Inhibition of Diabetic Nephropathy in Rats by an Oral Antidiabetic Material Extracted from Yeast

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Diabetic nephropathy is one of the major complications of diabetes. The glucose tolerance factor (GTF) is a dietary agent extracted from several natural sources; the richest among them is brewer’s yeast. Extraction and purification of an active and stable GTF preparation from brewer’s yeast previously was successful, and a remarkable decrease in plasma glucose and lipids from administration of GTF to animals with type 1 diabetes was demonstrated. The purpose of the present study was to examine whether GTF affects nephropathy in diabetic rats. The average urinary volume and protein excretion throughout the collection period in diabetic rats was 56.95 ± 2.2 ml/d and 5.42 ± 0.95 mg/d, respectively. These values were significantly (P < 0.001 versus baseline values) higher compared with healthy controls (average urine volume 15.12 ± 0.5 ml/d; average protein excretion 0.15 ± 0.08 mg/d). Treatment with GTF reduced average urine volume and protein excretion to 29.1 ± 1.94 ml/d (P < 0.01) and 1.55 ± 1.17 mg/d (P < 0.05), respectively. Kidney weight, which was elevated in diabetic rats, slightly decreased in diabetic animals that were treated with GTF, in association with reduction of lipid peroxidation levels in the renal cortex and the heart. Endothelial nitric oxide immunoreactivity in the renal cortex of both healthy and diabetic rats that were treated with GTF was remarkably lower than that found in renal cortex of untreated diabetic animals. This study demonstrates that yeast-derived material, GTF, can inhibit the development of nephropathy that is induced by diabetes.

Diabetes is the leading cause of ESRD. Approximately 30% of patients with diabetes experience diabetic nephropathy, which gradually develops to final renal failure (1). Fifty percent of the patients who need dialysis treatment in Western countries have diabetes, and the number is constantly growing (2). Large-scale studies have established that hyperglycemia, the defining metabolic abnormality, increases the risk for diabetic renal disease (3,4).

Although the complete mechanism of hyperglycemia that causes diabetic complications is not fully known, several biochemical pathways are involved in the pathogenesis, including increased formation of glucose-derived glycated end products, increased formation of reactive oxygen species (5–7), activation of aldose reductase pathway, and glucose-induced activation of protein kinase C. Reactive oxygen species exert their cytotoxic effects on membranes and cellular lipids, resulting in the formation of malondialdehyde (7,8). Oxidation of lipids in plasma lipoproteins and in cellular membranes is associated with the increased incidence of vascular disease in diabetes (9,10). Normally, protective mechanisms are present in the cell to prevent damage by free radicals (11). Enzymes such as superoxide dismutase, glutathione peroxidase, and catalase provide the detoxification steps for the oxidative products. It was shown that the activity of the antioxidant systems is decreased in people with diabetes (10,11).

The glucose tolerance factor (GTF) is a dietary agent that was extracted by Mertz and Schwarz (12,13) from brewer’s yeast. This natural compound reverses the impaired glucose tolerance of diabetic rats by increasing glucose transport in hepatocytes, adipocytes, and cardiomyocytes and reduces the elevated levels of lipid peroxidation products (12,13). In the present study, we investigated the protective effects of GTF on the renal and cardiovascular complications of type 1 diabetes in rats by measuring biochemical and enzymatic parameters and morphologic changes in the kidney.

Materials and Methods

Experiments were performed on 5-wk-old male Sprague-Dawley rats that weighed 120 to 130 g. Twenty-one rats were included in each protocol. The animals were housed in metabolic cages and maintained on a standard diet and water ad libitum. All experiments were performed according to the guidelines of the committee for the supervision of animal experiments, Technion, IIT.

Five animals served as healthy controls, and the rest received injections of streptozotocin (STZ; 60 mg/kg body wt, intraperitoneal) to induce diabetes. Half of the diabetic animals (n = 8) were used as diabetic controls, and the rest were treated, immediately after the injection of STZ, for 2 wk with oral doses of GTF (4 g/d), mixed in their food. In each group, blood glucose levels, GFR, urinary volume, and urinary excretion of protein (microalbuminuria and total daily protein excretion) were determined before and after the treatment period throughout the study.

