Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation1–4

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ABSTRACT
Background: Aging is associated with oxidative stress, but underlying mechanisms remain poorly understood.

Objective: We tested whether glutathione deficiency occurs because of diminished synthesis and contributes to oxidative stress in aging and whether stimulating glutathione synthesis with its precursors cysteine and glycine could alleviate oxidative stress.

Design: Eight elderly and 8 younger subjects received stable-isotope infusions of [2H2]glycine, after which red blood cell (RBC) glutathione synthesis and concentrations, plasma oxidative stress, and markers of oxidant damage (eg, F2-isoprostanes) were measured. Elderly subjects were restudied after 2 wk of glutathione precursor supplementation.

Results: Compared with younger control subjects, elderly subjects had markedly lower RBC concentrations of glycine (486.7 ± 28.3 compared with 218.0 ± 23.7 μmol/L; P < 0.01), cysteine (26.2 ± 1.4 compared with 19.8 ± 1.3 μmol/L; P < 0.05), and glutathione (2.08 ± 0.12 compared with 1.12 ± 0.18 mmol/L RBCs; P < 0.05); lower glutathione fractional (83.1 ± 6.43% compared with 45.8 ± 5.69%/d; P < 0.01) and absolute (1.73 ± 0.16 compared with 0.55 ± 0.12 mmol/L RBCs per day; P < 0.01) synthesis rates; and higher plasma oxidative stress (304 ± 16 compared with 346 ± 20 Carratelli units; P < 0.05) and plasma F2-isoprostanes (97.7 ± 8.3 compared with 136.3 ± 11.3 pg/mL; P < 0.05). Precursor supplementation in elderly subjects led to a 94.6% higher glutathione concentration, a 78.8% higher fractional synthesis rate, a 230.9% higher absolute synthesis rate, and significantly lower plasma oxidative stress and F2-isoprostanes. No differences in these measures were observed between younger subjects and supplemented elderly subjects.

Conclusions: Glutathione deficiency in elderly humans occurs because of a marked reduction in synthesis. Dietary supplementation with the glutathione precursors cysteine and glycine fully restores glutathione synthesis and concentrations and lowers levels of oxidative stress and oxidant damages. These findings suggest a practical and effective approach to decreasing oxidative stress in aging. Am J Clin Nutr 2011;94:847–53.

INTRODUCTION
Elevated oxidative stress has been linked to several aging-related illnesses, including the development of cataracts (1–3), macular degeneration (4), immune deficiencies (5, 6), neurodegenerative diseases (7), and increased DNA damage (8). The ability of a cell to resist oxidant damage is determined by a balance between the generation of reactive oxygen species and the defensive capacity to produce antioxidants. Glutathione (γ-glutamylcysteinylglycine) is the most abundant endogenous intracellular antioxidant present in millimolar quantities within cells. Glutathione plays a central role in antioxidant defenses, and irreversible cell damage supervenes when the cell is unable to maintain intracellular glutathione concentrations. Evidence from several animal (9–11) and human (4, 12–16) studies suggests that concentrations of glutathione decline with aging. Glutathione deficiency in aging results in an increased prooxidizing shift (17) and elevated oxidative stress (18), but underlying mechanisms are not well understood.

Glutathione is synthesized from the amino acids glutamate, cysteine, and glycine in 2 steps catalyzed by the enzymes glutamate cysteine ligase (also known as γ-glutamylcysteine synthetase) and γ-glutamyl-L-cysteine:glycine ligase (also known as glutathione synthetase). We hypothesized that blunted glutathione synthesis could be responsible for intracellular glutathione deficiency and consequent oxidative stress in aging. We further hypothesized that dietary supplementation with 2 amino acids, cysteine and glycine, fully restores the deficit.
Subjects and Methods

Subjects

The study was approved by and conducted in accordance with the ethical standards of the Institutional Review Board for Human Studies at Baylor College of Medicine. Eight healthy elderly humans (60–75 y) and 8 younger subjects (30–40 y) were recruited (initial recruitment in January 2005) and matched for sex (5 men and 3 women in each group). The elderly subjects did not use tobacco, had had no infections or illnesses for 6 mo, and were free of diabetes mellitus, thyroid disorders, hypercortisolemia, and liver or renal impairment. All subjects had sedentary lifestyles, and none consumed unusual diets or dietary supplements. The subjects had an initial visit to measure blood counts, glucose concentrations, and liver and renal profiles.

