS).

RESULTS The univariate predictors of IMT were age, body mass index, low-density lipoprotein cholesterol, triglycerides, high-density lipoprotein cholesterol, high-sensitivity C-reactive protein (hs-CRP), and Framingham risk score. Intima-media thickness was also higher in males and hypertensive subjects. Among the oxidative stress markers, GSH ($r = -0.39$, $p < 0.0001$), CySS ($r = 0.18$, $p = 0.049$), and E_h GSH/GSSG ($r = 0.34$, $p < 0.0002$) correlated with IMT. After adjusting for traditional risk factors and hs-CRP, only E_h GSH/GSSG remained an independent predictor of IMT. E_h GSH/GSSG predicted IMT in a manner that was both independent of and additive to Framingham risk score.

CONCLUSIONS Glutathione redox state (E_h GSH/GSSG), an in vivo measure of intracellular oxidative stress, is an independent predictor for the presence of early atherosclerosis in an otherwise healthy population. This finding supports a role for oxidative stress in the pathogenesis of premature atherosclerosis, and its measurement may help in the early identification of asymptomatic subjects at risk of atherosclerotic disease. (J Am Coll Cardiol 2006;47:1005–11) © 2006 by the American College of Cardiology Foundation

Oxidative stress is an imbalance between injurious oxidant and protective antioxidant events in which the former predominate (1,2). An overwhelming body of evidence indicates that oxidative events including modification of proteins and DNA, alteration in gene expression, promotion of inflammation, and deterioration in endothelial function in the vessel wall either trigger or exacerbate the atherosclerotic process (3–5). Although there is the strong etiologic link between increased vascular oxidative stress and subsequent development of atherosclerosis in experimental studies, clinical confirmation of this phenomenon has been impeded by the absence of reliable measures of oxidative stress in humans. Although some novel markers of oxidative stress have been identified recently (6,7), there remains an ongoing search for other markers, especially those that will be predictive of early stages of atherosclerosis.

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Manuscript received March 31, 2005; revised manuscript received September 13, 2005, accepted September 26, 2005.

Recent studies have demonstrated that quantification of the major intracellular and extracellular aminothiol compounds can be readily obtained from plasma, and provide a measure of in vivo oxidative stress (8,9). Intracellularly, glutathione (GSH) is a major antioxidant that helps eliminate peroxides and other oxidants (10). The oxidized disulfide form of GSH, GSSG, is formed during the reaction of glutathione peroxidase with hydrogen peroxide, or by a direct reaction of GSH with peroxynitrite and other oxidants. Glutathione released into plasma can be reliably measured, along with GSSG, as a marker of oxidative stress. Exposure to oxidative chemicals such as paraquat has been shown to be associated with decreased GSH levels and increased GSSG levels (11). The steady-state balance of GSH and GSSG can be expressed as the redox state (E_h) of the GSH/GSSG couple, calculated using the Nernst equation (12). Prior work suggests that the redox state of the plasma GSH/GSSG couple shows little variation, i.e., one SD of 9 mV (13), but there is evidence for progressive oxidation with aging after age 45 years (14–16). Increased oxidation of about 20 mV is associated with type 2 diabetes (16), high-dose chemotherapy (17), and cigarette smoking (18,19).

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March 7, 2006:1005–11

Abbreviations and Acronyms

BMI	= body mass index
CAD	= coronary artery disease
Cys	= cysteine
CySS	= cystine
E_h Cys/CySS	= cysteine redox state
E_h GSH/GSSG	= glutathione redox state
GPx-1	= glutathione peroxidase 1
GSH	= glutathione
GSSG	= oxidized form of glutathione
hs-CRP	= high-sensitivity C-reactive protein
IMT	= intima-media thickness

Glutathione is synthesized from cysteine (Cys), glycine, and glutamate, with Cys availability often being the rate-limiting factor (20). The predominant form of Cys in the plasma is the oxidized disulfide form, cystine (CySS), and the Cys/CySS redox state, E_h Cys/CySS, is calculated using the Nernst equation (13). Prior studies suggest that the Cys/CySS pool is an important antioxidant pool in human plasma (13), and can modulate cellular events including cell proliferation rate in culture medium (21,22) and resistance to apoptosis (23). The E_h Cys/CySS (about −80 mV) is considerably more oxidized than plasma E_h GSH/GSSG (about −140 mV) (13), and the latter is intermediate between tissue GSH/GSSG and plasma Cys/CySS redox. Recent experimental data suggest that intracellular proatherogenic events and cell adhesion are modulated in part by extracellular thiol/disulfide redox state (24).

We hypothesized that increased oxidative stress, estimated by measurement of the redox state of thiols, will predict early atherosclerosis, measured as carotid intima-media thickness (IMT) in healthy non-smoking subjects who are free from cardiovascular disease. The magnitude of common carotid IMT is a reliable surrogate measure of early sub-clinical atherosclerosis (25). It is also an important predictor of underlying carotid plaque, coronary atherosclerosis, and future cardiovascular events (26,27). Several studies have shown that even small changes in IMT during intervention are correlated with significant changes in the rates of future adverse cardiovascular events (28).

