Glutathione Synthesis Is Diminished in Patients With Uncontrolled Diabetes and Restored by Dietary Supplementation With Cysteine and Glycine

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OBJECTIVE — Sustained hyperglycemia is associated with low cellular levels of the antioxidant glutathione (GSH), which leads to tissue damage attributed to oxidative stress. We tested the hypothesis that diminished GSH in adult patients with uncontrolled type 2 diabetes is attributed to decreased synthesis and measured the effect of dietary supplementation with its precursors cysteine and glycine on GSH synthesis rate and oxidative stress.

RESEARCH DESIGN AND METHODS — We infused 12 diabetic patients and 12 nondiabetic control subjects with [$^2H_2$]-glycine to measure GSH synthesis. We also measured intracellular GSH concentrations, reactive oxygen metabolites, and lipid peroxides. Diabetic patients were restudied after 2 weeks of dietary supplementation with the GSH precursors cysteine and glycine.

RESULTS — Compared with control subjects, diabetic subjects had significantly higher fasting glucose (5.0 ± 0.1 vs. 10.7 ± 0.5 mmol/l; $P < 0.001$), lower erythrocyte concentrations of glycine (514.7 ± 33.1 vs. 403.2 ± 18.2 μmol/l; $P < 0.01$), and cysteine (25.2 ± 1.5 vs. 17.8 ± 1.5 μmol/l; $P < 0.01$); lower concentrations of GSH (6.75 ± 0.47 vs. 1.65 ± 0.16 μmol/g Hb; $P < 0.001$); diminished fractional (79.21 ± 5.75 vs. 44.86 ± 2.87%/day; $P < 0.001$) and absolute (5.26 ± 0.61 vs. 0.74 ± 0.10 μmol/g Hb/day; $P < 0.001$) GSH synthesis rates; and higher reactive oxygen metabolites (286 ± 10 vs. 403 ± 11 Carratelli units [UCarr]; $P < 0.001$) and lipid peroxides (2.6 ± 0.4 vs. 10.8 ± 1.2 pg/ml; $P < 0.001$). Following dietary supplementation in diabetic subjects, GSH synthesis and concentrations increased significantly and plasma oxidative stress and lipid peroxides decreased significantly.

CONCLUSIONS — Patients with uncontrolled type 2 diabetes have severely deficient synthesis of glutathione attributed to limited precursor availability. Dietary supplementation with GSH precursor amino acids can restore GSH synthesis and lower oxidative stress and oxidant damage in the face of persistent hyperglycemia.

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Diabetes is the leading worldwide cause of blindness, end-stage renal disease, and amputations. Diabetes also is associated with an elevated risk of macrovascular complications including myocardial ischemia and strokes. Although multiple pathways are involved in mediating tissue damage, including the polyol pathway, advanced glycation end product formation, protein kinase C activation, and the hexosamine pathway, a common feature is increased oxidative stress marked by elevated levels of reactive oxygen species (ROS) (1). The ability of a cell to resist damage caused by oxidative stress is determined by the capacity of an array of antioxidant defense systems, among which reduced glutathione (GSH) is the most ubiquitous and abundantly available within human cells. GSH is a tripeptide synthesized from glutamate, cysteine, and glycine in two steps catalyzed by γ-L-glutamyl-L-cysteine:glycine ligase and glutathione synthetase. Diabetes is associated with decreased cellular glutathione concentrations (2–5), but the cause of GSH deficiency currently is unknown.

Oxidative stress and ROS formation are markedly increased by uncontrolled hyperglycemia (2,6); conversely, lowering blood glucose concentrations lowers oxidative stress (7,8). Decreased oxidative stress could be an important mechanism whereby glycemic control diminishes the incidence of diabetic microvascular complications (9,10). However, there are practical limitations to blunting oxidative stress through glycemic control alone, despite strenuous attempts to implement evidence-based guidelines, a majority of patients are unable to achieve the glycemic goals (e.g., A1C <7%) advocated by the American Diabetes Association (11). Consequently, despite the clear message of landmark trials such as the Diabetes Control and Complications Trial and the UK Prospective Diabetes Study regarding the need for excellent glycemic control, diabetes remains the leading cause of blindness, renal failure, and amputations. There is an urgent need for novel strategies to reduce the rate of diabetes complications in patients unable to achieve stable glycemic control. We therefore investigated whether oxidative stress associated with low levels of GSH could be ameliorated through the alternative strategy of increasing cellular

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GSH levels in diabetic patients with uncontrolled hyperglycemia.

