ABSTRACT — Diabetic nephropathy (DN) is one of the complications of diabetes and is now the most common cause of end-stage renal disease. Fructose is a simple carbohydrate that is present in fruits and honey and is used as a sweetener because of its sweet taste. Fructose has been reported to have the potential to progress diabetes and DN in humans even though fructose itself does not increase postprandial plasma glucose levels. In this study, we investigated the effects of high fructose intake on the kidney of the Spontaneously Diabetic Torii (SDT) rats which have renal lesions similar to those in DN patients and compared these with the effects in normal SD rats. This study revealed that a 4-week feeding of the high fructose diet increased urinary excretion of kidney injury makers for tubular injury and accelerated mainly renal tubular and interstitial lesions in the SDT rats but not in normal rats. The progression of the nephropathy in the SDT rats was considered to be related to increased internal uric acid and blood glucose levels due to the high fructose intake. In conclusion, high fructose intake exaggerated the renal lesions in the SDT rats probably due to effects on the tubules and interstitium through metabolic implications for uric acid and glucose.

Key words: Fructose, Nephrotoxicity, Diabetic animal model, Uric acid, Hyperglycemia

INTRODUCTION

Diabetes mellitus increases the risk of long-term complications including nephropathy, vision problems (cataracts, retinopathy and eventual blindness), neuropathy and cardiovascular disease (Nathan, 1993). Diabetic nephropathy (DN) is one of the major complications and the population of DN patients is about 30% of diabetic patients (Yokoyama et al., 2016; Molitch et al., 2004). DN is now the commonest cause of end-stage renal disease (ESRD), which is total and permanent kidney failure (Molitch et al., 2004; Masakane et al., 2015). DN leads to increased mortality and diminished quality of life because ESRD can be lethal and ESRD patients need high-cost treatments including dialysis and a kidney transplant (Nakamura et al., 2017; Hörl et al., 1999). Therefore, early treatment for diabetes and DN is important to prevent the progression to ESRD.

Some recent trials report that strict blood glucose management using a carbohydrate-limited diet ameliorates hyperglycemia and such carbohydrate management is effective for delaying of the progression to DN (Delahanty and Halford, 1993; Kim, 2014). While the intake of glucose is one of the most important factors influencing the development of diabetes and DN, some studies revealed that other nutrients also contribute to the progression of diabetes and DN.

Fructose is a simple carbohydrate that is present in fruits and honey and is used as a sweetener because of its sweet taste. The major source of fructose is sucrose, which is a disaccharide that consists of 50% fructose and 50% glucose. Although some amount of fructose converts to glucose by its metabolism (Sun and Empie, 2012), fructose does not increase postprandial plasma glucose levels as high as does glucose (Bantle, 2009; Schaefer et al., 2009). In addition, fructose does not signal insulin release and can lower plasma glucose due to stimulation of hepatic glucose uptake (Moore et al., 2000; Shiota et al., 1998, 2002). Therefore, fructose used to be considered beneficial as an energy source for diabetic patients. Epidemiological studies, however, revealed that excessive fructose intake leads to increases in the rates of
obesity, diabetes, hypertension and kidney disease (Johnson et al., 2007). Some studies evaluating the short-term effects of fructose in humans showed that excessive fructose intake induced insulin resistance and hypertriglyceridemia (Lê and Tappy, 2006). Therefore, the American Diabetes Association made a proposal not to recommend fructose supplementation for diabetic subjects (American Diabetes Association position statement, 2002). Furthermore, there is evidence that high fructose-containing soft drinks are associated with an increased risk for renal disease as manifested by albuminuria in humans (Shoham et al., 2008).

In experimental animal studies, giving a high fructose diet causes the development of features of metabolic syndrome, including insulin resistance (Tobey et al., 1982; Hwang et al., 1987), elevation of triglycerides (Sleder et al., 1980; Bocarsly et al., 2010; Hwang et al., 1987), abdominal obesity (Bocarsly et al., 2010), elevation of blood pressure (Hwang et al., 1987) and hyperuricemia (Sanchez-Lozada et al., 2007; Nakagawa et al., 2006) in normal rats and enhances diabetic complications in diabetes model rats (Bell et al., 2000). Some studies focusing on the effect of fructose on the kidneys showed that excessive fructose intake causes renal injury, including glomerular hypertension, renal microvascular damage and tubulointerstitial injury in normal rats (Sanchez-Lozada et al., 2007; Nakayama et al., 2010) and increases oxidative stress in the kidneys of diabetes model rats (Bell et al., 2000). According to the evidence from these studies, fructose is considered to have the potential to exaggerate DN by enhancing metabolic syndrome. However, studies that reveal apparent adverse effects of fructose on the kidneys in diabetic patients and diabetic animal models are limited.