Metabolic Study Protocol

The subjects were studied in the adult General Clinical Research Center of Baylor College of Medicine after providing written informed consent. All subjects received intravenous infusions of stable isotopes to measure glutathione synthesis in the fasted state. Only the elderly subjects received oral treatment of 14 d with 0.81 mmol cysteine · kg⁻¹ · d⁻¹ (as N-acetylcysteine) and 1.33 mmol glycine · kg⁻¹ · d⁻¹ after which they were re-studied. The subjects were asked to consume their habitual diets and to avoid alcohol from 1 wk before beginning the study to the end of the study period. The subjects fasted for 10 h before the start of the stable-isotope infusions. The primary outcome variables were the FSRs and ASRs of glutathione, intracellular concentrations of glutathione, plasma F₂-isoprostane and lipid peroxide concentrations, and plasma oxidative stress measured as reactive oxygen metabolites.

Stable-Isotope Protocol

The erythrocyte glutathione rate of synthesis was measured from the rate of incorporation of [²H₂]glycine into the tripeptide by using erythrocyte free-glycine isotopic enrichment to represent the enrichment of the glycine precursor pool from which erythrocytes synthesize glutathione, as previously described (19–22). Sterile solutions of [²H₂]glycine (Cambridge Isotope Laboratories) were prepared. After the subjects fasted overnight for 10 h, intravenous catheters were inserted into the superficial veins of both arms, one for continuous infusion of the tracer solutions and the other for repeated blood sampling. The hand and forearm with the sampling catheter were wrapped in a heating pad to arterialize venous blood. After a 20-mL blood sample was drawn, a bolus intravenous infusion of [²H₂]glycine (20 μmol/kg) was given to prime the glycine pool, which was followed by a constant infusion of the same isotope at the rate of 15 μmol · kg⁻¹ · h⁻¹ for 8 h. Additional 5-mL blood samples were taken at 3, 4, 5, 6, 7, and 8 h for the measurement of erythrocyte glutathione-derived glycine isotopic enrichments. The same infusion protocol was repeated only in the elderly group after supplementation with cysteine and glycine for 14 d.

Sample Analyses

Blood Chemistry

Samples for plasma analyses were immediately centrifuged, and the plasma was separated, portioned into aliquots in tubes for the various assays, and frozen at −70°C for later analyses. Hemoglobin, hematocrit, and routine plasma variables were also measured.

Erythrocyte Glutathione Analyses

Erythrocyte glutathione concentration and isotopic enrichment were measured in duplicate 1-mL aliquots of whole blood. Briefly, a sample of blood was centrifuged, and plasma was removed and stored. The residual hematocrit was mixed immediately with 1 mL chilled, isotonic MBB (purchased from Calbiochem) buffer solution (pH 7.5) containing the following: 5 mmol MBB/L, 17.5 mmol Na₂EDTA/L, 50 mmol potassium phosphate/L, 50 mmol serine/L, and 50 mmol boric acid/L. This was subjected to 3 freeze-thaw cycles with liquid nitrogen to lyse RBCs. After being vigorously vortex-mixed, the whole blood–MBB mixture was incubated in the dark for the development of the glutathione–MBB derivative. Proteins were then precipitated with ice-cold 20% perchloric acid, and the supernatant fluid was stored at −70°C until further analyzed for glutathione. To determine GSSG concentrations, the reducing agent dithiothreitol (5 mmol/L) was added first to a blood sample to convert GSSG to glutathione, and the sample was processed as described above. The GSSG concentrations were obtained by subtracting the glutathione value from the glutathione + GSSG value.