Because circulating concentrations of a protein depend on the balance between its rates of production and consumption, we hypothesized that GSH deficiency in uncontrolled diabetes occurs because of diminished synthesis. We further hypothesized that short-term dietary supplementation of two key amino acid precursors of GSH, glycine and cysteine, would increase intracellular GSH synthesis and concentrations and thus lower oxidative stress, despite continuing hyperglycemia. To test these hypotheses, we used stable isotope methods to compare GSH synthesis rates and concentrations within erythrocytes, as well as plasma markers of oxidant damage, in adult patients with poorly controlled type 2 diabetes matched to nondiabetic control subjects. The diabetic patients were studied before and after 14 days of dietary supplementation with cysteine and glycine.

RESEARCH DESIGN AND METHODS

— The study was approved by the institutional review board for Human Studies at Baylor College of Medicine. Twelve adults with uncontrolled type 2 diabetes (A1C 8–10%) and 12 nondiabetic control subjects matched for age, sex, and BMI were recruited. Informed consent was obtained from all subjects. All subjects were free of thyroid disorders, hypercortisolemia, liver or renal impairment and malignancy, and had no infections or major illnesses during the preceding 6 months. All had sedentary lifestyles and none consumed unusual diets or dietary supplements. All subjects were instructed to abstain from alcoholic beverages during the study.

All patients received diabetic management from their primary physicians. To prevent acute swings of blood glucose, and to achieve comparable glycemic levels before and after supplementation with cysteine and glycine, we excluded patients receiving insulin therapy and recruited only subjects who recently were diagnosed and were being treated with either lifestyle modification or oral antidiabetic agents.

Subjects were studied in the adult general clinical research center (GCRC) of the Baylor College of Medicine. After measurement of blood counts, glucose concentrations, glycosylated hemoglobin, and liver and renal profiles, subjects participated in the first infusion study. The GCRC protocol consisted of intravenous infusions of stable isotopes to measure GSH synthesis in the fasted state. All subjects were studied under baseline conditions; only the diabetic subjects were studied again after 14 days of dietary supplementation with 0.81 mmol/kg/day of cysteine (given as *n*-acetylcysteine) and 1.33 mmol/kg/day of glycine. Subjects were asked to consume their usual habitual diets from 2 weeks before beginning the study to the end of the study period.

Sterile solutions of [$^2H_2$]glycine (Cambridge Isotope Laboratories, Woburn, MA) were prepared. After a 10-h fast, subjects were admitted to the GCRC for the study, where two intravenous catheters were inserted into superficial veins for continuous infusion of the tracer solutions and blood sampling. After a basal blood sample was drawn, a primed constant intravenous infusion of [$^2H_2$]glycine (prime dose 20 μmol/kg; infusion dose 15 μmol/kg/h) was maintained for 8 h. Additional blood samples were taken at 2, 3, 4, 5, 6, 7, and 8 h for measurement of erythrocyte GSH derived glycine isotopic enrichments.

The primary outcome variables were fractional and absolute synthesis rates of GSH within erythrocytes, erythrocyte GSH, cysteine, glycine and glutamate concentrations, plasma lipid peroxide levels, and plasma oxidative stress measured as reactive oxygen metabolites.

Sample analyses

Blood chemistries. Baseline plasma samples were aliquotted into tubes for the various assays, and stored at −80°C for later analyses. Hemoglobin, reactive oxygen metabolites (DROMS), (Diacron International, Grosetto Italy), plasma chemistries, and lipid peroxides were measured.

