Influence of Reduced Glutathione Infusion on Glucose Metabolism in Patients With Non–Insulin-Dependent Diabetes Mellitus

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To evaluate the relationship between oxidative stress and glucose metabolism, insulin sensitivity and intraerythrocytic reduced glutathione (GSH)/oxidized glutathione (GSSG) ratio were measured in 10 non–insulin-dependent diabetes mellitus (NIDDM) patients and 10 healthy subjects before and after the intravenous administration of GSH. In particular, after baseline insulin sensitivity was assessed by a 2-hour euglycemic hyperinsulinemic clamp, either glutathione (1.35 g · m^2 · min^{-1}) or placebo (saline) were infused over a period of 1 hour. The same protocol was repeated at a 1-week interval, in cross-over, according to a randomized, single-blind design. In healthy subjects, baseline intraerythrocytic GSH/GSSG ratio ($P < .0005$) and total glucose uptake ($P < .005$) were significantly higher than in NIDDM patients. In the same subjects, GSH infusion significantly increased total glucose uptake (from 37.1 ± 6.7 μmol · kg^{-1} · min^{-1} to 39.5 ± 7.7 μmol · kg^{-1} · min^{-1}, $P < .05$), whereas saline infusion was completely ineffective. In addition, the mean intraerythrocytic GSH/GSSG ratio significantly increased after GSH infusion (from 21.0 ± 0.9 to 24.7 ± 1.3, $P < .05$). Similar findings were found in diabetic patients, in whom GSH infusion significantly increased both total glucose uptake (from 25.3 ± 9.0 μmol · kg^{-1} · min^{-1} to 31.4 ± 10.0 μmol · kg^{-1} · min^{-1}, $P < .001$) and intraerythrocytic GSH/GSSG ratio (from 14.8 ± 4.1 to 21.7 ± 6.7, $P < .01$). Pooling diabetic patients and controls, significant correlations were found between intraerythrocytic GSH/GSSG ratio and total glucose uptake ($r = .425$, $P < .05$), as well as between increments of the same variables after GSH infusion ($r = .518$, $P < .05$). In conclusion, our data support the hypothesis that abnormal intracellular GSH redox status plays an important role in reducing insulin sensitivity in NIDDM patients. Accordingly, intravenous GSH infusion significantly increased both intraerythrocytic GSH/GSSG ratio and total glucose uptake in the same patients.

THE TRYPEPTIDE glutathione (GSH) is present in various human biological fluids and cells.[1,2] The pathophysiological relevance of this compound is related to its involvement in protein and nucleotide synthesis, as well as to its antioxidant properties.[3-6]

In this regard, it is well known that free radical production is increased in non–insulin-dependent diabetes mellitus (NIDDM) patients[7,8] and might play a role in the genesis of late diabetic complications.[9] Despite this, the mechanisms responsible for the linkage between increased oxidative stress and impaired glucose metabolism are still unclear.

In this context, pharmacological doses of vitamin E reduced oxidative stress and improved insulin action.[10] Moreover, changes in plasma GSH/oxidized glutathione (GSSG) ratio affected beta-cell response to glucose and improved peripheral insulin action in healthy subjects and in patients with NIDDM.[11] Thus, increased circulating GSH levels reflect a reduced oxidative stress and an improved insulin sensitivity.

However, it is noteworthy that GSH is the major redox buffer in cytosol,[12,13] whereas the GSH/GSSG ratio is crucial to maintain a normal cell metabolism.[14,15] Therefore, while intracellular GSH reflects the rate of activity of the GSH redox status, plasma GSH levels are merely secondary to trypeptide spill-over (ie, from cells into the bloodstream). Thus, the role of an altered GSH redox status in glucose metabolism could be better defined by evaluating intracellular GSH levels, rather than its circulating concentrations.

To address this topic, insulin sensitivity and the intraerythrocytic GSH/GSSG ratio were measured in both NIDDM patients and healthy subjects at baseline, as well as after changes in the ratio were obtained by intravenous administration of GSH.