A second aliquot of packed RBCs was washed 3 times with ice-cold normal saline, and the proteins were precipitated by adding 20% perchloric acid. The protein-free supernatant fluid containing the free amino acids was stored at −70°C until further analysis of erythrocyte free glycine enrichment and concentrations of glycine, cysteine, and glutamate.

Plasma amino acid concentrations were determined by ion exchange HPLC. The concentration measurement of erythrocyte-free cysteine and glutathione, and the isolation of erythrocyte-free glutathione was performed on a Waters HPLC system by using a 717plus autosampler complexed to a Waters 2475 fluorescent detector and a reversed-phase octadecysilane. Reversed-phase separation of thiol compounds was performed on a Waters Nova-Pak C₁₈ column (4 μm, 3.9 × 150 mm; Waters Inc). Elution of the thiois was accomplished over 35 min by a linear gradient of 3% acetonitrile to 13.5% acetonitrile in 1% acetic acid in water, pH 4.25, at a flow rate of 1.1 mL/min. Standards included known concentrations of cysteine and glutathione (Sigma) prepared and diluted in the same manner as were the samples. The glutathione-containing fractions were collected on a fraction collector and
processed for gas chromatography–mass spectrometry analysis of glutathione-bound glycine. The glutathione-containing fractions were dried, and the peptide was hydrolyzed for 4 h in 6 mol HCl/L at 110°C.

Erythrocyte free glycine was extracted from the protein-free supernatant fluid by cation-exchange chromatography. Erythrocyte free glycine and erythrocyte glutathione-derived glycine were converted to the n-propyl ester heptfluorobutyramide derivatives, and the isotope ratio of each was measured by negative chemical ionization gas chromatography–mass spectrometry on an Agilent 6980 gas chromatograph complexed to a 5973 mass spectrometer (Agilent Technologies), monitoring ions at an m/z of 293 to 295.

**Oxidative stress**

The reactive oxygen metabolites test was used to measure plasma hydroperoxide concentrations as an index of free radical formation. This test is based on the concept that the amount of organic hydroperoxides in plasma is related to the free radicals from which they are formed. Briefly, plasma is reacted with an acidic acetate buffer (pH 4.8), which liberates transition metal ions that catalyze the decomposition of the hydroperoxides to alkoxyl and peroxy radicals. These newly formed radicals in turn oxidize the spectrophotometric marker (N,N-diethyl-p-phenylenediamine), which is detectable by absorption at 505 nm as Carratelli units, where one unit is equal to 0.8 mg hydrogen peroxide/L. Oxidant markers were measured as plasma F2-isoprostanes and lipid peroxides as described next.

**F2-Isoprostanes**

Measurements were done as previously described (23). Briefly, blood was collected into a lithium-heparin tube containing indomethacin, centrifuged, and reacted with butylated hydroxytoluene, and the lipids were released with potassium hydroxide. After the addition of a formate buffer and centrifugation, the supernatant fluid was subject to solid-phase extraction with the addition of F2-isoprostane-d4 as an internal standard by using an Oasis HLB extraction cartridge preconditioned with methanol and 10 mmol formate buffer/L (pH 3.0) for isoprostane extraction. F2-Isoprostanes were eluted by washing the cartridge with 2 mL hexane-ethyl acetate-propan-2-ol and then analyzed by gas chromatography–mass spectrometry. The derivitization protocol used pentafluorobenzyl bromide and N,N-diisopropylethylamine to create pentafluorobenzyl-trimethylsilyl derivatives, which were analyzed on an Agilent 6890 series gas chromatograph (in NCI mode) coupled with a 5973 mass spectrometer (Agilent Technologies) by using methane as the reagent gas. Chromatography was carried out on a SPB-1701 column (30 m × 0.25 mm; film thickness, 0.25 μm; Supelco Inc) by using helium as the carrier gas. Selected ion monitoring was performed to monitor m/z 569 and 573 for F2-isoprostanes and the internal standard, respectively. Peak identification was based on the comparison of the relative retention indexes with the internal standard, and the concentration of F2-isoprostanes in the sample was calculated by using the ratio of the peak height of m/z 569 to that of m/z 573.