Erythrocyte GSH analyses. Erythrocyte GSH concentration and isotopic enrichment of GSH were measured as described next (12). Briefly, duplicate aliquots of 1 ml whole blood were centrifuged to separate packed cells and measure GSH and glutamate, cysteine, and glycine, respectively. The first aliquot of packed erythrocytes was washed thrice with normal saline and 1 ml of monobromobimane (MBB) buffer (5 mmol/l MBB, 17.5 mmol/l Na_2EDTA, 50 mmol/l potassium phosphate, 50 mmol/l serine, and 50 mmol/l boric acid) was added. Cells were immediately lysed by rapid freeze-thaw with liquid nitrogen, and the erythrocyte-MBB buffer mixture was shaken and left in the dark at room temperature for 20 min for development of the GSH-MBB derivative. After adding 0.5 ml of 20% perchloric acid, the sample was centrifuged, and the supernatant containing the MBB derivative was sealed and frozen at −80°C for later analysis of GSH. Concentrations of GSSG were measured by first converting oxidized glutathione to reduced glutathione with the addition of a reducing agent (5 mmol/l dithiothreitiol) and measuring this as total GSH; the calculated difference between total GSH and reduced GSH is the concentration of GSSG.

Erythrocyte GSH was isolated as an red blood cell-free aliquot, and the concentration measured high-performace liquid chromatography (Waters, Milford, MA) using a 717 Plus autosampler complexed to a 2475 fluorescent detector and equipped with a reverse-phase ODS Hypersil column (5 μm, 4.6 × 200 mm; Waters). Elution of GSH was accomplished with a 3–13.5% acetonitrile linear gradient in 1% acetic acid (pH 4.25) at a flow rate of 1.1 ml/min. The GSH eluate was collected using a fraction collector, dried, and hydrolyzed for 4 h in 4 mol/l HCl at 110°C.

Erythrocyte free amino acids

A 1-ml aliquot of blood was centrifuged, and erythrocytes were then washed thrice with 3 ml sodium chloride solution (9 g/l). RBCs were then lysed by freeze-thaw action with the use of liquid nitrogen, and cellular proteins were precipitated by using 10% perchloric acid solution. After centrifugation, the supernatant fluid was used for erythrocyte free amino acid analysis. Before derivatization for gas chromatographic–mass spectrometric analysis, erythrocyte-free glycine was isolated by cation-exchange (Dowex 200×; Bio-Rad Laboratories, Hercules, CA) chromatography. Samples of glycine derived from erythrocyte glutathione and plasma and erythrocyte-free glycine samples were converted to the *n*-propyl ester, heptafluorobutyramide derivative. The tracer-to-tracee ratio for glycine in various samples was determined by negative chemical ionization gas chromatographic–mass spectrometric analysis with selective monitoring of ions at mass-to-charge ratios of 293–295 on an Agilent 6980 gas chromatograph complexed to a 5973 mass spectrometer (Agilent Technologies, Wilmington, DE). Erythrocyte amino acid concentrations were determined by high-performace liquid chro-

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matography analysis with a Waters system (Millipore, Milford, MA).

Oxidant markers

To assess the level of oxidative stress, the derivatives of DROMs were determined in serum. 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 alkoxy and peroxyl 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 UCarr (where 1 UCarr is equal to 0.8 mg/l hydrogen peroxide).

Lipid peroxides

Briefly, this was measured using freshly prepared buffers containing ammonium ferrous sulfate, xylenol orange, H_2SO_4, BHT in 90% vol/vol methanol, and triphenylphosphine in methanol. Standard solutions were made using 30% H_2O_2. Heparinized blood was centrifuged, and 10 μl of 10 mmol/l TPP solution was added to 90 μl of plasma (control vials) then 10 μl of methanol was added (test vials), and this solution was incubated at room temperature for 30 min. After adding appropriate buffers, each vial was incubated at room temperature for 30 min, centrifuged, and the absorbance of the supernatant was determined by spectrophotometry. The hydroperoxide content was determined from test controls and assayed against the standard curve (13).

