Dietary Glutathione Protects Rats from Diabetic Nephropathy and Neuropathy

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ABSTRACT Recently, much attention has focused on the role of oxidative stress in the various forms of tissue damage in patients with diabetes. The aim of this study was to examine the involvement of oxidative stress in the progression of kidney dysfunction and neuropathy in diabetes and to evaluate the potential usefulness of glutathione (GSH) in diabetes. We examined the effect that treatment of streptozotocin (STZ)-induced diabetic rats with GSH has on the renal and neural functions. Diabetic rats were treated with 1 g/100 g GSH as a dietary supplement. GSH significantly suppressed the diabetes-induced increase in urinary 8-hydroxy-2'-deoxyguanosine, one of the markers of oxidative stress. It also prevented the diabetes-induced increases in albumin and creatinine in urine. The diabetes-induced increase in the tail flick reaction time to thermal stimuli also was normalized by treatment with dietary GSH. In conclusion, GSH treatment can beneficially affect STZ-induced diabetic rats, with preservation of in vivo renal and neural function. This suggests a potential usefulness of dietary GSH treatment to reduce diabetic complications. J. Nutr. 132: 897–900, 2002.

KEY WORDS: glutathione streptozotocin oxidative stress diabetes antioxidant rats

Hyperglycemia causes the autoxidation of glucose (1), the glycation of proteins (2) and the activation of polyol metabolism (3). These changes accelerate the generation of reactive oxygen species (ROS) and result in an increase in the oxidative modification of lipids, DNA and proteins in various tissues. Oxidative stress may play an important role in the development of complications in diabetes. An amino-carbonyl reaction, the so-called Maillard reaction, occurs in vivo as well as in vitro and is associated with the chronic complications of diabetes mellitus and aging in humans (1). Many different types of ROS and free radicals may be formed during the decomposition process of the Amadori rearrangement products.

Diabetes mellitus may be associated with increased lipid peroxidation caused by oxidative stress. Recently, a relationship between diabetic nephropathy and neuropathy and oxidative stress was reported (4,5), suggesting that oxidative stress affects the progress of diabetic complications. Therefore, antioxidants could ameliorate these complications.

Glutathione (GSH) is the most abundant nonprotein thiol and has many functions in vivo. The major role of GSH is the maintenance of cellular redox balance. It plays a role as a substrate of glutathione peroxidase, an antioxidant enzyme that scavenges various peroxides (6). The physiological role of GSH as an antioxidant has been described and substantiated in studies of numerous disorders reflecting the increased oxidation is a result of abnormal GSH metabolism (7,8). GSH is thought to be an important factor in cellular function and defense against oxidative stress, such as radiation and drug resistance (9,10). Many reports have demonstrated that GSH acts as an endogenous antioxidant. However, there have been no prior studies demonstrating a protective effect of dietary GSH against the diabetic nephropathy or neuropathy caused by oxidative stress.

In this study, we demonstrated for the first time that dietary GSH suppresses oxidative stress in vivo, and the impairment of renal function and diabetic neuropathy using streptozotocin (STZ)-induced diabetic rats.

MATERIALS AND METHODS

Chemicals. GSH was obtained from Kyowa Hakko Kogyo, (Tokyo, Japan). STZ was obtained from Wako Pure Chemical (Osaka, Japan).

Animals and diets. Male Wistar rats (7 wk old; Japan SLC, Hamamatsu, Japan), weighing 160–190 g were housed individually in stainless steel cages with screen bottoms. They were kept under controlled conditions with a 12-h light:dark cycle and at 21–25°C. All rats were fed commercial CE-2 (CLEA Japan, Tokyo, Japan) with free access to water for 1 wk to adapt to the new environment. The control diet, CE-2, contained (g/100 g): moisture 8.9, protein 25.4, fat 4.4, fiber 4.1, ash 6.9 and carbohydrate 50.3. and sufficient vitamins and minerals to maintain the health of the rats. GSH was added to the control diet (CE-2) at 1 g/100 g. The rats were divided into three groups (n = 8–9); two groups were injected with STZ (40 mg/kg body) as a freshly prepared solution (500 g/L) in 0.1 mol/L...
citrate buffer (pH 4.5) via the tail vain. These rats were immediately allowed free access to the experimental diets. One group was fed the control diet (diabetic group), and the other was fed the 1 g/100 g GSH diet (diabetic + GSH group). The control group was injected with citrate buffer instead of STZ and consumed the control diet. Experimental diets were fed for 60 d. All procedures were approved by the animal care committee of the Kyowa Hakko Tsukuba Research Laboratory.

