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Endothelial Function

Glutathione Reverses Endothelial Dysfunction and Improves Nitric Oxide Bioavailability

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OBJECTIVES We investigated whether glutathione (GSH), a reduced thiol that modulates redox state and forms adducts of nitric oxide (NO), improves endothelium-dependent vasomotion and NO activity in atherosclerosis.

BACKGROUND Endothelial dysfunction and reduced NO activity are associated with atherosclerosis and its clinical manifestations such as unstable angina.

METHODS In the femoral circulation of 17 patients with atherosclerosis or its risk factors, endothelium-dependent vasodilation with acetylcholine (ACH), and endothelium-independent vasodilation with nitroglycerin and sodium nitroprusside were studied before and after GSH. In 10 patients, femoral vein plasma cyclic guanylate monophosphate (cGMP) levels were measured during an infusion of ACH before and after GSH. Femoral artery flow velocity was measured using a Doppler flow wire and the resistance index (FVRI) calculated as mean arterial pressure ÷ flow velocity.

RESULTS Glutathione strongly potentiated ACH-mediated vasodilation; at the two doses, FVRI decreased by 47% and 56% before, and by 61% and 67% after GSH ($p = 0.003$). Glutathione also elevated cGMP levels in the femoral vein during ACH infusion from 17.6 ± 3 to 23.3 ± 3 pmol/ml ($p = 0.006$). Augmentation of ACH responses was only observed in patients with depressed endothelial function. Glutathione did not influence endothelium-independent vasodilation with either NO donor.

CONCLUSIONS Thiol supplementation with GSH selectively improves human endothelial dysfunction by enhancing NO activity. (J Am Coll Cardiol 1999;34:507–14) © 1999 by the American College of Cardiology

The endothelium, through the release of nitric oxide (NO), is central to the regulation of a variety of vascular functions including vasomotion, lipid transport and hemostasis (1). Abnormal function of endothelial cells is associated with reduced NO bioavailability, and is believed to be an early event in the pathogenesis of atherosclerosis (2). Though the exact mechanism leading to the reduction in NO activity remains unknown, extensive evidence from in vitro studies suggests that atherosclerosis and its risk factors such as hypercholesterolemia, hypertension and diabetes lead to increased generation of oxygen free radicals capable of degrading NO (3–5). Diminished NO activity enhances the expression of transcription factor–nuclear factor kappa B (6), which up-regulates the synthesis of inflammatory mediators such as cytokines and adhesion molecules. Furthermore, at sites of advanced atherosclerotic lesions, reduced NO activity predisposes to abnormal vasomotion, and to thrombus formation (by increasing platelet adhesion and aggregation). Thus, reversing endothelial dysfunction and improving NO bioavailability may be effective in treating ischemia by improving vasomotion, reducing thrombotic cardiovascular events and modifying the progression of atherogenesis.

Endogenous thiols, compounds with a sulfhydryl group (SH), form stable, biologically active adducts with NO in several human biologic systems (7). These S-nitrosothiols have half-lives considerably longer than that of NO, strongly activate guanylate cyclase and seem to be important intermediates in the action of NO (8). Though the precise cellular mechanisms remain unclear, it is likely that these S-nitrosothiols improve cytoplasmic and transmembrane transport of NO from the endothelium to vascular smooth muscle cells and platelets. Proteins, in particular albumin, are the major thiol in plasma; glutathione (GSH) serves as an important intracellular thiol (7,9,10). Glutathione also

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Abbreviations and Acronyms

ACH	= acetylcholine
ANOVA	= analysis of variance
cGMP	= cyclic guanosine monophosphate
CV	= coefficient of variation
FVRI	= femoral vascular resistance index
GSH	= glutathione
NO	= nitric oxide
NTG	= nitroglycerin
SH	= sulfhydryl group

plays an important role in cell defense by virtue of its antioxidant property (7). Glutathione-related antioxidant defenses have been shown to be relatively deficient in atherosclerotic plaques (11). Furthermore, male adolescents with a family history of premature coronary artery disease have depressed plasma GSH levels (12). Depletion of plasma and vascular GSH may result from increased oxidative stress and contribute to the pathogenesis of atherosclerosis (13). On the basis of the recent experimental studies demonstrating that exogenous reduced thiols potentiate the vasodilator (14) and antiplatelet (15,16) effects of NO, we hypothesized that thiol supplementation with GSH would improve endothelial dysfunction and NO bioavailability in patients with atherosclerosis or its risk factors. To obtain insights into the mechanisms underlying any improvement in endothelial function with GSH, we also studied the effects of GSH on the NO donors nitroglycerin and sodium nitroprusside, which release NO by thiol-dependent and -independent pathways in the circulation.