MATERIALS AND METHODS

Subjects

The study population consisted of 10 (seven male, three female) non-obese, nonhypertensive, NIDDM outpatients and 10 (seven male, three female) healthy drug-free subjects who were interval-matched for age and body-mass index (BMI).

Inclusion criteria for both diabetic patients and controls were as follows: (1) BMI greater than 18 and less than 26 kg/m^2; (2) age greater than 40 and less than 65 years; (3) supine systolic blood pressure less than 140 mm Hg; (4) supine diastolic blood pressure less than 90 mm Hg; (5) absence of microalbuminuria (ie, <20 μg/min on three consecutive 24-hour urine collections); (6) absence of fundus oculi abnormalities; and (7) absence of concomitant diseases. All subjects were white, nonsmokers and did not drink more than 10 g of alcohol per day. At entry, all subjects received the same weight-maintaining diet (2.5 kcal/d given as 50% carbohydrates, 30% proteins, and 20% lipids). NIDDM patients were in good metabolic control (hemoglobin A_{1c} [$HbA_{1c} < 7.5\%$, plasma glucose level <7.8 mmol/L at fast and <10.0 mmol/L after meals) and assumed glibenclamide 5 mg twice daily. Each subject was given a detailed description of the study, for which individual consent was obtained.

Experimental Design

In both NIDDM patients and control subjects, insulin sensitivity was determined by the euglycemic hyperinsulinemic clamp technique.[16] As required by this method,[16] basal insulin sensitivity was first assessed by a continuous insulin infusion over a period of 2 hours. Then, either glutathione (Tationil; Boehringer Mannheim, Milan, Italy; at a rate of 1.35 $g/m^2/h$) or placebo (isotonic saline at a rate of 10 mL/h) were infused for a further hour. The same protocol was repeated at a 1-week interval, in cross-over, according to a randomized, single-blind design.

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Thus, all patients and control subjects received both the gluthatione and the saline infusions at a 1-week interval.

Baseline Assessment of Insulin Sensitivity

For this purpose, each subject underwent a 2-hour euglycemic hyperinsulinemic clamp. In brief, a polytetrafluoroethylene cannula (Venflon; Viggo, Helsingor, Sweden) was inserted retrogradely in a distant forearm vein for blood sampling. A dorsal hand vein was cannulated in a retrograde fashion and placed in a heating device to facilitate sampling of arterialized venous blood. A second cannula was inserted in a contralateral antecubital vein for insulin and glucose infusion. Following venous cannulation, all subjects were kept quiet, at a constant room temperature of 22 C° in supine position. After 60 minutes, repeated baseline blood samples for glucose and insulin assays were taken at 5- and 20-minute intervals, respectively. Then, a priming insulin dose (Actrapid HM; Novo Nordisk A/S, Bagsvaerd, Denmark) was given over a period of 10 minutes, followed by a continuous infusion (40 mU/m^2/min) over a period of 170 minutes. Euglycemia was maintained by a variable-rate infusion of 20% dextrose solution. As a quantitative estimation of insulin sensitivity, we considered the mean glucose infusion rate during the steady-state of the euglycemic clamp (ie, total glucose uptake). The metabolic clearance rate of insulin (MCRI), expressed as milliliters per square meter body surface area per minute was calculated by the following formula: MCRI = Insulin infusion rate/Increase in plasma insulin concentration above basal.

Materials

Glutathione reductase was purchased from Boehringer Mannheim. 2-Vinylpyridine was obtained from Aldrich (Steinheim, Germany). GSH, GSSG, 5-sulfosalicylic acid, 5,5′-dithiobis-2-nitrobenzoic acid, and trietanolamine were obtained from Sigma (St Louis, MO). Radioimmunoassay kits for insulin and C-peptide were obtained from Ares Serono (Milan, Italy). The Beckman Glucose Analyzer II was derived from Beckman (Fullerton, CA).

Analytical Methods

Plasma glucose concentration was determined in duplicate by the glucose oxidase method. Insulin and C-peptide were measured by radioimmunoassay.