METHODS

One hundred twenty-five healthy non-smoking volunteers, aged 30 to 65 years, with no clinically manifest atherosclerosis were recruited. Subjects were excluded if they were known to have a history of diabetes, hypertension, or hyperlipidemia requiring treatment; had smoked in last three months; or were on any vasoactive medications, lipid-lowering agents, vitamins, or supplements. Pregnant female patients, those with acute or chronic illnesses, and those with elevation of high-sensitivity C-reactive protein (hs-CRP) (>10 mg/l, suggestive of acute illness [29]) were also excluded, leaving one hundred fourteen subjects for final analysis.

After routine physical examination, blood samples were drawn after an overnight fast and stored at −80°C. Plasma levels of total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, and hemoglobin A1c were measured. High-sensitivity C-reactive protein was measured by immunonephelometry (Dade Behring, Deerfield, Illinois). Institutional review board approval from Emory University and the Centers for Disease Control and Prevention was obtained before the study.

Measurement of thiol and disulfide forms of glutathione and cysteine and their redox state. The detailed procedures for the measurements of blood GSH and Cys have been previously described (13,14). Samples were collected directly into specially prepared tubes containing a preservative to reduce auto-oxidation, and centrifuged; the supernatant was frozen at −80°C. Samples were analyzed using high-performance liquid chromatography with fluorescence detection of damsel derivatives to quantitate GSH, GSSG, Cys, and CySS concentrations in plasma. On the basis of GSH and GSSG concentrations, E_h GSH/GSSG was calculated, and similarly, based on Cys and CySS concentrations, the E_h Cys/CySS was calculated using the Nernst equation. E_h GSH/GSSG and E_h Cys/CySS were expressed in mV, with higher (less negative) values being indicative of higher levels of oxidative stress.

Measurement of carotid IMT. Common carotid artery IMT was measured using ultrasonography and standard techniques (25,26). Longitudinal images of the distal 1.0 cm of both common carotid arteries, proximal to the carotid bulb were obtained using multiple scanning angles. The images were stored digitally, and measurements were made off-line using a semi-automated computerized analytical software (Carotid Tools, MIA Inc., Iowa City, Iowa), by two observers blinded to the test results. Average values of the IMT of each of the four segments of the distal 1.0 cm of both common carotid arteries (right near and far walls, and left near and far walls) were used as the IMT values for each subject. Inter-observer variability for carotid IMT was 0.03 ± 0.02 mm between measurements made in 20 subjects by 2 observers. Intra-observer variability was 0.02 ± 0.02 mm between 2 measurements made 1 week apart on 10 subjects.

Statistical considerations. Results are presented as mean ± SD for continuous variables and as proportions for categorical variables. Age, body mass index (BMI), hs-CRP, LDL, HDL, total cholesterol, and triglyceride levels were used as continuous variables. Hypertension, diabetes mellitus, smoking history, family history of coronary artery disease (CAD), and gender were taken as categorical variables. The presence of diabetes was defined as a fasting glucose level of >126 mg/dl or hemoglobin A1c of >7%. Hypertension was defined as elevated systolic (>140 mm Hg) or diastolic (>90 mm Hg) blood pressure on three separate measurements. On the basis of their IMT measurements, subjects were divided into four groups for analysis: those with IMT

<0.5 mm, >0.5 to 0.6 mm, >0.6 to 0.7 mm, and >0.7 mm. Linear trends were then assessed across the groups. Unadjusted linear regression and multivariate adjusted linear regression analyses were performed to ascertain whether any subject characteristics, presence of conventional risk factors, or markers of oxidative stress were independent determinants of IMT (S-Plus version 6.0.3, Insightful Corporation, Seattle, Washington). Separate multivariable models were run for each of the oxidative stress markers. Oxidative stress markers were divided into tertiles, and trends across the mean IMT were compared. The difference between the mean IMT for the various groups was assessed using the two-tailed Student *t* test. Only p values <0.05 were considered significant.

RESULTS

One hundred fourteen healthy subjects, 41% male, mean age 44 ± 8.5 years were included in the final analysis (Table 1). Although these subjects were themselves unaware, 11 (9.6%) were found to be hypertensive and 5 (4.3%) were diabetic. Serum cholesterol levels ranged between 130 and 303 mg/dl. The mean Framingham risk score for the study population was 0 (±6.3), and the mean common carotid IMT was 0.61 (±0.11) mm.

Plasma GSH, GSSG, Cys, and CySS levels and their redox (E_h) values were within the range observed previously in healthy subjects (12,13). There was a correlation between the redox values for the thiols, E_h GSH/GSSG and E_h Cys/CySS (r = 0.30, p = 0.001). As previously reported, there was a significant correlation between age and E_h GSH/GSSG (r = 0.23, p = 0.01), GSH (r = 0.30, p = 0.001), and CySS (r = 0.27, p = 0.004), but no relationship between age and any of the other markers. There were no gender differences in the levels of any of these novel markers. There was also no correlation between E_h GSH/GSSG and individual risk factors such as serum cholesterol level, blood pressure, and presence of diabetes, but there was a significant correlation with Framingham risk score (r = 0.25, p = 0.007).