METHODS

Patients

We studied 17 patients, 13 with atherosclerosis of the coronary artery and/or the iliofemoral circulation, four without angiographic atherosclerosis but with one or more risk factor (arterial pressure >140/90 mm Hg, cholesterol >200 mg/dl, presence of diabetes, age >60 years or history of current or recent smoking), who were undergoing diagnostic cardiac catheterization for investigation of chest pain or abnormal noninvasive tests. Patients with recent myocardial infarction, valvular heart disease, or occlusive peripheral vascular disease were excluded. The mean age was 56 ± 9 years (mean ± SD); 10 (59 %) were male, 7 were hypertensive, hypercholesterolemia was present in 14 patients, 4 were either current smokers or had smoked in the previous year, 5 had diabetes and 5 were older than 60 years of age.

Protocol

All cardiac medications were withdrawn at least 48 h before the study, and aspirin or other cyclooxygenase inhibitors were discontinued seven days before. The study was approved by the National Heart, Lung, and Blood Institute Investigational Review Board, and informed consent was obtained from all patients. A 6-F multipurpose A2 (Cordis, Miami, Florida) catheter was introduced retrogradely 1 cm beyond the end of a 7-F femoral artery sheath. A 0.018-in. (0.046-cm) Doppler flow wire (Cardiometrics, Mountain View, California) was introduced through the catheter and positioned 1 cm beyond the catheter tip to obtain an adequate flow velocity signal. All drugs were infused through the catheter 1 to 2 cm below the tip of the Doppler wire. A femoral angiogram was performed to assist with positioning of the wire and to visualize obstructive atherosclerotic plaques in the iliofemoral circulation, which may compromise blood flow measurements. Since diameter measurements were not made at the level of the Doppler wire with each intervention, we calculated femoral vascular resistance index (FVRI) as the mean arterial pressure ÷ femoral blood flow velocity.

Study 1: Effect of GSH on Endothelium-Dependent and -Independent Vasodilation

After measurement of baseline flow velocity and mean arterial pressure, endothelium-dependent vasodilation was estimated by performing a dose–response curve with incremental infusions of acetylcholine (ACH) at 150 and 300 μg/min for 2 min each. Endothelium-independent function was estimated with two NO donors: nitroglycerin (NTG) at 25 μg/min for 3 min and sodium nitroprusside at 20 μg/min for 2 min (Fig. 1).

In 15 patients, after a 5-min recovery period, GSH (Sigma, St Louis, Missouri), a reduced thiol, was infused intra-arterially at 50 mg/min for 10 min to achieve an estimated concentration of 1 mmol/liter. While continuing the infusion of GSH at 50 mg/min, ACH was coinfused at 150 μg/min and at 300 μg/min for 2 min each. After a 10-min recovery period, the GSH infusion was continued and coinfused with NTG at 25 μg/min for 3 min and sodium nitroprusside at 20 μg/min for 2 min. Blood flow velocity and mean arterial pressure were measured after each intervention.

Reproducibility. Reproducibility of the microvascular dilator response to repeated infusions of ACH and sodium nitroprusside was tested in six patients during infusion of 5% dextrose. The FVRIs during the first and second infusions were as follows: ACH (150 μg/min), 3.5 ± 0.6 and 4.0 ± 0.6, $p = 0.2$ (coefficient of variation [CV] 17.2%); ACH (300 μg/min), 3.1 ± 0.5 and 3.7 ± 0.6, $p = 0.2$ (CV 8.2%); sodium nitroprusside, 1.8 ± 0.1 and 1.8 ± 0.2, $p = 0.7$ (CV 8.7%). These findings are consistent with our previously described reproducibility of vascular responses in the femoral circulation (17).

Study 2: Effect of GSH on Endothelium-Derived NO

In 10 patients, femoral vein plasma cyclic guanosine monophosphate (cGMP) levels were measured as an indirect assay of NO. Measurements were made at baseline and during the infusion of ACH (300 μg/min), before and after GSH. After an intravenous loading dose of 15 mg/kg over 5 min, the infusion was continued at a rate of 60 mg/kg over

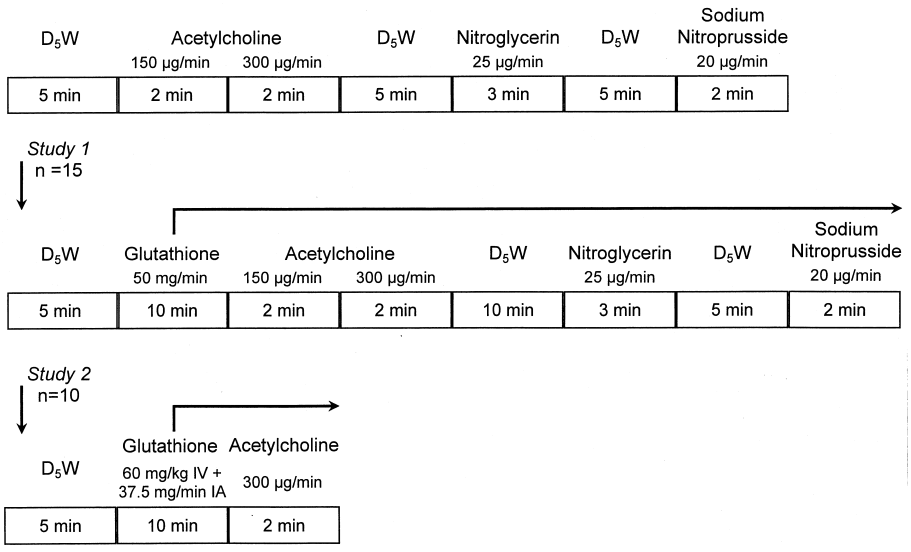

Figure 1. Protocol design. IA = intra-arterially; IV = intravenously; D_5W = 5% dextrose.

