Relationship Between Glutathione and Sorbitol Concentrations in Erythrocytes From Diabetic Patients

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Red blood cell (RBC) concentrations of sorbitol and reduced glutathione (GSH) were evaluated in 29 type II diabetic subjects and eight normal controls. In erythrocytes from diabetic subjects, sorbitol levels were higher (18.7 ± 1.33 *v* 11.2 ± 0.7 nmol/g hemoglobin [Hb], $P < .001$) and GSH levels were lower (5.48 ± 0.19 *v* 8.33 ± 0.24 μmol/g Hb, $P < .01$) than in nondiabetics. RBC sorbitol levels were positively correlated with fasting blood glucose ($r = .57$, $P < .001$) but not with HbA_{1c} ($r = .16$, P = NS). RBC GSH levels showed a negative correlation with fasting blood glucose ($r = -.35$, $P < .05$) and with HbA_{1c} ($r = -.34$, $P < .05$) and a significant negative correlation with RBC sorbitol levels ($r = -.62$, $P < .001$). Stepwise regression analysis highlighted the fact that the hyperglycemia-dependent increase in RBC sorbitol was significantly influenced by GSH concentrations (partial $F = 14.6$, $P < .001$). These data suggest the hypothesis that the hyperglycemia-induced enhanced activity of the polyol pathway leads to GSH depletion and, in turn, GSH depletion, reducing the glycolytic flux to pyruvate, enhances the rate of glucose metabolism through the polyol pathway. The overall effect is a progressive worsening of metabolic pseudohypoxia and depletion of GSH, resulting in lower defense against oxidative stress.

CHRONIC HYPERGLYCEMIA leads to an increased activity of the polyol pathway[1,2] and to an increased NADH/NAD ratio (metabolic pseudohypoxia).[3] An increase of the cytosolic NADH/NAD ratio is a peculiar feature of hypoxic tissues, because of impaired oxidation of NADH to NAD. In tissues exposed to elevated glucose levels, the metabolic imbalance associated with an increased production of NADH also leads to an increase in the NADH/NAD ratio despite normal tissue pO_2 (metabolic pseudohypoxia).[4] This redox imbalance is partially related to the accelerated oxidation of sorbitol to fructose by NAD-dependent sorbitol dehydrogenase and partially to increased free radical production from several different reactions.[4,5] Increased levels of lipid peroxides[6-8] and glycoxidation products[9,10] have been reported in the plasma of diabetic subjects, confirming the presence of a hyperglycemia-induced oxidative stress.

A decrease in the concentration of reduced glutathione (GSH) and an impairment of glutathione metabolism have been reported in erythrocytes from diabetic subjects[11,12] as a result of hyperglycemia-enhanced polyol pathway activity. GSH depletion has been attributed to the competition between aldose reductase and GSH reductase for NADPH cofactor and to increased oxidative stress (ie, an increased NADH/NAD ratio).[13,14] Attention has recently been focused on this oxidative stress, because it can play a major pathogenetic role in the development of chronic complications of diabetes.[15,16]

The aim of this study was to evaluate the relationship between erythrocyte concentrations of GSH and sorbitol, two key molecules in this pathway, and metabolic control.

SUBJECTS AND METHODS

Twenty-nine type II diabetic subjects and eight normal controls of similar age, gender, and body mass index ([BMI] weight in kilograms divided by height squared in meters) were studied (Table 1). Diabetic patients were treated by oral hypoglycemic agents (n = 15) or insulin (n = 14). Age at onset of diabetes and duration of disease were, respectively, 48.1 ± 2.1 years and 11.3 ± 1.4 years.

After a 12-hour overnight fast, venous blood was collected in heparin and immediately centrifuged. Packed red blood cells (RBCs) were washed three times with isotonic saline solution at 4°C and lysed by addition of 1 vol cold distilled water. Levels of hemoglobin (Hb), sorbitol, and GSH were estimated in each hemolysate. Sorbitol level was measured by a fluorometric enzymatic assay[17,18] in neutralized perchloric acid extracts, using sorbitol dehydrogenase (Sigma Chimica, Milan, Italy), and GSH was determined by spectrophotometric chemical assay.[19] Hb and glucose were determined using routine colorimetric methods (Sigma Chimica kits); HbA_{1c} (normal range, 2.9% to 4.6%) was evaluated by affinity column chromatography (Pierce, Prodotti Gianni, Milan, Italy). Sorbitol was expressed as nanomoles per gram Hb and GSH as micromoles per gram Hb.

The significance of differences between normal and diabetic groups was assessed by Student's *t* test for unpaired data. Relationships between two variables were analyzed by linear regression analysis. Multiple regression analysis followed by a stepwise procedure (partial F test) was used to study the relative dependence of both sorbitol and GSH concentration on fasting blood glucose, HbA_{1c}, age, and BMI.[20] The data are presented as the mean ± SEM.