Determination of glutathione. Heparinized venous blood samples from each subject were immediately chilled at 4°C, and 7 mL whole blood was mixed with 0.9% NaCl and layered on Hystopaque-1077 (Sigma, St Louis, MO) at baseline and at 180 minutes. After centrifugation, plasma and buffy coat were carefully removed. Then, red blood cells were washed three times with phosphate-buffered saline (pH 7.4), at 4°C, and used for glutathione estimation. Packed red blood cells (0.1 mL) were diluted with 0.1 mL isotonic saline and 0.5 mL HCl (10 mol/L). Then, erythrocytes were lysed in dry-ice acetone, thawed three times, and centrifuged for 10 minutes at 4°C. Supernatants were deproteinized with 10% 5-sulfosalicylic acid and used for total glutathione determination (GSH + GSSG) by the enzymatic method described by Anderson.[17] For GSSG determination, 0.1 mL of deproteinized supernatants (described earlier) was treated with 2 μL 2-vinylpyridine and neutralized with triethanolamine at a final pH of 6.5.[18] After 60 minutes of incubation, supernatants were used for GSH measurement. Recovery of both procedures ranged from 96% to 102% (mean, 98%). Interassay and intraassay variabilities were less than 10%.

Statistical Analysis

Statistical analysis was performed by Student's *t* tests for paired and unpaired data and the Wilcoxon signed-rank test. Repeated measures were tested by ANOVA followed by post hoc analysis (Bonferroni). Spearman rank correlation and Pearson coefficients were calculated to assess relations between variables, as appropriate. Data are presented as means ± SD. A *P* value less than .05 was regarded as statistically significant. A sample size of at least eight patients and eight controls was considered necessary to observe significant changes ($P < .05$) in total glucose uptake after GSH infusion. All calculations were made using the computer program Stat View II (Abacus Concepts, Berkeley, CA).

RESULTS

Healthy Subjects

The general characteristics, including fasting plasma glucose and insulin levels, baseline intraerythrocytic GSH and GSSG concentrations and GSH/GSSG ratio, and total glucose uptake are given in Table 1.

Steady-state plasma glucose (5.2 ± 0.8 mmol/L *v* 5.0 ± 0.2 mmol/L) and plasma insulin (641.9 ± 115.0 pmol/L *v* 635.0 ± 91.7 pmol/L) concentrations were similar between GSH and saline infusions, thus indicating that similar euglycemic and hyperinsulinemic levels were maintained in both the study sessions.

GSH infusion significantly increased total glucose uptake (from 37.1 ± 6.7 μmol · kg^{-1} · min^{-1} at baseline to 39.5 ± 7.7 μmol · kg^{-1} · min^{-1} at the end of GSH infusion, $P < .05$), while saline infusion was completely ineffective (from 37.2 ± 8.6 μmol · kg^{-1} · min^{-1} to 37.3 ± 8.0 μmol · kg^{-1} · min^{-1}, not significant [NS]).

After GSH infusion, the mean intraerythrocytic GSH/GSSG ratio significantly increased (from 21.0 ± 0.9 to 24.7 ± 1.3, $P < .05$), while intraerythrocytic GSSG significantly decreased (from 0.36 ± 0.02 μmol/gHb to 0.32 ± 0.04 μmol/g Hb, $P < .05$). Intraerythrocytic GSH content increased (from 7.6 ± 0.9 μmol/g Hb to 8.0 ± 0.8 μmol/gHb, $P < .05$) after GSH administration. As expected, saline infusion did not modify all of these variables. Therefore, compared with placebo, GSH infusion significantly increased the intraerythrocytic GSH/GSSG ratio ($P < .0001$) and total glucose uptake ($P < .005$) (Fig 1).