**Lipid peroxides**

Lipid peroxides were measured by using spectrophotometry as previously described (24).

**Calculations**

The FSR of erythrocyte glutathione (FSR_{GSH}) per day was calculated according to the precursor-product equation as described below:

\[
FSR_{GSH}(\% /d) = \frac{(IR_G - IR_N)}{(IR_{GSH} \times 1200)} (t_7 - t_5)
\]

where \(IR_G\) = IR_{GSH} is the increase in the isotope ratio of erythrocyte glutathione–bound glycine between the fifth and seventh hours of infusion, when the isotope ratio of erythrocyte free glycine, \(IR_{GSH}\), has reached a steady state.

The ASR of erythrocyte glutathione per day was calculated as follow:

\[
ASR = \frac{erythrocyte \ GSH \ concentration \times FSR}{6}
\]

ASR is expressed as mmol glutathione/L packed erythrocytes per day.

**Statistics**

The data are expressed as means ± SEs. The statistical analysis was performed by using one-factor ANOVA, and a post hoc Bonferroni correction was applied. The data were analyzed by using Statmate statistical software (GraphPad software version 4). The results were considered to be significant at \(P < 0.05\).

**RESULTS**

**Baseline characteristics**

The age range of the elderly subjects was 60–75 y, and that of the young control subjects was 20–40 y. The young subjects had a lower body mass index than did the elderly subjects. No differences in hematocrit, hemoglobin concentrations, renal function, or liver enzymes were found between the 2 groups. Younger subjects were euglycemic, but the elderly subjects had impaired glucose tolerance and higher concentrations of fasting glucose and glycated hemoglobin. No differences in hematologic variables, renal function, or liver enzymes were found between the 2 groups or before and after glutathione precursor supplementation in the elderly group (Table 1).

**RBC concentrations of glycine, cysteine, and glutamate**

Compared with younger control subjects, elderly subjects had 55.2% lower concentrations of cysteine (20.2 ± 1.3 mmol/L; \(P < 0.01\)) and 24.4% lower concentrations of glutamate (26.2 ± 1.4 mmol/L; \(P < 0.05\)) in RBCs. After the elderly subjects received cysteine and glycine supplementation, RBC glycine concentrations increased by 117.6% (218.0 ± 23.7 mmol/L; \(P < 0.01\)) and 24.4% lower concentrations of cysteine (26.2 ± 1.4 compared with 19.8 ± 1.3, \(P < 0.05\)) in RBCs. After the elderly subjects received cysteine and glycine supplementation, RBC glycine concentrations increased by 117.6% (218.0 ± 23.7 compared with 528.6 ± 33.5, \(P < 0.001\), and RBC cysteine concentrations increased by 55.1% (19.8 ± 1.3 compared with 30.6 ± 2.2 mmol/L; \(P < 0.01\)). No differences in RBC glutamate concentrations were found between the young and presupplemented elderly subjects (463.1 ± 69.0 compared with 464.0 ± 115.3 mmol/L; NS) (Table 2).