30 min to produce an approximate systemic plasma concentration of 0.25 mmol/liter (Fig. 1). As before, GSH was also infused directly into the femoral artery at the reduced rate of 37.5 mg/min during the last 10 min of the intravenous infusion. This combined regime of intravenous and intra-arterial GSH was estimated to produce a femoral artery plasma concentration of 1 mmol/liter. While continuing the GSH infusions, ACH (300 μg/min) was coinfused as described above (Fig. 1). Flow velocity measurements were made, and blood was collected from the femoral vein at baseline, and after ACH, before and after GSH, to determine cGMP concentration.

Measurement of plasma cGMP. Nine milliliters of blood was drawn into syringes containing 1 ml sodium citrate (final concentration 14.7 mmol/liter) and the specific cGMP phosphodiesterase inhibitor zaprinast (final concentration 10^{-5} mol/liter). Within 1 min of sampling, the blood was centrifuged at 140 *g* for 10 min. To two 1-ml aliquots of the resultant plasma, ice-cold 6% trichloroacetic acid was added to extract the cGMP. After neutralization by solvent partition with a mixture of Freon and tri-n-octylamine, cGMP was measured by radioimmunoassay (RPA 525, Amersham International, Chicago, Illinois).

Reproducibility. In four patients, reproducibility of arterial and venous plasma cGMP levels was tested during infusions of 5% dextrose and ACH (300 μg/min) twice in each patient. There was no difference between the femoral arterial and venous plasma cGMP levels under resting conditions (23.5 ± 3 and 24.8 ± 2.7 pmol/ml, p = 0.4, respectively, CV 17.5%). Similarly, there was no change in arterial cGMP content during infusion of ACH (22.5 ± 3 before and 21.4 ± 1 pmol/ml, p = 0.7 during ACH, CV 12%). Therefore, in the 10 study patients, only venous plasma cGMP content was measured.

Statistical Analysis

Data are expressed as mean ± SEM. Differences between means were compared by paired or unpaired Student *t* test, as appropriate. The effect of GSH on the ACH responses was compared by a two-way repeated measures analysis of variance (ANOVA) with appropriate interactions. All p values were two tailed, and a value <0.05 was considered of statistical significance. Correlation analysis was performed using Pearson correlation coefficient. Although percentage change in flow velocity and FVRI were calculated, except for the Pearson correlation, all statistical analyses were performed using absolute values.

RESULTS

Study 1: Femoral Vascular Response to GSH

After 10 min of intra-arterial GSH infusion there was a trend toward a reduction in vascular resistance (change in FVRI: 5.8 ± 0.4 to 5.4 ± 0.4 mm Hg·cm^{-1}·s, p = 0.07), but there was no change in flow velocity (18.3 ± 1.5 to 19.4 ± 1.6, p = 0.14) or mean arterial blood pressure (99 ± 4 mm Hg before and after, p = 0.9).

Effect of GSH on ACH responses. Acetylcholine infusions produced graded femoral microvascular dilation; at the two doses (150 and 300 μg/min), flow velocity increased by 97 ± 17% and 142 ± 22% and FVRI decreased by 47 ± 4% and 56 ± 4%, respectively. When coinfused with GSH, there was significant potentiation of ACH-induced vasodilation; flow velocity increased by 161 ± 23% and 197 ± 17% (p = 0.0016, ANOVA) and FVRI decreased by 61 ± 3% and 67 ± 2% (p = 0.005, ANOVA) respectively at the two doses (Fig. 2).

There was a significant negative correlation between the

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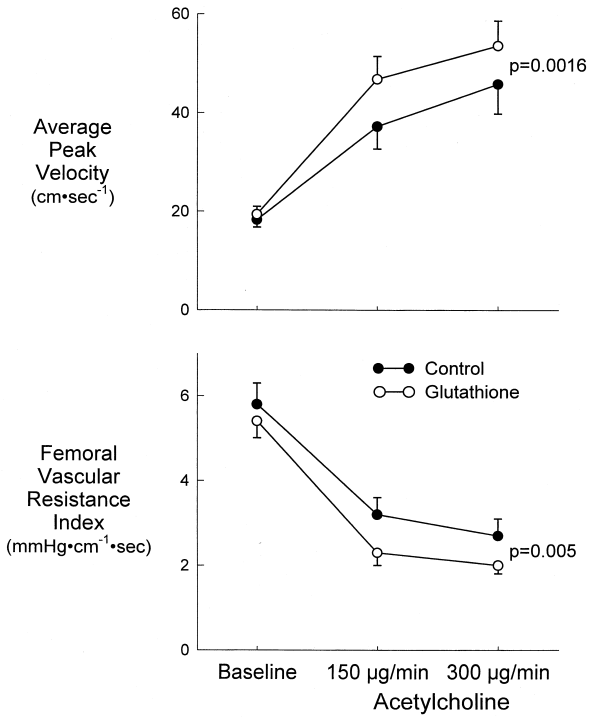