**Experimental design.** After 40, 50 and 60 d of feeding, the rats were placed in individual metabolic cages to collect their urine for 24 h. The collected urine was used to measure creatinine, albumin and 8-hydroxy-2'-deoxyguanosine (8-OHdG). At the end of the experiment, the rats were killed under anesthesia with sodium pentobarbital (50 mg/kg). The serum was obtained by centrifugation at 1600 × g for 10 min at 4°C. The separation of the serum was finished within 30 min, and it was immediately stored at −80°C until used.

**Biochemical analyses**

The concentrations of GSH and oxidized glutathione (GSSG) were estimated by using HPLC as described by Reeve et al. (11) and Mattia et al. (12). The concentration of 8-OHdG in the urine was measured using the 8-OHdG ELISA kit (Japan Institute for Control Analytical Science, Tokyo, Japan). Urinary albumin was determined with a commercial assay kit (EXOCELL Inc., Philadelphia, PA). The serum glucose concentration was enzymatically determined with a commercial kit (Wako Pure Chemical, Osaka, Japan).

**Tail flick reaction time.** To investigate the protective effect of dietary GSH on diabetic neuropathy, we measured the tail flick reaction time as described by Rani et al. (15). Using the techno-analgesiometer (MK-330B, Muromachikikai, Japan), the tail flick reaction time was determined after heat irradiation at 73°C at 40, 50 and 60 d after GSH treatment in the diabetic rats. The measurements were performed five times at 1-min intervals.

**Statistical analysis.** An overall difference among the groups was determined by one-way ANOVA. If the one-way ANOVA was significant, differences between individual groups were estimated using Fisher’s protected least significant difference test using StatView 5 for Macintosh (SAS Institute, Cary, NC). Differences were considered significant at P < 0.05.

### RESULTS

**Body weights.** The body weight of the STZ-induced diabetic rats was less than that of the nondiabetic control rats after the 60-d experiment, but the two diabetic groups did not differ (Table 1). GSH did not negatively affect food consumption (diabetic, 18.7 ± 2.3 g/d; diabetic + GSH, 18.8 ± 2.3 g/d).

<table>
<thead>
<tr>
<th>Group</th>
<th>Body weight (g)</th>
<th>Serum glucose (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>60</td>
</tr>
<tr>
<td>Control</td>
<td>170.3 ± 7.5</td>
<td>311.8 ± 21.03 ±</td>
</tr>
<tr>
<td>Diabetic</td>
<td>175.3 ± 5.4</td>
<td>137.5 ± 13.05 ±</td>
</tr>
<tr>
<td>Diabetic + GSH</td>
<td>171.1 ± 5.7</td>
<td>135.2 ± 18.37 ±</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 8–9. Values in a column with different letters differ, P < 0.05.

SERUM glucose. Serum glucose was three- to fourfold greater in the diabetic rats than in control rats (Table 1). Dietary GSH did not affect serum glucose.

**Oxidized and reduced glutathione.** Erythrocyte GSSG of the diabetic rat groups administrated GSH was greater than in the control diabetic rat group [diabetic, 1.25 ± 0.22 μmol/g hemoglobin (Hb); diabetic + GSH, 1.68 ± 0.40 μmol/g Hb]. However, erythrocyte GSH of diabetic rats was not affected by GSH (diabetic, 4.11 ± 1.02 μmol/g Hb; diabetic + GSH, 4.52 ± 1.36 μmol/g Hb).

**Urinary 8-OHdG, creatinine and albumin.** The level of 8-OHdG was greater in the urine of the untreated diabetic group compared with the control group and it was reduced by dietary GSH although not to control level (Table 2). Diabetic rats significantly increased the urinary creatinine and albumin levels compared with the control rats and levels were normalized in diabetic rats fed GSH (Table 2).

**Diabetic neuropathy.** The tail flick time was longer in the diabetic group than in the control group (Table 3). Dietary GSH completely normalized the reaction time in diabetic rats.

### DISCUSSION

Recently, there has been increasing interest in the protective function of dietary antioxidants. Several antioxidants, such as GSH, vitamin E and vitamin C, have been found to play important roles in protection against oxidative stress. To investigate whether dietary GSH is beneficial for diabetic complications, we examined whether dietary GSH could preserve renal and neural function in diabetic rats.

In mammalian cells and tissues, GSH is the most abundant nonprotein thiol; it is usually present in millimolar concentrations.