**RBC concentrations and kinetics of glutathione**

Compared with the young control subjects, the elderly subjects (before supplementation) had 46.2% lower RBC glutathione...
concentrations at baseline (2.08 ± 0.12 compared with 1.12 ± 0.18 mmol glutathione/L RBC; P < 0.05) (Figure 1A). The elderly subjects also had a 44.9% slower glutathione FSR (83.14 ± 6.43 compared with 45.80 ± 5.69%/d; P < 0.01) and a 68.2% slower ASR (1.73 ± 0.16 compared with 55.0 ± 0.12 mmol glutathione/L RBC/d; P < 0.01) at baseline (Figure 1, B and C). After treatment with cysteine and glycine for 14 d, compared with presupplementation values, the elderly subjects in the postsupplemented state had a 94.6% higher RBC glutathione concentration (from 1.12 ± 0.18 to 2.18 ± 0.35 mmol glutathione/L RBC; P < 0.05) and a 78.8% higher FSR (from 45.80 ± 5.69 to 81.91 ± 7.70%/d; P < 0.01), resulting in a 230.9% higher ASR (from 0.55 ± 0.12 to 1.82 ± 0.39 mmol glutathione/L RBC per day; P < 0.01). No statistical differences were found between young control and elderly subjects in the postsupplemented state for RBC glutathione concentrations, FSR, and ASR, and these values in the elderly group postsupplementation were similar to those seen in the young control subjects (Figure 1, A–C).

Compared with the young control subjects, the elderly also had significantly lower RBC glutathione:GSSG ratios at baseline before receiving supplements (16.1 ± 2.3 compared with 18.9 ± 2.1 with 0.15 ± 0.05 mmol GSSG/L RBC; P < 0.05). Supplementation the elderly group with cysteine and glycine (0.15 ± 0.05 compared with 0.13 ± 0.05 mmol GSSG/L RBC; NS) did not result in any changes. Correspondingly, when compared with young control subjects, the elderly subjects had significantly lower glutathione:GSSG ratios at baseline before receiving supplements (18.9 ± 2.1 compared with 7.4 ± 2.3; P < 0.01). After the elderly group received dietary supplementation, their glutathione:GSSG ratios were significantly increased (7.4 ± 2.3 compared with 16.1 ± 4.3, P < 0.05). The glutathione:GSSG ratios were not significantly different between young subjects (at baseline, without supplementation) and elderly subjects (post supplementation) (Table 2).

Because obesity per se has been linked to diminished antioxidant levels and increased oxidant damage (25, 26), a subgroup analysis was performed to exclude the impact of BMI on glutathione kinetics by comparing 5 BMI-matched elderly and young subjects. In this subgroup despite both groups of subjects having similar BMIs (in kg/m²; 26.7 ± 0.7 compared with 27.4 ± 0.6, P = NS), the elderly subjects still had significantly lower

### Table 1

Baseline clinical, hematologic, and biochemical characteristics before and after supplementation with glutathione precursors

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young control subjects</th>
<th>Elderly subjects (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before supplementation</td>
<td>After supplementation</td>
</tr>
<tr>
<td>Age (y)</td>
<td>39.8 ± 1.0²</td>
<td>70.3 ± 2.4</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.9 ± 2.1</td>
<td>82.9 ± 5.4</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.7 ± 0.6</td>
<td>29.8 ± 1.4</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td>14.2 ± 0.3</td>
<td>13.8 ± 0.3</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>42.4 ± 0.7</td>
<td>41.4 ± 0.8</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/L)</td>
<td>4.9 ± 0.2²</td>
<td>5.9 ± 0.3³</td>
</tr>
<tr>
<td>Hb A1c (%)</td>
<td>5.2 ± 0.1²</td>
<td>5.7 ± 0.1</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>13.8 ± 1.0</td>
<td>13.1 ± 1.0</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>1.0 ± 0.0</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>22.6 ± 3.2</td>
<td>28.1 ± 1.7</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>28.1 ± 4.4</td>
<td>25.8 ± 1.5</td>
</tr>
</tbody>
</table>

All values are means ± SEMs. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Hb A1c, glycated hemoglobin.