Calculations

The fractional synthesis rate (FSR) of erythrocyte GSH (FSR_{GSH}) was calculated according to the precursor-product equation: FSR_{GSH} (%/day) = $(IR_{t7} - IR_{t5})/(IR_{rbc} \times 1{,}200/t_7 - t_5)$, where $IR_{t7} - IR_{t5}$ is the increase in the isotope ratio of erythrocyte GSH-bound glycine between the fifth and seventh hours of infusion, when the isotope ratio of erythrocyte-free glycine, IR_{rbc}, had reached a steady state. The units of FSR are percentage per day (%/day). The absolute synthesis rate (ASR) of erythrocyte GSH per day was calculated as follows: ASR = erythrocyte GSH concentration × FSR. The units of ASR are expressed as micromols of GSH per gram of hemoglobin per day.

Statistics

Data are expressed as means ± SE. An independent unpaired *t* test was used to compute differences in means between the diabetic group presupplementation and the control group and also between the diabetic group postsupplementation and the control group. Differences in outcome measures in the diabetic patients studied pre- and postsupplementation was determined using a paired *t* test. Data analysis was performed with the Statmate statistical software (GraphPad Software, LA Jolla, CA). Results were considered to be statistically significant at $P < 0.05$.

Table 1—*Clinical, hematological, and biochemical characteristics of nondiabetic control subjects and pretreatment and posttreatment data for subjects with type 2 diabetes*

Parameters	Nondiabetic subjects	Diabetic subjects pretreatment	Diabetic subjects posttreatment	*P*
Age (years)	50.4 ± 3.8	51.0 ± 3.1	51.0 ± 3.1	NS
BMI (kg/m^2)	28.0 ± 0.9	30.4 ± 0.7	30.0 ± 0.9	NS
Hemoglobin (g/l)	14.2 ± 1.8	13.7 ± 1.4	13.7 ± 1.2	NS
Fasting plasma glucose (mmol/l)	5.0 ± 0.1	10.7 ± 0.5*	10.6 ± 0.4	<0.001*
A1C (%)	5.5 ± 0.1	9.1 ± 0.2*	9.0 ± 0.2	<0.001*
Blood urea nitrogen (mmol/l)	5.3 ± 0.3	5.4 ± 0.6	5.4 ± 0.4	NS
Creatinine (μmol/l)	88.4 ± 3.4	79.6 ± 4.2	79.6 ± 3.8	NS
Alanine aminotransferase (U/l)	20.4 ± 1.2	23.8 ± 5.0	24.4 ± 4.4	NS
Aspartate aminotransferase (U/l)	17.6 ± 1.4	18.2 ± 2.4	17.9 ± 2.6	NS
Glutamate (mmol/l)	530.1 ± 88.3	451.5 ± 120.7	482.4 ± 98.2	NS
Cysteine (μmol/l)	25.2 ± 1.5	17.8 ± 1.5*	25.5 ± 1.9†	<0.01* <0.05†
Glycine (μmol/l)	514.7 ± 33.1	403.2 ± 18.2*	521.6 ± 19.4†	<0.01* <0.01†
DROMs (UCarr)	286 ± 10	403 ± 11*	359 ± 10†‡	<0.001* <0.05† <0.01‡
Lipid peroxide (μmol/l)	2.6 ± 0.4	10.8 ± 1.2*	6.2 ± 0.9†‡	<0.001* <0.01† <0.05‡

*Nondiabetic control subjects versus diabetic subjects: pre-treatment. †Diabetic subjects: pretreatment versus posttreatment. ‡Diabetic subjects: posttreatment versus nondiabtic control subjects.

RESULTS

Baseline characteristics

The average ages of the control and diabetic subjects were 50.4 ± 3.8 and 51.0 ± 3.1 years, respectively (Table 1). There were no group differences in BMI, hematocrit and hemoglobin concentrations, renal functions, or liver enzymes. The control subjects were euglycemic, whereas the diabetic subjects had significantly higher fasting glucose concentrations and glycosylated hemoglobin. There were no differences in hematologic parameters, renal function, or liver enzymes before or after cysteine and glycine supplementation in the diabetic group.