At the end of the treatment period, the animals were killed and their plasma was collected and their kidneys and hearts were removed, weighed, and kept in -80°C for further determinations such as endothelial nitric oxide synthase (eNOS) immunoreactivity in renal cortex.
Chemical Analysis

The urinary concentration of protein was determined by spectrophotometry, after 3% sulfosalicylic acid precipitation of urine that was collected from rats that were housed individually in metabolic cages for 24 h throughout the experimental period. Plasma glucose concentrations were determined using glucometer (Ascensia Elite XL; Bayer, Dublin, Ireland). Thiobarbituric acid reactive substance (TBARS) was determined by measuring the products of lipid oxidation on the basis of colorimetric reaction, as described previously (14). Products of lipid oxidation were measured in the presence (induced) and absence (non-induced) of FeSO₄.

Immunohistochemistry

Immunofluorescence analysis of eNOS in the renal cortex and heart tissue was performed in the different experimental groups as well as in normal rats. Kidneys were removed, sliced in half, fixed in buffered solution of 4% paraformaldehyde, and embedded in paraffin. Longitudinal 5-μm sections were cut and mounted on glass slides that were precoated with poly-L-lysine (Sigma Chemicals, St. Louis, MO). After deparaffinization in xylene and rehydration in decreased concentrations of ethanol, sections were permeabilized for 5 min in 1% SDS in tris-buffered saline (TBS; 100 mM Tris [pH 7.4], 138 mM NaCl, and 27 mM KCl). To prevent nonspecific binding of Ig, sections were incubated for 30 min with 1% BSA in TBS. Then, eNOS primary mAb (Oxis International Inc., Portland, OR), diluted to 1:250 in TBS, were added. Immunohistochemical reaction was performed according to the streptavidin-biotin-peroxidase technique by using Histostain Plus kit (Zymed, Dublin, Ireland). Thiobarbituric acid reactive substance (TBARS) was determined by measuring the products of lipid oxidation on the basis of colorimetric reaction, as described previously (14). Products of lipid oxidation were measured in the presence (induced) and absence (non-induced) of FeSO₄.

Statistical Analyses

One-way ANOVA for repeated measures, followed by the Dunnett test, was used for comparison of treatment values with baseline value in each group. For comparison of the graphs representing control and experimental groups, two-way ANOVA was used. P < 0.05 was considered statistically significant. Data are presented as mean ± SEM.

Results

Blood Glucose in Untreated and GTF-Treated Diabetic Rats

A single dose of GTF (4 g) administered orally to STZ-induced diabetic rats resulted in an immediate decrease in blood glucose. The maximal glucose reduction was achieved within 120 to 180 min and lasted for several hours (Table 1).

<table>
<thead>
<tr>
<th>Group</th>
<th>Glucose Level</th>
</tr>
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<tbody>
<tr>
<td>Healthy</td>
<td>102 ± 4</td>
</tr>
<tr>
<td>Diabetic</td>
<td>397 ± 34</td>
</tr>
<tr>
<td>Diabetic + GTF</td>
<td>315 ± 15b</td>
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*A single dose of glucose tolerance factor (GTF; 4 g) was administered orally to streptozocin-induced diabetic rats. Blood glucose was measured after 120 to 180 min. 

These results confirm the antidiabetic effect of GTF as was reported by us previously (12,13).

Urinary Volume and Proteinuria in Diabetic Rats

Urinary volume and protein excretion were determined in healthy, diabetic, and diabetic GTF-treated rats. Figure 1 depicts the urinary volume of the various experimental groups and shows an increase of approximately threefold (P < 0.001) in urinary volume of the untreated diabetic rats as compared with healthy control animals.

Treatment of diabetic rats with GTF reduced significantly (approximately 50%; P < 0.01) the average urinary flow rate throughout the whole experiment. The beneficial effect of GTF was evident on the third day of the treatment and lasted for the 2 wk of the treatment period.