### Table 2

Red blood cell concentrations in young control (unsupplemented) and elderly (before and after supplementation with glutathione precursors) subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young control subjects</th>
<th>Elderly subjects (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before supplementation</td>
<td>After supplementation</td>
</tr>
<tr>
<td>Glycine (μmol/L)</td>
<td>486.7 ± 28.3²</td>
<td>218.0 ± 23.7⁴</td>
</tr>
<tr>
<td>Cysteine (μmol/L)</td>
<td>26.2 ± 1.4²</td>
<td>19.8 ± 1.3³</td>
</tr>
<tr>
<td>Glutamate (μmol/L)</td>
<td>463.1 ± 69.0</td>
<td>464.0 ± 115.3</td>
</tr>
<tr>
<td>GSSG (mmol/L)</td>
<td>0.11 ± 0.04⁴</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>Glutathione:GSSG ratio</td>
<td>18.9 ± 2.1⁴</td>
<td>7.4 ± 2.3⁵</td>
</tr>
</tbody>
</table>

All values are means ± SEMs. GSSG, oxidized glutathione.

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2 ¹ Significantly different from the elderly before supplementation (1-factor ANOVA and Bonferroni-corrected t tests).

3 P < 0.01, 4 P < 0.05.

5 ² Significantly different from the elderly after supplementation (1-factor ANOVA and Bonferroni-corrected t tests).

6 P < 0.001, 7 P < 0.01, 8 P < 0.05.
different from the elderly before supplementation, and lower ASRs (1.50 \( \pm \) 0.2 compared with 0.7 \( \pm \) 0.4 mol glutathione/L RBC; \( P < 0.05 \)), a lower glutathione FSR (80.1 \( \pm \) 8.9 compared with 33.0 \( \pm \) 5.2% /d, \( P < 0.05 \)), a lower ASR (1.9 \( \pm \) 0.3 compared with 0.2 \( \pm \) 0.1 mmol glutathione/L RBC/d; \( P < 0.01 \)), and higher oxidant damage (1.6 \( \pm \) 0.5 compared with 8.1 \( \pm \) 0.4 \( \mu \)mol lipid peroxides/L plasma; \( P < 0.01 \)). This suggests that the alterations in glutathione kinetics in the elderly group are the result of aging per se and not the result of glycemic changes.

Oxidant variables

The slower rates of glutathione synthesis in the elderly subjects at baseline were associated with significantly higher concentrations of markers of oxidative damage (plasma reactive oxygen metabolites, plasma F₂-isoprostanes, and lipid peroxides; Table 3), than those in the young control subjects. After the elderly subjects received 14 d of treatment with dietary cysteine and glycine supplements, a significant decrease in these variables was observed, which resulted in post-supplementation values that were similar to those of the young control subjects.

DISCUSSION

In this study we investigated the kinetics of glutathione, which are critical to the development and prevention of oxidative stress in aged humans. First, we measured the effect of aging on glutathione synthesis and on oxidative stress by comparing in vivo synthesis and concentrations of erythrocyte glutathione between young and elderly humans. We then correlated these to plasma markers of oxidant damage. Second, we measured the ability of elderly humans to correct the defect in glutathione synthesis and decrease oxidant damage after supplementing their diets with the glutathione precursor amino acids cysteine and glycine for 14 d. We found that the rate of intracellular glutathione synthesis and glutathione concentrations were markedly lower in the elderly than in the young humans. This glutathione deficiency was associated with increased oxidant stress, because plasma markers of oxidant damage were elevated in the elderly. Treating elderly subjects with brief oral supplementation with cysteine and glycine improved both the FSR and ASR of glutathione to levels observed in young humans. An important consequence of this increase in glutathione synthesis was a significant decline in both oxidative stress and plasma markers of oxidant damage in the elderly to levels similar to those in the young control subjects. These data indicate that a major contributor to glutathione deficiency and associated oxidative stress in aging humans is a diminished rate of glutathione synthesis, which in turn is due to low availability of its precursor amino acids. Replenishing the supply of cysteine and glycine by oral supplementation is effective at restoring glutathione concentrations and reducing oxidative stress to levels observed in young healthy humans.