Figure 2. Effects of acetylcholine on femoral blood flow velocity and femoral vascular resistance index, before and after glutathione. Data represent mean ± SEM; p values represent results of two-way analysis of variance.

patient's endothelial function measured as the decrease in FVRI with ACH (300 μg/min) at baseline, and the magnitude of improvement of ACH response with GSH ($r = -0.81$, $p < 0.001$), indicating that patients with a depressed dilator response with ACH had greater improvement with thiol supplementation. We therefore examined the effect of GSH on the ACH responses in patients whose endothelial function was within the range (mean ± 2 SD) of changes observed in normal volunteers and those whose responses were below this level. In a previous study, normal subjects without risk factors for atherosclerosis had a 68 ± 6.5% reduction in FVRI with 300 μg/min ACH (18). Thus, eight patients with ACH-mediated reduction in FVRI >55% were considered to be within the "normal" range, and the remaining seven patients with FVRI decrease <55% were examined as a group with "depressed endothelial function" (Fig. 3). Significant improvement in ACH-mediated femoral microvascular vasodilation with GSH was only apparent in patients with depressed endothelium-dependent vasodilation ($p = 0.003$ for change in FVRI by ANOVA) (Fig. 3). In contrast, patients with "normal" endothelial function had no significant change (Fig. 3).

Effect of GSH on the response to NTG and sodium nitroprusside. Nitroglycerin increased femoral flow velocity by 41 ± 6% and reduced FVRI by 30 ± 3% compared with baseline ($p < 0.001$, both). After GSH, the 42 ± 6% increase in flow velocity and 33 ± 3% reduction in FVRI were not significantly different (Fig. 4). Sodium nitroprusside increased femoral flow velocity by 127 ± 14% and reduced FVRI by 58 ± 3% compared with baseline ($p < 0.001$, both). After GSH, the 120 ± 12% increase in flow velocity and 59 ± 3% reduction in FVRI were not significantly different (Fig. 4). Thus, GSH did not potentiate vasodilation in response to either NO donor.

Study 2: Effect of GSH on Plasma cGMP

The vasodilator responses to ACH during the infusion of intra-arterial GSH alone and during the combined intravenous and intra-arterial infusions were similar. In the 10 patients who had the combined infusion of GSH in study 2, baseline FVRI was 6.1 ± 0.3 and remained unaltered by GSH. Acetylcholine (150 and 300 μg/min) reduced FVRI by 51 ± 7% and 59 ± 5% before and by 61 ± 4% and 65 ± 4%, respectively, after GSH ($p = 0.04$, ANOVA).

Glutathione did not alter basal venous plasma cGMP concentration (18.6 ± 3.2 to 19.9 ± 2.9 pmol/ml, $p = 0.5$, baseline vs. after GSH). However, it strongly potentiated plasma cGMP level during infusion of ACH (300 μg/min); femoral vein plasma cGMP level increased by 20.7 ± 12% from 17.6 ± 3.2 to 23.3 ± 3.2 pmol/ml ($p = 0.006$). The magnitude of increase in venous cGMP level correlated with the magnitude of improvement in vasodilation with ACH (measured as the percentage change in FVRI with ACH before vs. after GSH) ($r = -0.68$, $p = 0.032$). Thus, patients with greater improvement in dilation with ACH after GSH also had a greater increase in plasma cGMP.

DISCUSSION

We have previously shown that endothelial dysfunction, present in both the coronary and peripheral circulations of patients with atherosclerosis or its risk factors, is accompanied by a reduction in basal and stimulated activity of NO (19–21) and is likely to be responsible, at least in part, for the abnormal vasomotion that accompanies atherosclerosis (18,22,23). Thus, pharmacologic interventions that reverse endothelial dysfunction and enhance NO activity may be of therapeutic value in ischemic syndromes by reducing vasoconstriction and inhibiting platelet activity. An even more attractive possibility is that such therapy may prevent atherogenesis or inhibit its progression if used early in the disease process. In this study, we have demonstrated that acute administration of GSH improves endothelial dysfunction in patients with atherosclerosis or its risk factors by enhancing NO activity but without augmenting the vasodilator action of the NO donors, NTG and sodium nitroprusside.