IMT and traditional risk factors. Baseline clinical characteristics of the subjects, divided into four groups on the basis of their IMT (IMT ≤0.5 mm, 0.51 to 0.60 mm, 0.61 to 0.70 mm, and >0.7 mm), are shown in Table 1. Significant univariate correlations were evident between IMT and increasing age (r = 0.57, p < 0.0001), BMI (r = 0.35, p = 0.0002), LDL cholesterol (r = 0.19, p = 0.04), triglycerides (r = 0.28, p = 0.002), and lower HDL cholesterol (r = −0.23, p = 0.01). Intima-media thickness was also significantly higher in males (IMT 0.64 vs. 0.59 mm, p = 0.02), and hypertensives (IMT 0.70 vs. 0.60 mm, p = 0.02). There was also a trend towards higher IMT in subjects with a prior smoking history (IMT 0.66 vs. 0.60 mm, p = 0.07) or elevated total cholesterol (r = 0.18, p = 0.06). There was no difference in IMT in subjects with and without a family history of premature atherosclerosis. Interestingly, when the definition of family history was modified to include family members with coronary heart disease irrespective of age at onset, then a family history was associated with a higher IMT (IMT 0.63 vs. 0.60 mm, p = 0.05). The traditional risk factors accounted for 45% of the observed variability in carotid IMT (r^2 = 0.45, p < 0.001). The relationship between IMT and Framingham risk score was also assessed. There was a significant correlation between Framingham risk score and IMT (r = 0.43, p < 0.0001). After multivariate analysis that included all risk factors and hs-CRP in the analysis, age (p < 0.0001), gender (p = 0.01), and BMI (p = 0.03) remained independent predictors of IMT.

IMT and markers of oxidative stress. The relationships between markers of oxidative stress and IMT are shown in Figures 1 and 2 and in Table 2. Linear regression analyses demonstrated significant correlations between carotid IMT and GSH, E_hGSH/GSSG, and CySS, indicating that a higher level of oxidative stress was associated with increasing IMT (Fig. 1, Table 2). Similarly, increasing IMT also correlated with higher hs-CRP level (Fig. 1, Table 2). Figure 2 demonstrates the unadjusted relationship between

Table 1. Clinical Characteristics and Their Relationship to Carotid IMT

	IMT Groups (mm)					
	Mean n = 114	**<0.5 n = 18**	**0.51–0.6 n = 46**	**0.61–0.7 n = 39**	**>0.7 n = 21**	**p Value**
Age (yrs)	44 ± 8.5	39 ± 6.4	41 ± 7.0	45 ± 7.8	52 ± 8.9	0.008
Male gender (%)	41.2	33.3	32.6	44.8	61.9	0.03
LDL (mg/dl)	122 ± 36	120 ± 38	115 ± 33	124 ± 34	135 ± 43	0.11
HDL (mg/dl)	50 ± 14	51 ± 13	53 ± 14	47 ± 11	46 ± 17	0.16
Triglycerides (mg/dl)	109 ± 63	92 ± 35	92 ± 47	129 ± 86	132 ± 61	0.10
Cholesterol (mg/dl)	191 ± 33	188 ± 34	185 ± 34	196 ± 27	202 ± 39	0.07
BMI	27 ± 5.4	24 ± 4.0	25 ± 4.1	28 ± 4.4	30 ± 7.8	0.04
Hypertension (%)	13.2	0	6.5	10.3	42.9	0.05
Diabetes (%)	7.0	5.6	8.7	0	14.3	0.53
Prior smoking (%)	22.8	11.1	17.4	31.0	33.3	0.06
Family history of CAD (%)	13.2	11.1	13.0	20.7	4.8	0.64
Framingham risk score	0.0 ± 6.3	−2.6 ± 6.2	−1.9 ± 6.3	1.6 ± 5.7	4.4 ± 4.7	0.03

BMI = body mass index; CAD = coronary artery disease; HDL = high-density lipoprotein; IMT = intima-media thickness; LDL = low-density lipoprotein.