Several diseases of aging are associated with oxidative stress and increased oxidant damage (1–7), but the underlying mechanisms are not well understood. Although glutathione is the largest component of intracellular antioxidants, and glutathione concentrations between young control subjects and un-supplemented elderly subjects (104.3 \( \pm \) 1.2 compared with 106.0 \( \pm \) 3.6 mg/dL; NS), the elderly subjects still had a significantly lower concentration of glutathione (2.3 \( \pm \) 0.2 compared with 0.7 \( \pm \) 0.4 mol glutathione/L RBC; \( P < 0.05 \)), a lower glutathione FSR (80.1 \( \pm \) 8.9 compared with 33.0 \( \pm \) 5.2% /d, \( P < 0.05 \)), a lower ASR (1.9 \( \pm \) 0.3 compared with 0.2 \( \pm \) 0.1 mmol glutathione/L RBC/d; \( P < 0.01 \)), and higher oxidant damage (1.6 \( \pm \) 0.5 compared with 8.1 \( \pm \) 0.4 \( \mu \)mol lipid peroxides/L plasma; \( P < 0.01 \)). This suggests that the alterations in glutathione kinetics in the elderly group are the result of aging per se and not the result of glycemic changes.

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FIGURE 1. Mean (±SEM) glutathione (GSH) concentrations (A), GSH fractional synthesis rates (B), and GSH absolute synthesis rates (C) in young control (n = 8) and elderly (n = 8) subjects before and after supplementation with cysteine or glycine. Values are significantly different at \( P < 0.05 \) on the basis of 1-factor ANOVA and Bonferroni-corrected \( t \) tests. A: *Significantly different from the elderly before supplementation, \( P < 0.05 \); †not significantly different from the elderly after supplementation; ‡significantly different from the elderly before supplementation, \( P < 0.05 \). B: †Significantly different from the elderly before supplementation, \( P < 0.01 \); ‡not significantly different from the elderly after supplementation; ‡significantly different from the elderly after supplementation, \( P < 0.01 \). C: †Significantly different from the elderly before supplementation, \( P < 0.01 \); ‡not significantly different from the elderly after supplementation; ‡significantly different from the elderly after supplementation, \( P < 0.01 \). RBC, red blood cells.

concentrations of glutathione (2.0 \( \pm \) 0.1 compared with 1.2 \( \pm \) 0.2 mmol glutathione/L RBC; \( P < 0.05 \)), lower FSRs of glutathione (74.2 \( \pm \) 5.8 compared with 47.3 \( \pm \) 8.8%/d; \( P < 0.05 \)), and lower ASRs (1.50 \( \pm \) 0.2 compared with 0.6 \( \pm \) 0.2 mmol glutathione/L RBCs/d; \( P < 0.01 \)), and higher oxidant damage (1.8 \( \pm \) 0.3 compared with 5.4 \( \pm \) 1.3 \( \mu \)mol lipid peroxides/L plasma; \( P < 0.05 \)). This suggests that the changes in glutathione kinetics in the elderly group are the result of aging per se and not to their overall higher BMI.