MCRI was not altered by either placebo (from 595.0 ± 140.0 mL/m^2/min to 620.0 ± 90.0 mL/m^2/min, NS) or GSH infusions

Table 1. Clinical and Laboratory Features of NIDDM Patients and a Group of Age-, Sex-, and BMI-Matched Healthy Controls

Feature	Healthy Subjects (n = 10)	Diabetic Patients (n = 10)
Age (yr)	48 ± 10	52 ± 7
BMI (kg/m^2)	24.3 ± 1.7	25.1 ± 0.8
Sex (male/female)	7/3	7/3
Fasting blood glucose (mmol/L)	4.9 ± 0.1	6.4 ± 0.7‡
Fasting plasma insulin (pmol/L)	94.9 ± 24.6	119.5 ± 53.5*
HbA_{1c} (%)	4.3 ± 0.9	6.0 ± 0.6‡
Systolic blood pressure (mm Hg)	130 ± 10	122 ± 18
Diastolic blood pressure (mm Hg)	82 ± 7	87 ± 3
Heart rate (bpm)	78 ± 5	81 ± 8
Total glucose uptake (μmol · kg^{-1} · min^{-1})	37.1 ± 6.7	25.3 ± 9.0†
GSH (μmol/g Hb)	7.6 ± 0.9	5.8 ± 1.4†
GSSG (μmol/g Hb)	0.36 ± 0.02	0.45 ± 0.27
GSH/GSSG ratio	21.0 ± 0.9	14.8 ± 4.1‡

NOTE. Data are means ± SD.

*$P < .05$, †$P < .005$, ‡$P < .0005$ *v* healthy subjects.

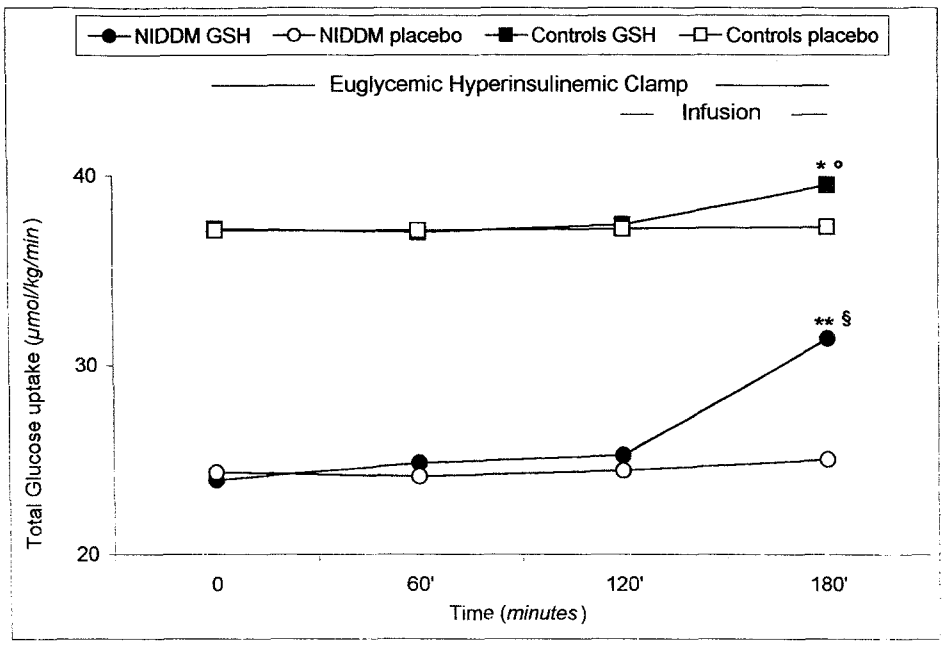

Fig 1. Total glucose uptake before and after 1-hour GSH or placebo infusions. Standard deviations are omitted for clarity. *$P < .05$ 180 *v* 120 minutes; °$P < .05$ *v* saline; **$P < .001$ 180 *v* 120 minutes; §$P < 0.03$ *v* saline.

(from 644.0 ± 104.0 mL/m²/min to 587.0 ± 77.0 mL/m²/min, NS).

Diabetic Patients

The general characteristics of NIDDM patients are given in Table 1. As shown, these patients had lower baseline total glucose uptake ($P < .005$), intraerythrocytic GSH content ($P < .005$), and GSH/GSSG ratios ($P < .0005$) than healthy controls.

During the clamp studies, steady-state plasma glucose (5.1 ± 0.3 mmol/L *v* 5.0 ± 0.1 mmol/L) and insulin concentrations (683.0 ± 138.0 pmol/L *v* 732.0 ± 7.7 pmol/L) were similar between GSH and saline infusions, thus indicating the maintenance of identical euglycemic and hyperinsulinemic conditions in both study sessions.