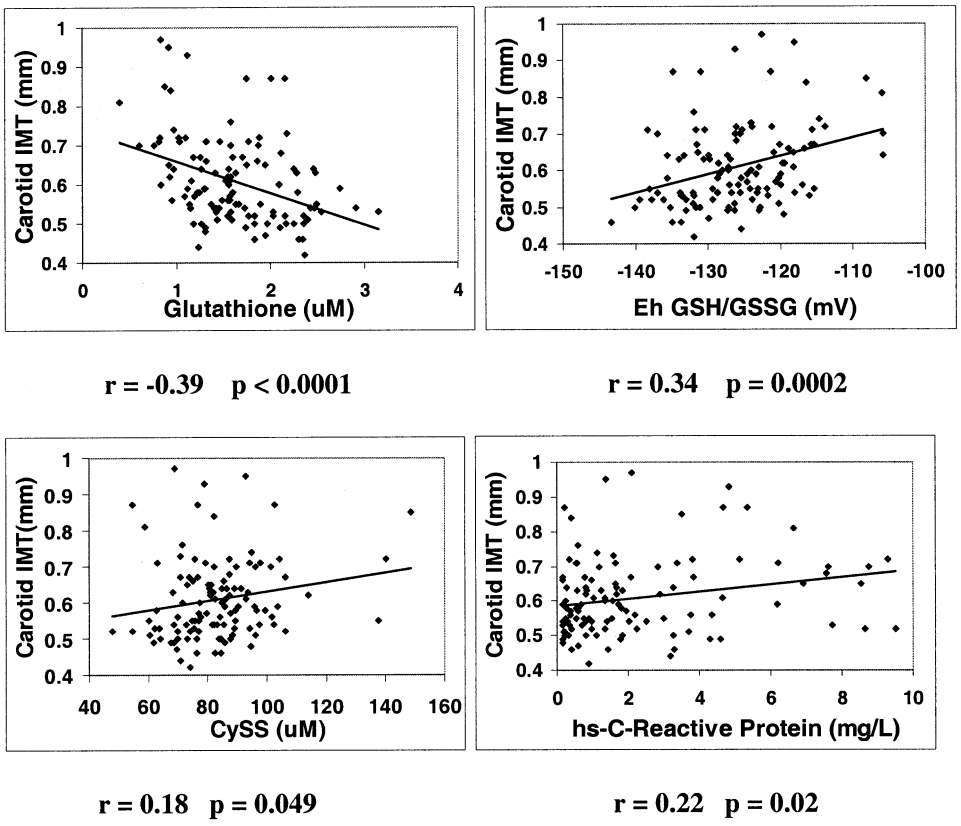

Figure 1. Pearson correlations between the markers of oxidative stress and high-sensitivity C-reactive protein (hs-CRP) and carotid intima-media thickness (IMT). **(Top row, right)** E_h GSH/GSSG; **left:** GSH; **(bottom row, left)** CySS; **right:** hs-CRP. CySS = cystine; E_h GSH/GSSG = glutathione redox state.

carotid IMT and E_h GSH/GSSG with subjects divided into equally sized tertiles.

Multivariate analysis was performed to investigate whether these novel markers contributed to carotid IMT, in addition to the predictive value of traditional risk factors. After multivariate analysis adjusting for demographics, individual risk factors, and hs-CRP, the oxidative stress marker E_h GSH/GSSG ($p = 0.02$) was an independent predictor of IMT. When multivariate analysis was repeated with the Framingham risk score, both E_h GSH/GSSG (partial correlation coefficient, 0.24; $p = 0.005$) and the Framingham risk score (partial correlation coefficient, 0.37; $p < 0.0001$) were independent predictors of carotid IMT. Thus, when subjects were divided into low and high oxidative stress groups based on the median value for E_h GSH/GSSG ($<$ or >-126.4 mV), and into low and high risk groups based on Framingham risk score (≤ 0 or >0), those with low oxidative stress and low Framingham risk score had the lowest IMT, whereas subjects with both high levels of oxidative stress and high Framingham risk score had the highest IMT. Subjects with either high oxidative stress or high IMT had intermediate IMT ($p = 0.01$ for trend and $p < 0.0001$ for high-risk vs. low-risk group) (Fig. 3).

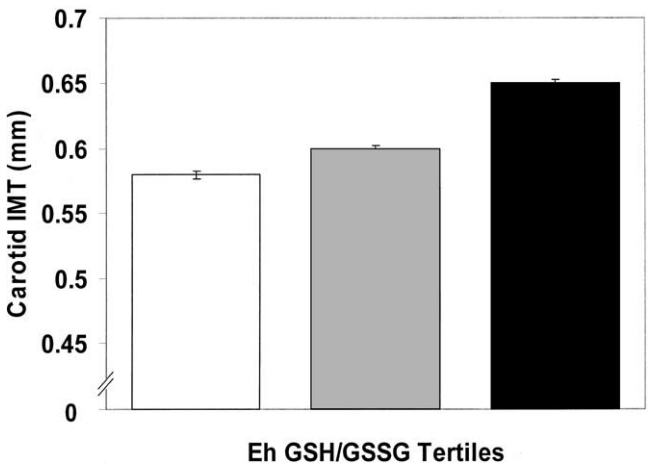

Figure 2. Relationship between E_h GSH/GSSG tertiles and carotid IMT: unadjusted relationship between carotid IMT (mean ± SEM) and E_h GSH/GSSG tertiles ($p = 0.005$ for trend, and $p = 0.004$ highest vs. lowest E_hGSH/GSSG tertiles, $n = 38$ in each group). Abbreviations as in Figure 1.