Because hyperglycemia per se has been reported to decrease glutathione synthesis (27, 28), we performed a second subgroup analysis to exclude the effect of elevated fasting glucose on glutathione kinetics by comparing 3 elderly and young subjects matched for fasting blood glucose in the impaired glucose tolerant range. In this subgroup comparison, despite similar fasting glucose concentrations between young control subjects and un-supplemented elderly subjects (104.3 \( \pm \) 1.2 compared with 106.0 \( \pm \) 3.6 mg/dL; NS), the elderly subjects still had a significantly lower concentration of glutathione (2.3 \( \pm \) 0.2 compared with 0.7 \( \pm \) 0.4 mol glutathione/L RBC; \( P < 0.05 \)), a lower glutathione FSR (80.1 \( \pm \) 8.9 compared with 33.0 \( \pm \) 5.2% /d, \( P < 0.05 \)), a lower ASR (1.9 \( \pm \) 0.3 compared with 0.2 \( \pm \) 0.1 mmol glutathione/L RBC/d; \( P < 0.01 \)), and higher oxidant damage (1.6 \( \pm \) 0.5 compared with 8.1 \( \pm \) 0.4 \( \mu \)mol lipid peroxides/L plasma; \( P < 0.01 \)). This suggests that the alterations in glutathione kinetics in the elderly group are the result of aging per se and not the result of glycemic changes.
deficiency has been reported in the aging (29), no previous study has directly measured the FSR and ASR of glutathione to determine the reasons for its diminished intracellular concentration. Glutathione is a tripeptide of glutamate, cysteine, and glycine. Glutamate concentrations are known to be adequate in elderly humans because amino acid nitrogen is recycled through glutamate as an intermediary. However, little published data on cysteine and glycine in elderly humans are available.

The flux of a nonessential amino acid consists of its release from protein breakdown and from de novo synthesis. The diminished intracellular concentrations of cysteine and glycine in elderly subjects could be a result of slower body protein turnover or decreased de novo synthesis. There is evidence for a deceleration of overall protein turnover with aging (30–34), which suggests that the supply of cysteine and glycine could be decreased. In addition, in vivo studies have shown that when healthy adults are fed diets either deficient in sulfur amino acids (35) or containing reduced amounts of total protein (22), glutathione turnover is suppressed. Furthermore, animals fed diets lacking glutathione precursor amino acids, especially cysteine, develop glutathione deficiency (36–38). Collectively, these data, together with the current findings, indicate that intracellular glutathione deficiency in aging is due in large part to decreased in vivo synthesis secondary to a decreased supply of the precursor amino acids, cysteine and glycine. The metabolic mechanisms underlying the decreased availability of cysteine and glycine in aging need to be elucidated.

Because it has been suggested that obesity per se could influence oxidant-antioxidant homeostasis (25, 26), and because the BMI of the 2 groups differed significantly, we performed a subgroup analysis matching subjects for BMI and comparing glutathione kinetics. The results show that elderly humans still had lower glutathione concentrations, significantly slower glutathione FSRs and ASRs, and increased oxidant damage. Hence it appears that increased age, not obesity, was the primary reason for the altered glutathione kinetics in our elderly subjects.

In elderly humans, a deficiency of glutathione precursors predisposes not only to glutathione deficiency, but also to oxidative stress and oxidant damage. The observation that improving glutathione synthesis and concentrations resulted in a reduction in oxidant damage suggests that the primary reason for oxidative stress in aging is glutathione deficiency. Furthermore, the supplements used were not associated with any adverse effects and did not include any alterations in renal function or liver enzymes. These data could provide a sound basis to develop a simple, safe, and inexpensive nutritional intervention to improve glutathione production and reduce oxidant damage in aging humans. Long-term studies using this simple intervention are necessary to determine whether these effects are sustained and could attenuate the progression of chronic diseases associated with aging.

In conclusion, severe glutathione deficiency is a critical mechanism underlying the oxidative stress and oxidant damage associated with human aging. Our data suggest that the glutathione deficiency in aged humans occurs in large part because of a markedly diminished supply of the precursors cysteine and glycine rather than a diminished capacity to synthesize glutathione. Supplementing the diets of elderly humans with these amino acids for 14 d stimulated glutathione synthesis to rates comparable with those of younger subjects and restored intracellular concentrations. This in turn was associated with a significant reduction in oxidant damage. These findings may have important clinical implications for the treatment of diseases in elderly humans that are linked to increased oxidative stress.

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