Effect of GSH on endothelial function and endogenous NO activity. Previously, we have shown that femoral microvascular dilation to ACH is endothelium-dependent and predominantly NO-mediated (18). In the present study, microvascular dilation with ACH was undoubtedly enhanced by GSH, a finding that is consistent with a recent

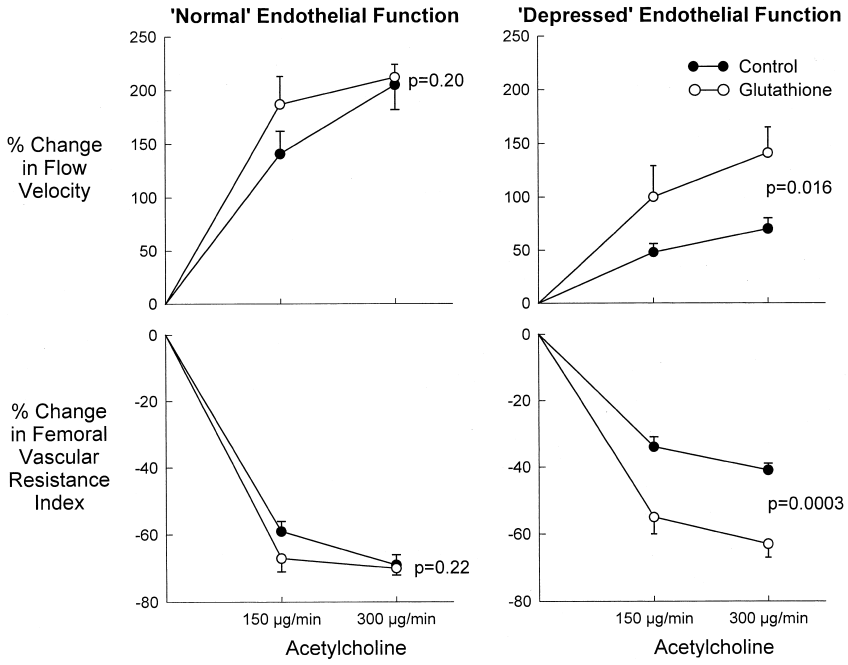

Figure 3. Effects of acetylcholine on flow velocity and femoral vascular resistance index in patients with normal **(left)** and depressed **(right)** endothelial function, before and after glutathione. Data represent mean ± SEM; p values represent results of two-way analysis of variance.

report demonstrating that GSH also improves abnormal ACH responses in epicardial coronary arteries of patients with risk factors for atherosclerosis (24). The beneficial effect of GSH appeared to be restricted to patients with worse endothelial dysfunction demonstrable as a lower vasodilator response to ACH, and is consistent with a previous study in normal volunteers in whom exogenous N-acetylcysteine did not potentiate endothelium-dependent vasodilation (25).

Although ACH stimulates release of other relaxing fac-

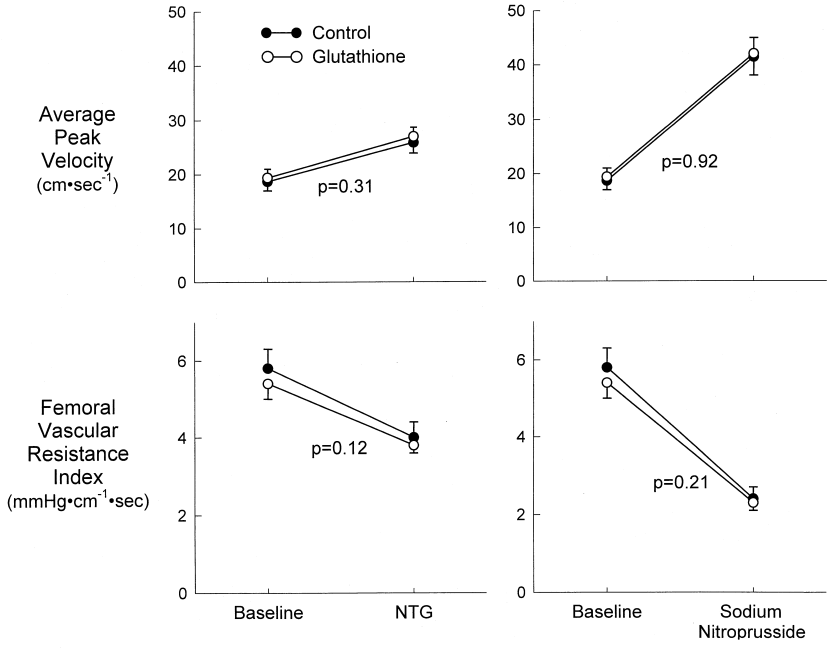

Figure 4. Effects of nitroglycerin (NTG) and sodium nitroprusside on femoral blood flow velocity and femoral vascular resistance index, before and after glutathione. Data represent mean ± SEM.

tors such as endothelium-derived hyperpolarizing factor and prostacyclin in addition to NO, only NO increases soluble cGMP in the vascular wall and platelets. The significant increase in venous plasma cGMP with ACH and GSH in our study, with a higher increase in patients with a greater potentiation of ACH-mediated smooth muscle dilation, confirms enhancement of the NO pathway with GSH in patients with endothelial dysfunction.