In order to investigate the predictive value of markers of oxidative stress in subjects free of traditional modifiable risk factors for atherosclerosis, we analyzed the subset of subjects who did not have hypertension, diabetes, or serum cholesterol level >250 mg/dl. When this subset of 96 healthy subjects free of risk factors for atherosclerosis was divided into two equal groups on the basis of the E_h GSH/GSSG levels, carotid IMT was significantly greater than in those with higher oxidative stress (0.62 vs. 0.57 mm, $p = 0.01$), indicating that this marker of oxidative stress predicts the

Table 2. Relationship Between Markers of Oxidative Stress and Inflammation Groups and Carotid IMT

	IMT Groups (mm)					
	Mean n = 114	<0.5 n = 18	0.51–0.6 n = 46	0.61–0.7 n = 39	>0.7 n = 21	p Value
GSH (μM)	1.6 ± 0.5	1.9 ± 0.4	1.7 ± 0.5	1.6 ± 0.5	1.3 ± 0.5	0.01
GSSG (μM)	0.11 ± 0.05	0.10 ± 0.03	0.11 ± 0.05	0.12 ± 0.06	0.08 ± 0.06	0.64
E_h GSH/GSSG (mV)	−126 ± 7.5	−131 ± 6.0	−127 ± 6.2	−124 ± 8.0	−123 ± 8.6	0.02
Cys (μM)	10 ± 2.6	10 ± 2.7	9 ± 2.2	11 ± 2.7	10 ± 3.0	0.93
CySS (μM)	83 ± 15.9	76 ± 9.1	82 ± 16.0	85 ± 10.2	87 ± 23.7	0.02
E_h Cys/CySS (mV)	−71 ± 7.9	−73 ± 7.6	−70 ± 8.0	−73 ± 7.1	−69 ± 8.7	0.48
hs-CRP (mg/l)	2.2 ± 2.4	1.6 ± 1.5	1.8 ± 2.2	2.6 ± 2.7	3.0 ± 2.6	0.03

Cys = cysteine; CySS = cystine; E_h Cys/CySS = cysteine redox state; E_h GSH/GSSG = glutathione redox state; GSH = glutathione; GSSG = oxidized form of glutathione; hs-CRP = high-sensitivity C-reactive protein; IMT = intima-media thickness.

presence of early sub-clinical atherosclerosis in otherwise healthy subjects.

DISCUSSION

Our study was designed to assess the relationship between the specific markers of oxidative stress and the presence of early sub-clinical atherosclerosis in humans as assessed by carotid IMT. In this study, traditional risk factors for atherosclerosis, including male gender, increasing age, hypertension, BMI, LDL cholesterol and triglycerides, and lower HDL cholesterol, as well as the Framingham risk score and higher hs-CRP, were significant univariate predictors of early atherosclerosis measured as carotid IMT. These data confirmed that our population of healthy volunteers had the expected associations with early atherosclerosis, and that the constellation of factors that constitute the cardiovascular metabolic syndrome appeared to be significant predictors of increased carotid IMT. Even though the traditional risk factors predicted carotid IMT, their combined presence accounted for 45% of the variability in IMT in these healthy volunteers. This supports data from several larger studies in diverse populations that demonstrate that not all cardiovascular disease severity is explained by traditional risk factors (29,30). Such observations have fueled the search for novel risk factors, genetic predisposition, and other mechanisms as potential contributors to atherosclerosis (31).

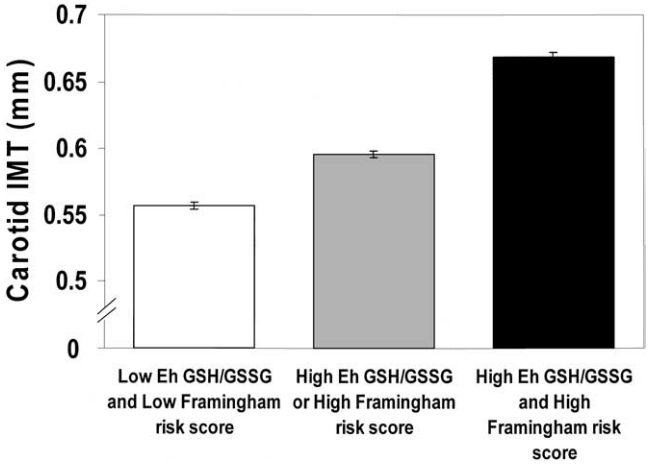

Figure 3. Combined predictive value of Framingham risk score and E_h GSH/GSSG: subjects with higher levels of both oxidative stress and Framingham risk score had higher IMT (mean ± SEM) compared to subjects with lower levels of both markers ($p < 0.001$ highest vs. lowest group and $p = 0.01$ for trend). Abbreviations as in Figure 1.