Figure 2 presents the urinary daily excretion of protein in healthy, diabetic, and diabetic GTF-treated rats and demonstrates that diabetic rats have significantly higher proteinuria (approximately 30-fold; P < 0.001) compared with healthy controls. GTF administration remarkably and significantly (P < 0.05) reduced urinary protein excretion in diabetic rats. Daily administration of GTF to diabetic animals significantly reduced the protein excretion throughout the whole study (Figure 2). Kidney and heart weights of the various groups were increased significantly (P < 0.05) at the end of the study compared with the relevant values in the beginning (Table 2). This increase was more substantial in the diabetic rats as compared with healthy controls. Treatment with GTF for 2 wk slightly and nonsignificantly reduced kidney weight.

Lipid Peroxidation in the Plasma and Kidneys

The levels of the lipid peroxidation products, TBARS, in the plasma and kidneys were performed in the different experimental groups as well as in normal rats. Kidneys were removed, sliced in half, fixed in buffered solution of 4% paraformaldehyde, and embedded in paraffin. Longitudinal 5-μm sections were cut and mounted on glass slides that were precoated with poly-L-lysine (Sigma Chemicals, St. Louis, MO). After deparaffinization in xylene and rehydration in decreased concentrations of ethanol, sections were permeabilized for 5 min in 1% SDS in tris-buffered saline (TBS; 100 mM Tris [pH 7.4], 138 mM NaCl, and 27 mM KCl). To prevent nonspecific binding of Ig, sections were incubated for 30 min with 1% BSA in TBS. Then, eNOS primary mAb (Oxis International Inc., Portland, OR), diluted to 1:250 in TBS, were added. Immunohistochemical reaction was performed according to the streptavidin-biotin-peroxidase technique by using Histostain Plus kit (Zymed, South San Francisco, CA). Negative controls were treated with nonspecific immune serum instead of primary antibody and processed simultaneously. All sections were counterstained with hematoxylin, and representative photographs were taken.

Table 1. Plasma glucose levels in the different animal groups*  

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A single dose of glucose tolerance factor (GTF; 4 g) was administered orally to streptozocin-induced diabetic rats. Blood glucose was measured after 120 to 180 min.

Figure 1. Daily urinary volume of diabetic rats (n = 8) and glucose tolerance factor (GTF)-treated diabetic rats (n = 8) and healthy controls (n = 5). *P < 0.01, **P < 0.05 versus untreated diabetic animals; ***P < 0.001, ****P < 0.01, ***P < 0.05 versus healthy controls.
significantly higher than that in healthy animals, and the level of lipid peroxides in the kidney of diabetic rats that were treated with oral doses of GTF was very low, similar to the level found in healthy animals (Figure 3A). Similar results were obtained when the lipid peroxidation products were determined in the cardiac tissue (Figure 3B). Kidney weight, which was significantly elevated in diabetic rats, decreased slightly in diabetic animals that were treated with GTF (Table 2).

eNOS in Renal Cortex

eNOS immunoreactivity was measured in the renal cortex and medulla of healthy and untreated diabetic rats or GTF-treated animals. eNOS immunoreactivity in untreated diabetic rats was remarkably higher than that found in renal cortex and medulla of normal animals (Figure 4). Treatment with GTF restored the amount of eNOS in the cortical and medullary tissues of diabetic rats to normal levels.

Discussion

This study conducted on Streptozotocin induced DM rats, describes the renal protective effect of a new dietary agent. GTF is known as an antioxidant factor and improves the glucose transport in muscle and adipocyte cells (12,13).

Both urine volume and urinary excretion of protein of diabetic rats were higher compared with the control group and were reduced after treatment with GTF. The levels of the lipid

Table 2. Kidney, heart, and liver weights of the various experimental groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean Body Weight (g)</th>
<th>Mean Liver Weight (g)</th>
<th>Mean Kidney Weight (g)</th>
<th>Mean Heart Weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beginning of study</td>
<td>147 ± 2.3</td>
<td>7.8 ± 0.2</td>
<td>0.88 ± 0.03</td>
<td>0.66 ± 0.01</td>
</tr>
<tr>
<td>Healthy</td>
<td>251 ± 7.7a</td>
<td>11.95 ± 1.0a</td>
<td>1.3 ± 0.08a</td>
<td>0.88 ± 0.05a</td>
</tr>
<tr>
<td>Diabetic untreated</td>
<td>214 ± 10a</td>
<td>11.7 ± 0.8a</td>
<td>1.6 ± 0.08a,b</td>
<td>0.78 ± 0.04</td>
</tr>
<tr>
<td>Diabetic + GTF</td>
<td>219 ± 2.3a</td>
<td>13.2 ± 0.5a</td>
<td>1.53 ± 0.05a,b</td>
<td>0.81 ± 0.02</td>
</tr>
</tbody>
</table>