Erythrocyte GSH kinetics and concentration of glycine, cysteine, glutamate, and GSH

Compared with nondiabetic control subjects, subjects with poorly controlled diabetes had 73.8% lower erythrocyte-reduced glutathione concentrations (6.75 ± 0.47 vs. 1.65 ± 0.16 μmol/g Hb; $P < 0.001$) (Fig. 1*A*) and higher concentrations of erythrocyte-oxidized GSSG (0.10 ± 0.01 vs. 0.33 ± 0.07 μmol/g Hb; $P < 0.05$). Compared with control subjects, total glutathione concentrations (6.75 ± 0.47 vs. 1.65 ± 0.16 μmol/g Hb; $P < 0.001$) and the ratio of GSH to GSSG were both significantly lower in diabetic subjects (59.15 ± 4.12 vs. 6.30 ± 1.30; $P < 0.001$). Diabetic subjects also had 43.4% slower GSH FSR (79.21 ± 5.75 vs. 44.86 ± 2.87%/day; $P < 0.001$) and 85.5% slower ASR (5.26 ± 0.61 vs. 0.74 ± 0.10 μmol/g Hb/day; $P < 0.001$) (Figs. 1*B* and *C*). Compared with control subjects, diabetic subjects also had significantly lower RBC concentrations of glycine (514.7.7 ± 33.1 vs. 403.2 ± 18.2 μmol/l; $P < 0.01$) and cysteine (25.2 ± 1.5 vs. 17.8 ± 1.5, μmol/l; $P < 0.01$) but not glutamate (530.1 ± 88.3 vs. 451.5 ± 120.7 μmol/l; $P = 0.61$).

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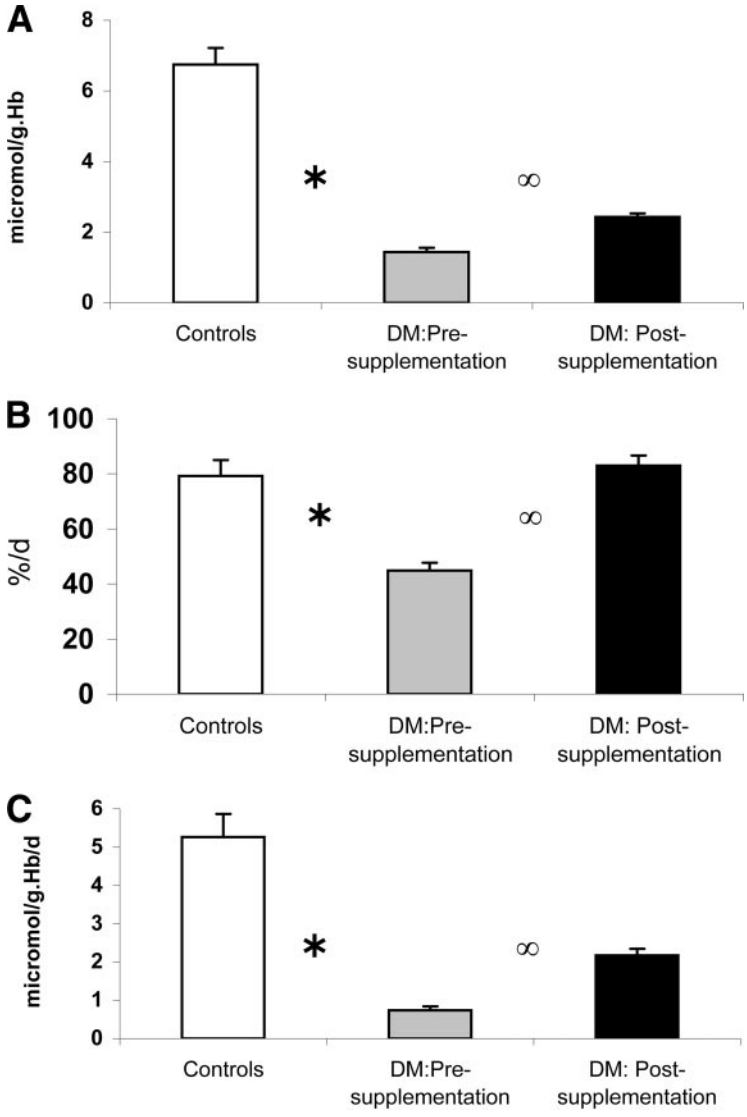

Figure 1—A: *Erythrocyte GSH concentrations (μmol GSH/g Hb).* B: *GSH fractional synthesis rate (%/day).* C: *GSH absolute synthesis rate (μmol GSH/g Hb /day). RBC = erythrocytes. *Control subjects versus diabetes pretreatment,* $P < 0.001$; ∞*Diabetes pretreatment versus diabetes posttreatment,* $P < 0.001$).