We reasoned that no matter which risk factors were causative, they would lead to atherosclerosis by promoting increased oxidative stress in the vasculature. This was confirmed by the observation that the Framingham risk score correlated with the GSH redox. Moreover, when several thiol markers of oxidative stress were evaluated, carotid IMT was independently predicted by the GSH redox that predominantly measures intracellular oxidative stress, but not by the cysteine redox that predominantly measures extracellular oxidative stress. E_h GSH/GSSG remained predictive of early atherosclerosis even after adjusting for the presence of traditional risk factors and inflammation measured as hs-CRP level. In addition, E_h GSH/GSSG appeared to predict IMT in a manner that was both independent of and additive to the predictive value of the Framingham risk score. Thus, for any given Framingham risk score, IMT was greater in those with increased oxidative stress and vice versa. When we specifically excluded subjects with modifiable risk factors, E_h GSH/GSSG remained a significant predictor of IMT. Importantly, these observations illustrate the potential etiologic role of oxidative stress in the pathogenesis of atherosclerosis in patients without traditional modifiable risk factors.

This is the first demonstration of a direct relationship between this measure of oxidative stress and atherosclerosis. A prior study has shown a relationship between GSH levels in adolescent males and parental atherosclerosis (32), and a correlation between vascular endothelial function and the markers GSH, GSSG, and GSH/GSSG ratio was reported in patients with chronic renal failure (33). Another recent study measured red cell levels of glutathione peroxidase 1 (GPx-1), a ubiquitous intracellular enzyme that uses GSH to reduce hydrogen peroxide, lipid peroxides, and peroxynitrite. A lower level of red-cell GPx-1 activity was associated with increased cardiovascular events (34). Similarly, the functional variants of the GPx-1 gene were associated with increased IMT and risk of macrovascular disease in a group of Japanese type 2 diabetic patients (35). These studies

together with our observations provide additional evidence supporting the biologic importance of GSH-based antioxidant systems in development of both early vascular disease and late cardiovascular complications. Other markers of oxidative stress have also been evaluated previously, but their predictive value for detection of early atherosclerosis has been variable. Tsimikas et al. (36) recently demonstrated a relationship between oxidized LDL and CAD, and a recent study demonstrated that patients with CAD had higher oxidized LDL compared with age-matched control subjects (7). However, the Atherosclerosis Risk In Community study (37) showed no relationship between IMT and either LDL susceptibility to oxidation, or auto antibodies against oxidized LDL. Although some studies have found urinary isoprostanes to be associated with risk factors for atherosclerosis and predictors of coronary disease (6,38), others have failed to confirm any relationship between isoprostanes and IMT (39), indicating that isoprostanes may not be good predictors of early atherosclerosis. Finally, Drueke et al. (40) reported a relationship between advanced protein oxidation products and IMT in patients with end-stage renal disease, and others have demonstrated an inverse relationship between IMT and the antioxidant lycopene (41) and antioxidant vitamin levels (37,42).

Unlike most of the other studies mentioned above, our study evaluated a relatively healthy low-risk population with a mean Framingham risk score of 0, in which 90% of the subjects were between 30 and 55 years of age and were current non-smokers, and the vast majority was free of traditional risk factors. Our data confirm the heterogeneity of the oxidative process in the bloodstream and tissues that can affect vascular health and function. It is likely that a panel of markers that includes these and others will be required for disease prediction. As these oxidant mechanisms affect a diverse number of intracellular and extracellular compartments, it has become clear in this study that specific pools of markers of oxidative stress are needed to capture the full picture. The process is further complicated because some potential markers of oxidative stress that can be measured in the blood may not reflect vascular oxidative stress, but rather represent oxidant injury in other organ systems (43–48).

The prediction of IMT by inflammatory biomarkers such as hs-CRP has been tested previously. Although the results vary by the population studied, it appears that after adjustment for traditional risk factors, there is often a weak or insignificant correlation between hs-CRP and carotid IMT (49–52). In our study, CRP was a univariate predictor of IMT but was not independently predictive of IMT after adjustment was made for all the traditional risk factors. Moreover, the addition of hs-CRP to the analysis did not affect the predictive value of novel oxidative stress marker E_h GSH/GSSG.

Study limitations. The study, by nature of its cross-sectional design, demonstrates a correlation between a marker of early atherosclerosis and oxidative stress, but does not establish causality. The observed correlation between the marker of oxidative stress and atherosclerosis may imply either that oxidative stress is a precipitating factor for development of atherosclerosis, or that atherosclerosis causes elevation of these markers. Moreover, we were unable to study the relationship between these markers of oxidative stress and temporal progression of atherosclerosis or occurrence of future cardiovascular events. These issues need to be further addressed in other populations, including those with more advanced disease. The study also cannot conclusively prove that oxidant stress measured by these markers reflects changes in the arterial wall exclusively.

Conclusions. Plasma glutathione redox (E_h GSH/GSSG), a marker of oxidative stress, may be helpful in identifying healthy individuals at risk for early atherosclerosis, independent of traditional risk factor assessment and presence of inflammation. Identifying at-risk individuals with elevated oxidative stress from a relatively healthy low-risk population may allow earlier targeting of specific therapy, with the ultimate aim of reducing future adverse outcomes. This study adds to the growing body of evidence supporting the relationship between oxidative stress and atherosclerosis in humans.