Nitric oxide–stimulated plasma cGMP may originate from both platelets and the vessel wall. We designed the second study with the aim of increasing femoral vascular and circulating platelet GSH levels. Although the intra-arterial GSH infusion would be expected to elevate local femoral plasma concentrations of GSH, systemic levels would not have changed significantly. Consequently, platelets outside of the femoral circulation would not be exposed to high levels of GSH, and the rapid transit through the femoral circulation would also be inadequate to achieve a steady state. Therefore, we also infused GSH intravenously to ensure that the circulating platelets were adequately exposed to exogenous GSH.

Potential mechanisms underlying the improvement in NO bioavailability. At least two mechanisms may be potentially responsible for the observed beneficial effects of GSH on the endothelial function. First, it may enhance the bioavailability of NO by forming a stable, biologically active S-nitrosoglutathione adduct (10). Although NO has a very short half-life, on the order of 0.1 to 1 s, recent evidence suggests that it circulates in the plasma primarily as S-nitrosoalbumin after reacting with the SH of cysteine 34 (26), and in red blood cells as nitrosyl-hemoglobin and S-nitrosohemoglobin. S-Nitrosoalbumin is biologically active but has minimal intracellular access and probably serves as a circulating reservoir of NO (9,16). Low molecular weight thiols such as cysteine form less diffusion-limited NO adducts that may transport NO to target sites within vascular smooth muscle cells and platelets (10). Glutathione is the major intracellular nonprotein source of the SHs, with tissue levels ranging between 0.5 and 10 mmol/liter, and is synthesized intracellularly from cysteine, glutamate and glycine. It is continuously and irreversibly transported out of cells to augment extracellular thiol availability. Glutathione is metabolized by the membrane-bound enzyme gamma-glutamyl transpeptidase, present in abundant amounts on endothelial cells, to release cysteine. As cysteine is rapidly metabolized, GSH acts as the storage and transport form of cysteine (27).

The second potential mechanism for the action of GSH may relate to its antioxidant properties (7,28). Experimental studies suggest that atherosclerosis, hypertension, hypercholesterolemia and diabetes result in increased vascular generation of oxygen free radicals (3–5). Similarly, increased oxidative stress has been detected in the plasma of smokers (29). These oxygen free radicals are capable of inactivating NO and inducing endothelial dysfunction. Because GSH itself is not transported intracellularly, exogenously administered GSH in this study is most likely to have acted by supplementing plasma GSH levels to reduce luminal oxidant stress, and thereby increase NO bioavailability in patients with endothelial dysfunction.

Glutathione and NO donors. Nitroglycerin requires enzymatic metabolism in the presence of SH-containing compounds to release NO. Recent studies indicate that the enzyme responsible for metabolizing NTG is localized mainly in the cell membrane fraction of vascular smooth muscle cells (30). Thus, exogenously administered thiols would need to elevate vascular interstitial levels to potentiate NTG. In our study, NTG-mediated microvascular vasodilation was not potentiated by GSH. This is consistent with the observation in vitro that exogenous GSH does not potentiate NTG-mediated microvascular dilation in canine coronary arteries (31). Though GSH is a major source of the SH radical, exogenous GSH cannot be transported intracellularly and is thus unlikely to acutely influence NTG responses (26). However, as mentioned above, GSH does elevate levels of plasma cysteine, which can be transported intracellularly, but the relatively short infusions used in this study may have been insufficient to raise cysteine levels in the vascular interstitium, the site at which NO is generated from NTG. In contrast, N-acetylcysteine, a pharmacologic precursor of cysteine, potentiates NTG-mediated vasodilation in the canine coronary circulation, and in human peripheral and coronary circulations (32–35). Unlike GSH, N-acetylcysteine can be rapidly transported into the vascular tissue and generate GSH, possibly accounting for the observed difference between the two thiols.

In vitro studies have shown that sodium nitroprusside releases NO by an endothelium-independent mechanism in the presence of a variety of reducing agents present in plasma and tissue extracts (36,37). These include thiols such as cysteine and GSH. However, our study demonstrates that GSH does not augment sodium nitroprusside–mediated peripheral microvascular dilation in vivo. Although this may be explained by the poor vascular permeability of GSH, a previous study in humans showed that N-acetylcysteine also does not potentiate the systemic or pulmonary hemodynamic effects of sodium nitroprusside (38). These data suggest that thiols are not rate-limiting, perhaps due to the availability of other reducing agents, in the peripheral vasodilator activity of sodium nitroprusside in patients with atherosclerosis or its risk factors. Alternatively, any potentiation of NO release from nitroprusside by GSH in the blood may have been obscured by binding of NO to reduced hemoglobin (36).

Study limitations. We have not studied normal volunteers without risk factors for atherosclerosis in this study and therefore cannot comment on whether the observed improvement in patients would be present in normal subjects. However, the improvement in ACH-mediated vasodilation and plasma cGMP levels observed in patients with endo-

thelial dysfunction, and the finding that those with ACH-mediated dilation within the range observed in normal subjects had no improvement, suggest that the effect of GSH is likely to be observed in those in whom stimulated NO activity is most compromised. It is also unlikely that the greater improvement observed in patients with the worst baseline function is artifactual, because measurements of increase in cGMP levels, which provided an independent index of luminal NO release, were also greater in this subset.