Diabetic subjects received treatment with cysteine and glycine supplementation for 14 days, and this led to 85.1% increase in erythrocyte GSH FSR (44.86 ± 2.87 vs. 83.03 ± 3.66%/day; $P < 0.001$), resulting in a 64.4% increase in erythrocyte GSH concentrations (1.65 ± 0.16 vs. 2.72 ± 0.15 μmol/g Hb; $P < 0.001$) and a 193.8% increase in GSH ASR (0.74 ± 0.10 vs. 2.17 ± 0.17 μmol GSH/g Hb/day; $P < 0.001$). Precursor supplementation increased GSH FSR to the level of nondiabetic control subjects and led to significant increases in GSH concentrations and GSH ASR. However, when GSH concentrations and ASR were compared between diabetic subjects and control subjects diabetic after supplementation, they remained at 59.7% (2.72 ± 0.15 vs. 6.75 ± 0.47 μmol GSH/g Hb; $P < 0.001$) and 58.7% (0.74 ± 0.10 vs. 5.26 ± 0.61 μmol GSH/g Hb/day; $P < 0.001$) lower, respectively, in the diabetic subjects postsupplementation (Figs. 1*A–C*). Compared with presupplementation values, the post supplementation values of erythrocyte concentrations of GSSG (0.33 ± 0.07 vs. 0.28 ± 0.07 mmol/g Hb; P = NS) and the ratio of GSH to GSSG (6.30 ± 1.30 vs. 12.63 ± 3.15; P = NS) did not change significantly.

Plasma oxidant parameters

The slower rates of GSH synthesis in the diabetic subjects at baseline were associated with significantly higher concentrations of markers of oxidative damage (plasma DROMs and lipid peroxides) (Table 1) than in control subjects. After 14 days of cysteine and glycine supplementation, there was a significant fall in these parameters in the diabetic subjects, although not to the levels observed in the nondiabetic control subjects.

CONCLUSIONS — The results of this study demonstrate that intracellular concentrations of GSH, as well as of its precursor amino acids cysteine and glycine, are decreased in adult patients with poorly controlled type 2 diabetes compared with nondiabetic subjects. Whereas previous studies also have found that diabetic patients have deficient cellular levels of GSH (3–5), the present data go further to demonstrate that the GSH deficiency seems to be attributed to a markedly lower synthetic rate of GSH and is associated with increased oxidant stress and elevated plasma markers of oxidant damage. Fourteen days of oral dietary supplementation with cysteine and glycine in diabetic subjects restored the fractional synthesis rates of GSH to those observed in nondiabetic control subjects. This was accompanied by significant declines in both oxidative stress and plasma markers of oxidant damage.

Suboptimal restoration of the absolute synthesis rate of GSH after cysteine and glycine supplementation, despite full normalization of its fractional synthesis, suggests that there is also persistent, accelerated consumption of GSH in diabetic patients with uncontrolled hyperglycemia. Furthermore, although the levels of the oxidative stress markers were significantly diminished after 2 weeks of precursor supplementation, they did not attain the low levels observed in the nondiabetic control subjects. A longer duration of intervention may be required to normalize synthesis and intracellular concentrations of GSH.

GSH is a tripeptide of glutamate, cysteine, and glycine, and measurement of these amino acids within erythrocytes showed low levels of cysteine and glycine but not of glutamate. Deficiency of cysteine and glycine in diabetic humans also has been reported previously in the literature (14). Because catabolic processes of virtually all amino acids cycle through glutamate production, it is not surprising that glutamate levels were not different from those of nondiabetic subjects. The deficiency in cysteine and glycine is in-

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triguing because both these amino acids traditionally are considered to be "nonessential," meaning that they can be synthesized endogenously. Why are patients with uncontrolled type 2 diabetes unable to synthesize cysteine and glycine adequately? The plasma flux of a nonessential amino acid results from the sum of its rate of release from protein breakdown, de novo synthesis, and dietary absorption. Studies evaluating protein turnover in diabetic patients have reported abnormal overall protein balance (15), and disruption of metabolic pathways resulting from hyperglycemia could impose higher dietary protein requirements (15). Hence, a combination of impaired protein turnover and dietary deficiency could underlie inadequate availability of cysteine and glycine for GSH synthesis in type 2 diabetes. Additional studies are needed to evaluate the mechanisms underlying decreased availability of cysteine and glycine in type 2 diabetes.