RESULTS

The diabetic subjects showed poor metabolic control, with elevated fasting blood glycemia (9.46 ± 0.69 mmol/L; range, 4.44 to 18.3) and HbA_{1c} (6.93% ± 0.30%; range, 3.9% to 11.2%) (Table 1).

RBC sorbitol concentrations were significantly higher in diabetic subjects than in controls (18.7 ± 1.33 *v* 11.2 ± 0.7 nmol/g Hb, $P < .001$).

GSH levels in erythrocytes from diabetic subjects were significantly lower than in nondiabetics (5.48 ± 0.19 *v* 8.33 ± 0.24 μmol/g Hb, $P < .01$).

In all subjects, RBC sorbitol levels were positively corre-

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Table 1. Clinical and Metabolic Characteristics of the Study Subjects

Characteristic	Controls	Type II Diabetics	*P*
Sex (M/F)	4/4	15/14	
Age (yr)	60.1 ± 6.0	58.9 ± 2.3	—
BMI (kg/m²)	26.8 ± 2.2	27.5 ± 1.0	—
HbA_{1c} (%)	4.14 ± 0.10	6.93 ± 0.30	<.001
FBG (mmol/L)	5.48 ± 0.27	9.46 ± 0.69	<.001
GSH (μmol/g Hb)	8.33 ± 0.24	5.48 ± 0.19	<.005
Sorbitol (nmol/g Hb)	11.2 ± 0.70	18.7 ± 1.33	<.001

Data are expressed as the mean ± SE.
Abbreviation: FBG, fasting blood glucose.

lated with fasting blood glucose ($r = .57$, $P < .001$; Fig 1) but not with HbA_{1c} ($r = .16$, P = NS). RBC GSH levels showed a negative correlation with fasting blood glucose ($r = -.35$, $P < .05$) and with HbA_{1c} ($r = -.34$, $P < .05$) and a significant negative correlation with RBC sorbitol levels ($r = -.62$, $P < .001$; Fig 2). Stepwise regression analysis made it clear that the hyperglycemia-dependent increase in RBC sorbitol levels was significantly influenced by GSH concentrations (partial F = 14.6, $P < .001$), whereas the sorbitol-dependent decrease in GSH was not influenced by fasting blood glucose concentrations (partial F = 0.1, P = NS) or HbA_{1c} (partial F = 3.79, P = NS). No influence of age or BMI was observed in these relationships.

DISCUSSION

Both glutathione synthesis and regeneration processes are important factors in maintaining GSH at concentrations that are effective in protecting cells against oxidative stress and xenobiotics.[21,22] Depletion of tissue GSH, as described in diabetes, is determined partly by a lower activity of glutathione reductase,[12,23] the enzyme that regenerates oxidized glutathione in a NADPH-dependent reaction, and partly by increased polyol formation through aldose reductase, which also requires NADPH[13,14] (Fig 3). In diabetic subjects, the production of NADPH is low because of the lower activity of the hexose monophosphate shunt,[24] and the competition for NADPH between aldose

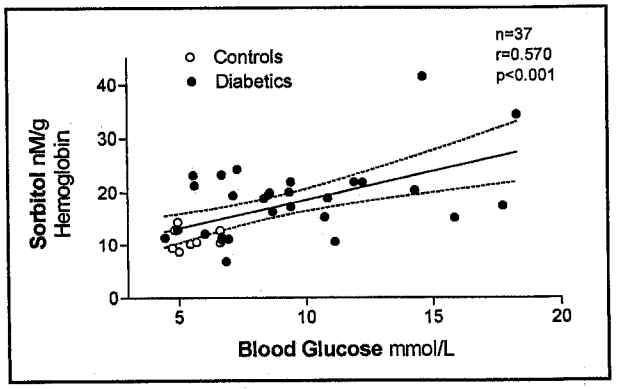

Fig 1. Plot of RBC concentration of sorbitol against fasting blood glucose in type II diabetic and normal subjects. (——) Linear regression (y = 7.71 + 1.086x, $P < .001$); (---) 95% confidence intervals.