Plasma cGMP elevation with GSH after ACH compared with ACH alone confirmed increased production of NO, but we are unable to measure the contribution of platelets versus the vascular wall to the observed enhancement because the NO pathway is active in both. Recent studies demonstrating potent inhibition of platelets with S-nitrosoglutathione (39,40) suggest that GSH-induced increase in plasma cGMP is a reflection also of its potential antiplatelet aggregative action.

Conclusions and implications. In this study, we demonstrate that depressed peripheral vascular endothelium-dependent vasodilation, present in patients with atherosclerosis and those with its risk factors, is selectively improved by GSH. The observation that NO bioavailability is enhanced offers a potential cellular mechanism by which thiol supplementation may be of clinical benefit. This acute improvement in endothelial function, and the recent demonstration that S-nitrosoglutathione has platelet inhibitory effects in humans, indicate that thiols such as GSH and cysteine may have therapeutic potential (39,40). In a previous randomized double-blind study, the combination of NTG and N-acetylcysteine significantly reduced the incidence of myocardial infarction compared with NTG alone in patients with severe unstable angina (41). Furthermore, an inhibitory effect of S-nitrosoalbumin on neointimal proliferation in an animal model of vascular injury suggests that long-acting NO adducts may also have the potential to prevent restenosis after angioplasty (42) and retard progression of atherosclerosis. However, GSH is completely metabolized by intestinal and hepatic gamma glutaryl transferase and is therefore unsuitable for oral therapy. L-2-Oxothiazolidine-4-carboxylic acid, a cysteine prodrug, can be administered orally and repletes cellular glutathione levels. Data from a double-blind placebo-controlled study suggests that L-2-oxothiazolidine-4-carboxylic acid acutely improves flow-mediated endothelium-dependent brachial artery vasodilation in patients with coronary artery disease (43), and suggests that thiols may have antiatherogenic properties. However, further studies are required to establish their mechanism of action and clinical efficacy.

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REFERENCES

1. Rubyani CM. The role of endothelium in cardiovascular homeostasis and diseases. J Cardiovasc Pharmacol 1993;22 Suppl 4:S1–14.
2. Ross R. The pathogenesis of atherosclerosis: a prospective for the 1990s. Nature 1993;362:801–9.
3. OharaY, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993;91:2546–51.
4. Witztum JL. The oxidation hypothesis of atherosclerosis. Lancet 1994;344:793–5.
5. Cosentino F, Hishikawa K, Katusic ZS, Lüscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. Circulation 1997;96:25–8.
6. Barnes PJ, Karin M. Nuclear factor-κB—a pivotal transcription factor in chronic inflammatory diseases. N Engl J Med 1997;336:1066–71.
7. Stamler JS, Slivka A. Biological chemistry of thiols in the vasculature and in vascular-related disease. Nutr Rev 1996;54:1–30.
8. Myers PR, Minor RL Jr, Guerra R, Jr, et al. Vasorelaxant properties of the endothelium-derived relaxing factor more closely resemble S-nitrosocysteine than nitric oxide. Nature 1990;345:161–3.
9. Keaney JF Jr, Simon DI, Stamler JS, et al. NO forms an adduct with serum albumin that has endothelium-derived relaxing factor-like properties. J Clin Invest 1993;91:1582–9.
10. Scharfstein J, Keaney JF Jr, Slivka A, et al. In vivo transfer of nitric oxide between a plasma protein-bound reservoir and low-molecular weight thiols. J Clin Invest 1994;94:1432–9.
11. Lapenna D, de Gioia S, Ciofani G, et al. Glutathione-related antioxidant defenses in human atherosclerotic plaques. Circulation 1998;97:1930–4.
12. Morrison JA, Jacobsen DW, Sprecher DL, et al. Serum homocysteine, glutathione and risk of coronary heart disease in adolescent males. Circulation 1997;96:I-101.
13. Aucoin MM, Barhoumi R, Kochevar DT, et al. Oxidative injury of coronary venular endothelial cells depletes intracellular glutathione and induces HSP 70mRNA. Am J Physiol 1995;268:H1651–8.
14. Cooke JP, Stamler J, Andon N, et al. Flow stimulates endothelial cells to release a nitrosovasodilator that is potentiated by reduced thiol. Am J Physiol 1990;259:H804–12.
15. Stamler J, Mendelsohn ME, Amarante P, et al. N-acetylcysteine potentiates platelet inhibition by endothelium-derived relaxing factor. Circ Res 1989;65:789–95.
16. Simon DI, Stamler JS, Jaraki O, et al. Antiplatelet properties of protein S-nitrosothiols derived from nitric oxide and endothelium-derived relaxing factor. Arterioscler Thromb 1993;13:791–9.
17. Husain S, Andrews NP, Mulcahy D, et al. Aspirin improves endothelial dysfunction in atherosclerosis. Circulation 1998;97:716–20.
18. Dakak N, Husain S, Mulcahy DM, et al. Contribution of nitric oxide to reactive hyperemia: impact of endothelial dysfunction and effect of L-arginine. Hypertension 1998;32:9–15.
19. Quyyumi AA, Dakak N, Mulcahy D, et al. Nitric oxide activity in the atherosclerotic human coronary circulation. J Am Coll Cardiol 1997; 29:308–17.
20. Quyyumi AA, Dakak N, Andrews NP, et al. Nitric oxide activity in the human coronary circulation. J Clin Invest 1995;95:1747–55.
21. Panza JA, Casino PR, Kilcoyne CM, Quyyumi AA. Role of endothelium-derived nitric oxide in the abnormal endothelium-dependent vascular relaxation of patients with essential hypertension. Circulation 1993;87:1468–74.
22. Ludmer PL, Selwyn AP, Shook TL, et al. Paradoxical vasoconstriction induced by acetylcholine in atherosclerotic coronary arteries. N Engl J Med 1986;315:1046–51.
23. Quyyumi AA, Dakak N, Andrews NP, et al. Contribution of nitric oxide to metabolic coronary vasodilation in the human heart. Circulation 1995;92:320–6.
24. Kugiyama K, Ohgushi M, Motoyama T, et al. Intracoronary infusion of reduced glutathione improves endothelial vasomotor response to