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Time, d</th>
<th>Body weight</th>
<th>Serum glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>60</td>
<td>7</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetic + GSH</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Effect of dietary glutathione (GSH) on body weights and serum glucose of diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>8-OHdG (nmol/24 h)</th>
<th>Creatinine (μmol/24 h)</th>
<th>Albumin (nmol/24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.89 ± 0.34 ±</td>
<td>73.0 ± 8.65 ±</td>
<td>13.68 ± 4.07 ±</td>
</tr>
<tr>
<td>Diabetic</td>
<td>6.33 ± 0.90 ±</td>
<td>123.5 ± 27.0 ±</td>
<td>21.94 ± 5.24 ±</td>
</tr>
<tr>
<td>Diabetic + GSH</td>
<td>4.72 ± 0.80 ±</td>
<td>92.8 ± 22.0 ±</td>
<td>15.72 ± 3.76 ±</td>
</tr>
</tbody>
</table>

1 Values are means ± SD, n = 8–9. Values in a column with different letters differ, P < 0.05.
TABLE 3

Effect of dietary glutathione (GSH) on tail flick reaction time in diabetic rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Tail flick reaction time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>40</td>
</tr>
<tr>
<td>Control</td>
<td>19.8 ± 6.2b</td>
</tr>
<tr>
<td>Diabetic</td>
<td>27.4 ± 5.6b</td>
</tr>
<tr>
<td>Diabetic + GSH</td>
<td>20.8 ± 5.8b</td>
</tr>
</tbody>
</table>

1 Data are means ± SD, n = 8–9. Values in a column with different letters differ, P < 0.05.

DIETARY GLUTATHIONE IMPROVES DIABETIC COMPLICATIONS

The main function of exogenous GSH is to suppress lipid peroxidation, which occurs in the plasma membrane and damages the membrane's structure and permeability. It has been unclear whether dietary GSH beneficially affects diabetic nephropathy and neuropathy, but in this study, we showed for the first time that dietary GSH can improve renal and neural functions in diabetic rats. Previous studies have documented that hyperglycemia is associated with excessive free radical generation and oxidative stress and poor antioxidant status (19,20). Free radicals are highly reactive and unstable chemical species that have been implicated in mediating vascular and tissue damage in several diseases (21,22). Hyperglycemia-induced free radical generation may be derived from the polyol pathway of the glucose metabolism (23), nonenzymatic glycation (24), glucose autoxidation (25), and enhanced arachidonic acid metabolism (26). Urinary 8-OHdG is an index of systemic oxidative DNA damage that has been repaired (27), passing freely into the urine by glomerular filtration and serving as an index of whole-body oxidative stress (28). Oxidative DNA damage, however, has been shown to be related to the peroxidation of membrane fatty acids and low antioxidant status (29), both present in diabetes. In this study, the level of GSH in the erythrocytes of diabetic rats was not changed by the administration of GSH. However, diabetes increased urinary 8-OHdG excretion, and this was ameliorated by dietary GSH, indicating that dietary GSH improves diabetes-induced oxidative stress in vivo.

Diabetic nephropathy is a serious microvascular complication of diabetes mellitus. The natural history of diabetic nephropathy is well known, i.e., dipstick-positive proteinuria and the development of renal failure (30) follow the appearance of microalbuminuria. The production of peroxynitrite increases in the proximal tubules of patients with diabetic nephropathy, suggesting that oxidative injury of the proximal tubules may be an important part in the pathogenesis of diabetic nephropathy (31). Antioxidant treatment may have a potential role for the prevention of diabetic nephropathy. Treating diabetic rats with GSH significantly suppressed the diabetes-induced elevation of urinary albumin and creatinine levels. Thus, dietary GSH improved renal dysfunction in diabetic rats through its antioxidant function, and the urinary 8-OHdG excretion data support these results.

Neuropathy is the most common complication of diabetes mellitus (32) with autonomic and/or peripheral neuronal components. Peripheral neuropathy may be either painful or painless. Diabetic neuropathy is associated with a decrease in nerve conduction velocity (32). Diabetes-induced oxidative stress and the generation of superoxides may be responsible in part for the development of vascular and neural complications (33). In our studies, dietary GSH prevented the diabetes-induced impairment in the tail-flick reaction time. Therefore, our present results support the hypothesis that ROS, provoked by hyperglycemia in vivo, play an important role in neural dysfunction.

In conclusion, our present results show for the first time that dietary GSH can exert beneficial effects on diabetic complications in STZ-induced diabetic rats. Thus, a sufficient supply of dietary GSH may prevent or delay renal and neural dysfunctions in diabetes by providing protection against oxidative stress.

LITERATURE CITED