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REFERENCES

1. Sies H. Oxidative stress: introductory comments. In: Sies H, editor. Oxidative Stress. London: Academic Press, 1985;1–8.
2. McCord JM. The evolution of free radicals and oxidative stress. Am J Med 2000;108:652–9.
3. Chevion M, Berenshtein E, Stadtman ER. Human studies related to protein oxidation: protein carbonyl content as a marker of damage. Free Radic Res 2000;33 Suppl:S99–108.
4. Tahara S, Matsuo M, Kaneko T. Age-related changes in oxidative damage to lipids and DNA in rat skin. Mech Ageing Dev 2001;122:415–26.
5. Stocker R, Keaney JF. Role of oxidative modifications in atherosclerosis. Physiol Rev 2004;84:1381–478.
6. Keaney JF, Larson MG, Vasan RS, et al. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham study. Arterioscler Thromb Vasc Biol 2003;23:434–9.
7. Holvoet P, Harris TB, Tracy RP, et al. Association of high coronary heart disease risk status with circulating oxidized LDL in the well-functioning elderly: findings from the Health, Aging, and Body Composition study. Arterioscler Thromb Vasc Biol 2003;23:1444–8.
8. Jones DP. Redox potential of GSH/GSSG couple: assay and biological significance. Methods Enzymol 2002;348:93–112.
9. Norris RL, Eaglesham GK, Shaw GR, et al. A sensitive and specific assay for glutathione with potential application to glutathione disulphide, using high-performance liquid chromatography-tandem mass spectrometry. J Chromatogr B Biomed Sci Appl 2001;762:17–22.
10. Buettner GR. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch Biochem Biophys 1993;300:535–43.
11. Schafer FQ, Buettner GR. Redox environment of the cell as viewed through the redox state of the glutathione disulfide/glutathione couple. Free Radic Biol Med 2001;30:1191–212.
12. Jones DP, Carlson JL, Mody VC, Cai J, Lynn MJ, Sternberg P. Redox state of glutathione in human plasma. Free Radic Biol Med 2000;28:625–35.