acetylcholine in human coronary circulation. Circulation 1998;97: 2299–301.
25. Creager MA, Roddy M-A, Boles K, Stamler JS. N-acetylcysteine does not influence the activity of endothelium-derived relaxing factor in-vivo. Hypertension 1997;29:668–72.
26. Stamler JS, Jaraki O, Osborne J, et al. Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. Proc Natl Acad Sci USA 1992;89:7674–7.
27. Meister A. Selective modification of glutathione metabolism. Science 1983;220:472–7.
28. Shan X, Aw TY, Jones DP. Glutathione-dependent protection against oxidative injury. Pharmacol Ther 1990;47:61–71.
29. Morrow JD, Frei B, Longmire AW, et al. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. N Engl J Med 1995;332:1198–203.
30. Seth P, Fung H-L. Biochemical characterization of a membrane-bound enzyme responsible for generating nitric oxide from nitroglycerin in vascular smooth muscle cells. Biochem Pharmacol 1993;46:1481–6.
31. Wheatley RM, Dockery SP, Kurz MA, et al. Interaction of nitroglycerin and sulfhydryl-donating compounds in coronary microvessels. Am J Physiol 1994;266:H291–7.
32. Kurz MA, Lamping KG, Bates JN, et al. Mechanisms responsible for the heterogenous coronary microvascular response to nitroglycerin. Circ Res 1991;68:847–55.
33. Horowitz JD, Henry CA, Syrjanen ML, et al. Combined use of nitroglycerin and N-acetylcysteine in the management of unstable angina pectoris. Circulation 1988;77:787–94.
34. Winniford MD, Kennedy PL, Wells PJ, Hillis D. Potentiation of nitroglycerin-induced coronary dilation by N-acetylcysteine. Circulation 1986;73:138–42.
35. Boesgaard S, Poulsen HE, Aldershvile J, et al. Acute effects of nitroglycerin depend on both plasma and intracellular sulfhydryl compound levels in vivo. Circulation 1993;87:547–53.
36. Bates JN, Baker MT, Guerra R Jr, Harrison DG. Nitric oxide generation from nitroprusside by vascular tissue. Evidence that reduction of the nitroprusside anion and cyanide loss are required. Biochem Pharmacol 1991;42 Suppl:S157–65.
37. Gruetter DY, Gruetter CA, Barry BK, et al. Activation of coronary arterial guanylate cyclase by nitric oxide, nitroprusside, and nitrosoguanidine-inhibition by calcium, and other cations, enhancement by thiols. Biochem Pharmacol 1980;29:2943–50.
38. Daley WL, Loscalzo J, Vita JA, et al. The effect of thiol on the hemodynamic profile of nitroprusside: mechanistic implication in vivo. Circulation 1992;86 Suppl I:I-489.
39. DeBelder AJ, MacAllister R, Radomski MW, et al. Effects of S-nitrosoglutathione in the human forearm circulation: evidence for selective inhibition of platelet activation. Cardiovasc Res 1994;28: 691–4.
40. Langford EJ, Brown AS, Wainwright RJ, et al. Inhibition of platelet activity by S-nitrosoglutathione during coronary angioplasty. Lancet 1994;344:1458–60.
41. Horowitz JD, Antman EM, Lorell BH, et al. Potentiation of the cardiovascular effects of nitroglycerin by N-acetylcysteine. Circulation 1983;68:1247–53.
42. Marks DS, Vita JA, Folts JD, et al. Inhibition of neointimal proliferation in rabbits after vascular injury by a single treatment with a protein adduct of nitric oxide. J Clin Invest. 1995;96:2630–8.
43. Vita JA, Frei B, Holbrook M, et al. L-2-Oxothiazolidine 4 carboxylic acid improves endothelial dysfunction in patients with coronary artery disease. J Clin Invest 1998;101:1408–14.