GSH infusion significantly increased total glucose uptake (from 25.3 ± 9.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ to 31.4 ± 10.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, $P < .001$), while saline infusion did not modify the same parameter (from 24.3 ± 2.6 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ to 25.0 ± 3.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, NS) (Fig 1).

GSH infusion induced a significant increase of the intraerythrocytic GSH/GSSG ratio (from 14.8 ± 4.1 to 21.7 ± 6.7, $P < .01$) and a decrease of intraerythrocytic GSSG (from 0.45 ± 0.27 µmol/g Hb to 0.33 ± 0.18 µmol/g Hb, $P < .01$). Intraerythrocytic GSH content also increased during the GSH infusion (from 5.8 ± 1.4 µmol/g Hb to 6.2 ± 1.0 µmol/g Hb $P < .05$). By contrast, placebo infusion did not induce significant modifications of the intraerythrocytic GSH/GSSG ratio or GSSG and GSH contents. Therefore, as observed in healthy subjects, compared with placebo, GSH infusion significantly increased the GSH/GSSG ratio ($P < .0001$) and total glucose uptake ($P < .005$) (Fig 1).

Mean MCRI did not change during placebo (from 495.0 ± 69.0 mL/m²/min to 471.0 ± 50.0 mL/m²/min, NS) and GSH infusions (from 529.0 ± 157.0 mL/m²/min to 525.0 ± 94.0 mL/m²/min, NS).

Between-Group Comparison

Compared with healthy subjects, NIDDM patients showed lower total glucose uptake, intraerythrocytic GSH levels, and GSH/GSSG ratios (Table 1). No other significant differences were found, except for percentage increments of total glucose uptake (25.8% in NIDDM patients *v* 6.4% in control subjects, $P < .001$) and GSH/GSSG ratios (47.5% in NIDDM patients *v* 18.6% in control subjects, $P < .01$) after GSH infusion.

Correlations

No significant correlations between the evaluated variables were found in healthy subjects and NIDDM patients. By contrast, analysis of pooled basal data from healthy and diabetic subjects showed significant correlations between intraerythrocytic GSH/GSSG ratio and total glucose uptake ($r = .425$ $P < .05$) (Fig 2), as well as between percentage changes of

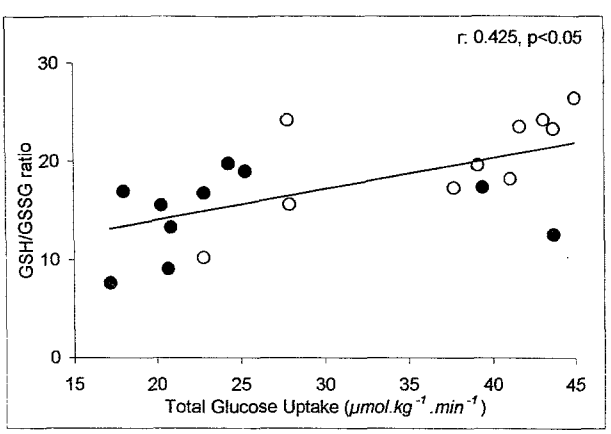

Fig 2. Correlation between baseline total glucose uptake and intraerythrocytic GSH/GSSG ratio in NIDDM patients (n = 10, ●) and healthy subjects (n = 10, ○).

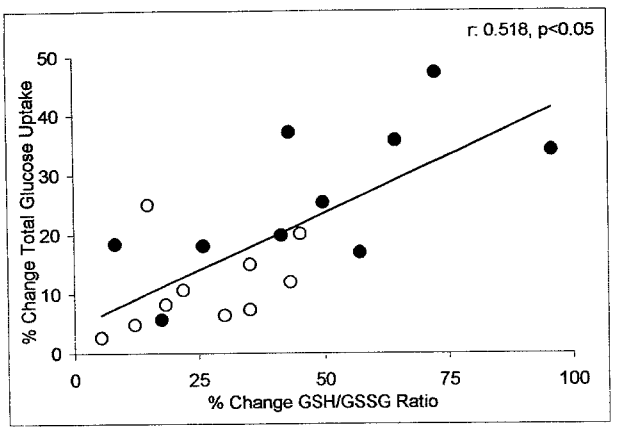

Fig 3. Correlation between changes (%) in intraerythrocytic GSH/GSSG ratio and total glucose uptake after 1-hour glutathione infusion in NIDDM patients (n = 10, ●) and healthy subjects (n = 10, ○).

intraerythrocytic GSH/GSSG ratio and total glucose uptake after GSH infusion ($r = .518$ $P < .05$) (Fig 3).