13. Jones DP, Mody VC Jr., Carlson JL, Lynn MJ, Sternberg P Jr. Redox analysis of human plasma allows separation of pro-oxidant events of aging from decline in antioxidant defenses. Free Radic Biol Med 2002;33:1290–300.
14. Kretzschmar M, Mueller D. Aging, training and exercise. A review of effects on plasma glutathione and lipid peroxides. Sports Med 1993; 15:196–209.
15. Samiec PS, Drews-Botsch C, Flagg EW, et al. Glutathione in human plasma. Decline in association with aging, age-related macular degeneration and diabetes. Free Rad Biol Med 1998;24:699–704.
16. Jonas CR, Puckett AB, Kurtz JC, et al. High-dose chemotherapy decreases plasma glutathione (GSH) in patients undergoing bone marrow transplantion (BMT). FASEB J 1997;11:A649.
17. Wang NP, Lim PS, Chen TT, Thien LM, Wang TH, Hsu WM. Smoking is associated with alterations of blood thiol-group related antioxidants in patients on emodialysis. Nephron 2002;92:772–9.
18. Moriarty SE, Shah JH, Lynn M, et al. Oxidation of glutathione and cysteine in human plasma associated with smoking. Free Radic Biol Med 2003;35:1582–8.
19. Cotgreave IA, Gerdes RG. Recent trends in glutathione biochemistry-glutathione-protein protein interactions: a molecular link between oxidative stress and cell proliferation. Biochem Biophys Res Commun 1998;242:1–9.
20. Miller LT, Watson WH, Kirlin WG, Ziegler TR, Jones DP. Oxidation of the glutathione/glutathione disulfide redox state is induced by cysteine deficiency in human colon carcinoma HT29 cells. J Nutr 2002;132:2303–6.
21. Jonas CR, Ziegler TR, Gu LH, Jones DP. Extracellular thiol/disulfide redox state affects proliferation rate in a human colon carcinoma (Caco2) cell line. Free Radic Biol Med 2002;33:1499–506.
22. Jiang S, Moriarty SE, Miller NR, Jones DP, Sternberg P Jr. Regulations of human retinal pigment epithelial (hRPE) cell growth and cellular glutathione/glutathione disulfide (GSH/GSSG) redox status by changing extracellular cysteine/cystine (Cys/CySS) (abstr). Invest Ophthalmol Vis Sci 2001;42:B75.
23. Go YM, Jones DP. Intracellular proatherogenic events and cell adhesion modulated by extracellular thiol/disulfide redox state. Circulation 2005;111:2973–80.
24. Pignoli P, Tremoli E, Poli A, Oreste P, Paoletti R. Intimal plus medial thickness of arterial wall: a direct measurement with ultrasound imaging. Circulation 1986;74:1399–406.
25. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997;96: 1432–7.
26. Burke GL, Evans GW, Riley WA, et al. Arterial wall thickness is associated with prevalent cardiovascular disease in middle-aged adults. The Atherosclerosis Risk in Communities (ARIC) Study. Stroke 1995;26:386–91.
27. Lonn EM, Yusuf S, Dzavik V, et al. Effects of ramipril and vitamin E on atherosclerosis: the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E (SECURE). Circulation 2001;103:919.
28. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499–511.
29. Khot UN, Khot MB, Bajzer CT, et al. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA 2003;290:898–904.
30. Greenland P, Knoll MD, Stamler J, et al. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA 2003;290:891–7.
31. Ridker PM, Brown NJ, Vaughan DE, Harrison DG, Mehta JL. Established and emerging plasma biomarkers in the prediction of first atherothrombotic events. Circulation 2004;109 Suppl 1:IV6–19.
32. Morrison JA, Jacobsen DW, Sprecher DL, Robinson K, Khoury P, Daniels SR. Serum glutathione in adolescent males predicts parental coronary heart disease. Circulation 1999;100:2244–7.
33. Annuk M, Zilmer M, Lind L, Linde T, Fellstrom B. Oxidative stress and endothelial function in chronic renal failure. J Am Soc Nephrol 2001;12:2747–52.
34. Blankenberg S, Rupprecht HJ, Bickel C, et al., the AtheroGene Investigators. Glutathione peroxidase 1 activity and cardiovascular events in patients with coronary artery disease. N Engl J Med 2003;349:1605–13.
35. Hamanishi T, Furuta H, Kato H, et al. Functional variants in the glutathione peroxidase-1 (GPx-1) gene are associated with increased intima-media thickness of carotid arteries and risk of macrovascular diseases in Japanese type 2 diabetic patients. Diabetes 2004;53:2455–60.
36. Tsimikas S, Brilakis ES, Miller ER, et al. Oxidized phospholipids, Lp(a) lipoprotein, and coronary artery disease. N Engl J Med 2005; 353:46–57.
37. Iribarren C, Folsom AR, Jacobs DR Jr., Gross MD, Belcher JD, Eckfeldt JH. Association of serum vitamin levels, LDL susceptibility to oxidation, and autoantibodies against MDA-LDL with carotid atherosclerosis. A case-control study. The ARIC Study Investigators. Atherosclerosis Risk in Communities. Arterioscler Thromb Vasc Biol 1997;17:1171–7.
38. Schwedhelm E, Bartling A, Lenzen H, et al. Urinary 8-iso-prostaglandin F2alpha as a risk marker in patients with coronary heart disease: a matched case-control study. Circulation 2004;109:843–8.
39. Moussavi N, Renier G, Roussin A, Mamputu JC, Buithieu J, Serri O. Lack of concordance between plasma markers of cardiovascular risk and intima-media thickness in patients with type 2 diabetes. Diabetes Obes Metab 2004;6:69–77.
40. Drueke T, Witko-Sarsat V, Massy Z, et al. Iron therapy, advanced oxidation protein products, and carotid artery intima-media thickness in end-stage renal disease. Circulation 2002;106:2212–17.
41. Gianetti J, Pedrinelli R, Petrucci R, et al. Inverse association between carotid intima-media thickness and the antioxidant lycopene in atherosclerosis. Am Heart J 2002;143:467–74.
42. Dwyer JH, Paul-Labrador MJ, Fan J, Shircore AM, Merz CN, Dwyer KM. Progression of carotid intima-media thickness and plasma antioxidants: the Los Angeles Atherosclerosis Study. Arterioscler Thromb Vasc Biol 2004;24:313–9.
43. Droge W. Oxidative stress and aging. Adv Exp Med Biol 2003;543: 191–200.
44. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408:239–47.
45. Klein JA, Ackerman SL. Oxidative stress, cell cycle, and neurodegeneration. J Clin Invest 2003;111:785–93.
46. Rottkamp CA, Nunomura A, Raina AK, Sayre LM, Perry G, Smith MA. Oxidative stress, antioxidants, and Alzheimer disease. Alzheimer Dis Assoc Disord 2000;14:S62–6.
47. Trotti R, Carratelli M, Barbieri M, et al. Oxidative stress and a thrombophilic condition in alcoholics without severe liver disease. Haematologica 2001;86:85–91.
48. Cahill A, Cunningham CC, Adachi M, et al. Effects of alcohol and oxidative stress on liver pathology: the role of the mitochondrion. Alcohol Clin Exp Res 2002;26:907–15.
49. Folsom AR, Pankow JS, Tracy RP, et al. Association of C-reactive protein with markers of prevalent atherosclerotic disease. Am J Cardiol 2001;88:112–7.
50. Sitzer M, Markus HS, Mendall MA, Liehr R, Knorr U, Steinmetz H. C-reactive protein and carotid intimal medial thickness in a community population. J Cardiovasc Risk 2002;9:97–103.
51. Wang TJ, Nam BH, Wilson PW, et al. Association of C-reactive protein with carotid atherosclerosis in men and women: the Framingham Heart Study. Arterioscler Thromb Vasc Biol 2002;22:1662–7.
52. van der Meer IM, de Maat MP, Bots ML, et al. Inflammatory mediators and cell adhesion molecules as indicators of severity of atherosclerosis: the Rotterdam Study. Arterioscler Thromb Vasc Biol 2002;22:838–42.