*P < 0.05 versus values in the beginning of the study.

*p < 0.05 versus healthy controls.
peroxidation products, TBARS, in the kidney and the heart were significantly higher in untreated diabetic rats than in healthy animals, whereas the level of lipid peroxides in the kidney and the heart of diabetic rats that were treated with GTF was very low, comparable to the level found in healthy animals. Kidney weight, which was elevated in diabetic rats, decreased slightly in diabetic animals that were treated with GTF. eNOS immunoreactivity in renal cortex and medulla were higher in diabetic rats compared with normal controls and remarkably decreased after the administration of GTF for 2 wk. This study is of interest because diabetes is the leading cause of ESRD. The number of deaths among dialysis-treated patients with diabetes is much higher than in dialysis patients without diabetes (2,15). Type 1 diabetes was induced by intraperitoneally injected STZ, and half of the animals were treated with GTF or vehicle for 2 wk. Untreated diabetic rats developed severe hyperglycemia with polyuria as a result of osmotic diuresis. However, the diabetic rats that were treated with GTF showed significant decrease in urinary volume along the study, probably as a result of the normalization of plasma glucose level or synergistic effect with insulin as shown in other studies (12,13).

GTF-treated diabetic rats showed an impressive decrease in the amount of proteinuria in parallel with the decrease in urinary volume. Proteinuria, a hallmark feature of early glomerular damage in patients with diabetes, is associated with renal hemodynamic and histologic changes (1,2). It is widely known that hyperglycemia can induce microalbuminuria by a hyperfiltration mechanism that is related to NO metabolism alteration (3,16), increased synthesis of reactive oxygen species, or loss of nephrin in podocytes as shown by different investigators (17). Early treatment with GTF can decrease proteinuria in these diabetic rats by improving the exaggerated oxidative state that is expressed by the decrease in lipid peroxidation and TBARS in the kidney tissue (18).

More impressive was the improvement in the morphologic changes that were expressed by the enzymatic expression of eNOS in the renal kidneys of rats that were treated early with GTF. It is widely known that the early morphologic changes that are involved in the diabetic kidney are related to the NO system (19). Various studies have shown that in the early stages of diabetes, there is an increase in superoxide production in the kidney with concomitant decrease in NO function by increased degradation (19). This decrease in NO function can cause an increase in capillary hydrostatic pressure and hyperfiltration and a compensational increase in NO synthesis in the renal parenchyma with exaggerated production of free radicals and glomerular damage with proteinuria (19). As we showed in Figure 4, there is an increase in eNOS expression in the cortex of diabetic rats, which returned to basal levels when the animals were treated with GTF.

Conclusion
Our results demonstrate that GTF treatment has a beneficial impact on the consequences of hyperglycemia and glomerular permeability expressed by proteinuria and increased oxidative stress. On this basis, we propose that GTF expresses insulin-like effect at the cellular level and decreases the synthesis of free radicals that damage the glomerular basement membrane.

Acknowledgment
This research was partially supported by the Russell Berrie Foundation and D-CURE.

References
3. Cosentino F, Hishikawa K, Katusic ZS, Luscher TF: High glucose increases nitric oxide synthase expression and su-

Figure 4. (A) Endothelial nitric oxide synthase (eNOS) immunostaining in kidney cortex of controls (n = 5), diabetic rats (n = 8), and diabetic rats that were treated with GTF (n = 8). (B) eNOS immunostaining in the renal medulla of controls (n = 5), diabetic rats (n = 8), and diabetic rats that were treated with GTF (n = 8). Magnification, ×100.