DISCUSSION

The current study confirms our previous report,[22] showing significant reductions of baseline intraerythrocytic GSH content and GSH/GSSG ratio in NIDDM patients compared with healthy subjects. Further, it demonstrates that a 1-hour intravenous GSH infusion increases the GSH/GSSG ratio in red blood cells from healthy subjects and NIDDM patients, and simultaneously leads to a significant improvement of whole-body insulin-mediated glucose uptake.

In this regard, previous studies suggested that GSH does not penetrate the cell membranes[19] and that the putative effect of GSH on glucose metabolism reflects GSH-induced modifications of cell membrane permeability.[11] Therefore, it seems difficult to understand the mechanism responsible for the observed effect of GSH administration on total glucose uptake and the intraerythrocytic GSH/GSSG ratio.

In this context, the more marked increments of the intraerythrocytic GSH/GSSG ratio after GSH infusion observed in NIDDM patients compared with control subjects should merely reflect the marked reduction of baseline intraerythrocytic GSH/GSSG ratio, which was present in the first group (Table 1). On the other hand, the relatively modest increment of intraerythrocytic GSH content, which was observed in both NIDDM patients and healthy subjects after GSH infusion, is probably due to the short period of GSH infusion. Indeed, a more prolonged GSH infusion could lead to more pronounced increments of intraerythrocytic GSH content. Unfortunately, it was impossible for us to perform such prolonged GSH infusions, due to obvious concerns about patient compliance.

In this study, we also demonstrated that the baseline intraerythrocytic GSH/GSSG ratio directly correlated with total glucose uptake (Fig 2). Furthermore, we showed that intravenous GSH administration increased insulin-mediated glucose uptake in NIDDM patients and, to a lesser degree, in normal subjects (Fig 1), thus supporting GSH infusion was responsible for the improvement of both the intraerythrocytic GSH/GSSG ratio and whole-body insulin-mediated glucose uptake. In this regard, we also observed a direct correlation between increments of total glucose uptake and the intraerythrocytic GSH/GSSG ratio after GSH infusion (Fig 3). Although this is not a prove of causality, this finding allows to suggest that the increase of the intraerythrocytic GSH/GSSG ratio manifested by NIDDM patients after GSH infusion might have improved glycolytic enzyme activities. Indeed, these latter enzymes are inhibited by free radicals.[23] Thus, an abnormal GSH redox status could impair glucose degradation through excess free radical production. By contrast, GSH administration might improve glucose homeostasis by reducing intracellular free radical concentrations. In keeping with this hypothesis, changes in GSH redox status are known to interfere with other intracellular metabolic pathways.[15] For instance, the redox state of sulfydryl protein is dependent on the GSH/GSSG ratio, which in turn can be modified by a number of physiological stimuli.[24] Therefore, the oxidation-reduction processes seem to regulate several intracellular functions by affecting protein structure and activity.

As an alternative explanation of our results, it could be speculated that the increased intraerythrocytic GSH/GSSG ratio after GSH infusion could have positively affected cellular glucose metabolism by altering the MCRI, which is known to be strongly influenced by glutathione.[25,26] In contrast to this hypothesis, our results did not show modifications of the MCRI after GSH infusion in both NIDDM and control groups.

In conclusion, the current study indicates that a 1-hour GSH infusion simultaneously improves the intraerythrocytic GSH/GSSH ratio and insulin sensitivity in NIDDM patients. Further investigations are needed to expand on these findings and clarify the molecular mechanisms responsible for GSH action on glucose metabolism.

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