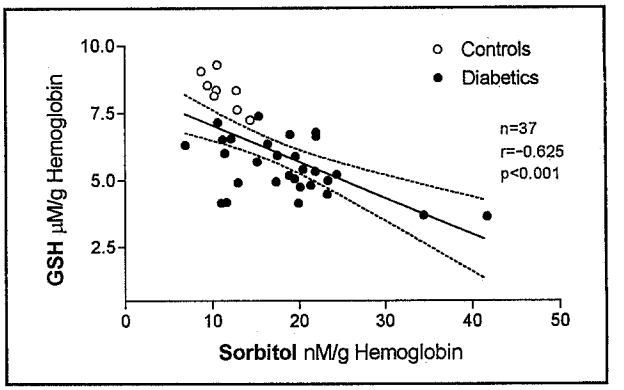

Fig 2. Plot of GSH concentration against sorbitol concentration in erythrocytes from type II diabetic and normal subjects. (——) Linear regression (y = 8.38 − 0.135x, $P < .001$); (---) 95% confidence intervals.

reductase and glutathione reductase plays an important role in producing the lower activity of GSH regeneration. On the other hand, the conversion of sorbitol to fructose, catalyzed by sorbitol dehydrogenase, produces NADH, increasing the NADH/NAD ratio.

This pattern—increased consumption of NADPH, increased NADH/NAD ratio, and increased depletion of GSH—leads to a higher level of cellular oxidative stress. During hyperglycemia, the Maillard reaction may also be greatly activated, leading to the formation of advanced Maillard end products that are, at least in part, responsible for diabetic complications and for a worsening of redox balance[15,16] (Fig 3).

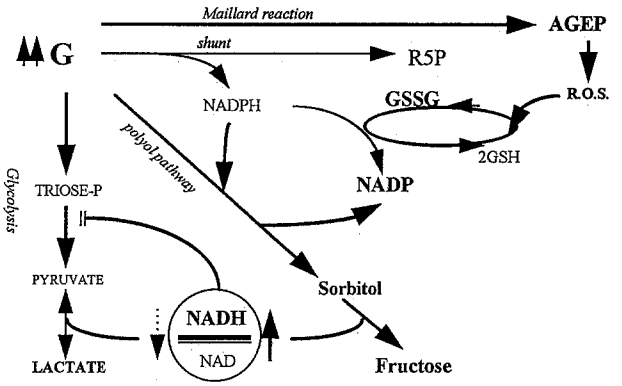

Fig 3. Some aspects of glucose and glutathione metabolism in diabetic subjects. Chronic hyperglycemia increases activity of the polyol pathway and of the Maillard reaction. The increased rate of formation of advanced glycation end products (AGEP) and reactive oxygen species (ROS) leads to an increase in the oxidation of glutathione. Both reduction of glucose to sorbitol by aldose reductase and reduction of oxidized glutathione (GSSG) to GSH by glutathione reductase are NADPH-dependent. A relative depletion of NADPH, secondary to excessive consumption and to reduced production through the pentose cycle, impairs the regeneration of GSH and leads to a depletion of this important free radical scavenger. Reduction of sorbitol to fructose by sorbitol dehydrogenase increases the rate of formation of NADH. The increase in the cytosolic NADH/NAD ratio and the depletion of GSH inhibit the oxidation of triose-P to pyruvate.

In our study, the actual blood glucose level is able to affect the level of sorbitol in RBCs, unlike the long-term control of glucose (HbA_{1c} %), which seems to have no influence on it.

The negative relationship between RBC sorbitol and GSH concentrations strongly suggests that hyperglycemia enhances polyol pathway activity to the detriment of regeneration of glutathione. Multiple regression analysis further supports the hypothesis that the decrease of glutathione in RBCs diabetic subjects mainly depends on the increase of sorbitol concentration.

Finally, the depletion of glutathione hampers membrane-bound glyceraldehyde-3-phosphate dehydrogenase,[25,26] an enzyme that catalyzes the oxidation of glycero aldehyde phosphate (GAP) to 1,3-di phospho glycerate (DPG). This reaction is also inhibited by an increase in the free cytosolic NADH/NAD ratio.[27] In this case, the glycolytic flux from glucose to pyruvate is reduced, and probably more glucose is switched to sorbitol and fructose formation, worsening the depletion of NADPH and the metabolic pseudohypoxia.

Stepwise regression analysis indicated that the increase in RBC sorbitol concentration was significantly dependent both on the increase in blood glycemia and on the decrease in RBC GSH concentrations. These data suggest the hypothesis that the hyperglycemia-induced enhanced activity of the polyol pathway leads to GSH depletion, and in turn the GSH depletion, reducing the glycolytic flux to pyruvate, enhances the rate of glucose metabolism through the polyol pathway. The overall effect is a progressive worsening of metabolic pseudohypoxia and depletion of GSH, leading to a lower defense against oxidative stress and to a higher susceptibility to hypoxic and ischemic injury.

Thus, the metabolic alterations observed in diabetes and the typical metabolic cellular imbalance linked with hyperglycemia (pseudohypoxia) are the mediators of early vascular and neural dysfunction and favor late-onset complications.[4]

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