Although the exact mechanisms underlying cysteine and glycine deficiency in type 2 diabetes are not clear, the net result of this deficiency could have a domino effect leading to oxidative stress and tissue damage. First, GSH synthesis would be blunted, and this would result in a critical imbalance between GSH-driven antioxidant protection and the harmful effects of unopposed elevated oxidative stress in uncontrolled diabetes. Deficiency of sulfur amino acids (16) or protein content (17) in the diets of healthy humans has been previously shown to result in suppression of GSH turnover in vivo. Further, animals fed diets specifically lacking GSH precursor amino acids, especially cysteine, develop GSH deficiency (18–21). Together with the present findings, these data indicate that an important underlying cause for intracellular GSH deficiency in diabetes is decreased in vivo GSH synthesis because of a reduced availability of the precursor amino acids cysteine and glycine.

Could the lower concentrations of GSH be a result of impaired cycling between its oxidized and reduced isoforms, with a greater proportion of glutathione trapped as the oxidized form? To answer this question, we also measured concentrations of total GSH by converting oxidized glutathione to its reduced isoform with a reducing agent, and found that subjects with diabetes still had significantly lower concentrations of total glutathione. This suggests that in poorly controlled type 2 diabetes, there is a true deficiency of glutathione.

Sustained hyperglycemia is linked to increased oxidative stress, and with an increased risk of diabetic microvascular and macrovascular complications. Mechanisms implicated in hyperglycemia-driven tissue damage in diabetes include abnormal signaling through protein kinase C, elevated advanced glycation end products, and the aldose reductase pathway (1). ROS is known to stimulate these pathways by activation of aldose reductase, protein kinase C isoforms, and nuclear factor-κB and induction of diacylglycerol and advanced glycation end-product formation (22). Lowering levels of mitochondrial ROS (and thereby oxidative stress) successfully prevents activation and induction of these mechanisms (23). Because ROS production is increased by hyperglycemia, optimizing glycemic control in diabetic subjects should decrease ROS, but, in clinical practice, normalizing glycemia remains a major challenge in diabetes management. It is estimated that up to 55% of diabetic patients in the U.S. do not attain the American Diabetes Association's recommended glycemic goals, and 67% do not attain the more stringent glycemic targets of the American Association of Clinical Endocrinologists. Therefore, interventions directly aimed at lowering ROS-mediated oxidative stress are needed to prevent diabetic tissue damage even in the presence of hyperglycemia. The present data suggest that increasing GSH levels with oral precursor supplementation is a viable intervention to target diabetic oxidative stress directly and could constitute a novel, safe, and inexpensive form of nutritional treatment. When used as an adjunct to standard glycemic management, this approach could significantly attenuate tissue damage due to oxidative stress in patients with diabetes.

In conclusion, these data show that an important reason underlying elevated oxidative stress in type 2 diabetes is deficiency of glutathione, which occurs because of the decreased synthesis caused by limited availability of the precursor amino acids cysteine and glycine. Dietary supplementation of these amino acid precursors restores fractional synthesis of GSH and significantly reduces oxidative stress and markers of oxidant damage. Providing supplemental cysteine and glycine in the diet could be a safe, inexpensive, and innovative approach to increase GSH synthesis and concentrations and thus combat oxidative stress and prevent chronic complications in patients with diabetes.

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R.V.S. was responsible for study design, conduction, supervision of the study, sample and data analyses, manuscript preparation, and submission. S.V.M. contributed to study conduction and sample analyses. S.G.P. analyzed samples. A.G.P. recruited subjects and conducted studies. V.T.R. analyzed samples. A.B. reviewed and edited the manuscript. F.J. contributed to study design and manuscript review.

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