The Spontaneously Diabetic Torii (SDT) rat is known as a useful model for non-obese type 2 diabetes and spontaneously develops hyperglycemia and glucose intolerance resulting from impaired insulin secretion due to β cell degeneration in the pancreas (Masuyama et al., 2004). It has been reported that the male SDT rats develop diabetes from about 20 weeks of age and that renal lesions accompanied by increases in urine volume and renal function parameters (urinary protein and urinary albumin) are observed from about 24 weeks old (Sasase et al., 2013). The renal lesions and alteration of the renal function parameters observed in the SDT rats are enhanced with the progress of diabetes and the renal lesions are improved by blood glucose control with insulin (Ohta and Sasase, 2011; Ohta et al., 2007). Therefore, the renal lesions observed in the SDT rats are considered to result from exposure to high blood glucose. In addition, the SDT rats develop various histopathological changes in the kidney with aging, including severe tubular lesions, diffuse glomerular lesions and glomerular nodular lesions (Sasase et al., 2013). These renal changes are very close to those seen in DN patients, therefore, the SDT rat is a useful model for investigating DN in humans.

In this study, 27-week-old male SDT rats, which are expected to have already developed diabetes and mild nephropathy accompanied by proteinuria, were fed high fructose diet for 4 weeks and the effects of a high fructose diet on the kidneys were examined by measuring renal function parameters and kidney injury markers and by histopathological examination of the kidneys.

MATERIALS AND METHODS

Diet

The commercial diet, CRF-1, was purchased from Oriental Yeast Co., Ltd (Tokyo, Japan) and was used as the basal diet. High fructose diet, D11707R (65% fructose diet), was purchased from Research Diets, Inc. (New Brunswick, NJ, USA).

Animals

Male Crl:CD (SD) and Jcl:SDT (SDT) rats were purchased from the suppliers (SD rats: Charles River Laboratories Japan, Inc., Kanagawa, Japan, SDT rats: CLEA Japan, Inc., Shizuoka, Japan) and six of each strain animals were used for the study. The rats were allowed free access to the basal diet (CRF-1) ad libitum before allocation. At 27 weeks of age, the animals of each strain were divided into 2 groups and then assigned to either a high fructose (D11707R) or a standard (CRF-1) diet group. There were 4 groups with three animals each for the CRF-1 fed-SD rats (C-SD) group, D11707R fed-SD rats (HF-SD) group, CRF-1 fed-SDT rats (C-SDT) group and D11707R fed-SDT rats (HF-SDT) group. The composition of the D11707R and CRF-1 diets is shown in Table 1. The rats were allowed free access to their assigned diets. Tap water was available for drinking ad libitum. The animals were housed individually in wire-mesh cages kept in an air-conditioned room with a 12-hr light-dark cycle (lighting from 7:00 a.m. to 7:00 p.m.) at a temperature of 23 ± 1°C, a relative humidity of 55 ± 5% and a ventilation rate of about 15 times per hour. All the animal experimental procedures were approved by the Institutional Animal Care and Use Committee of the Toxicological Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc. This study was conducted in accordance with Japanese Law for the Humane Treatment and Management of Animals (Law No. 105, as revised in
Table 1. The composition of the CRF-1 and D11707R diets.

<table>
<thead>
<tr>
<th></th>
<th>Standard Diet CRF-1 (g/100 g)</th>
<th>High Fructose Diet D11707R (g/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>22</td>
<td>20</td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Mineral</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>(Calcium)</td>
<td>1.22</td>
<td>0.52</td>
</tr>
<tr>
<td>(Phosphate)</td>
<td>0.81</td>
<td>0.4</td>
</tr>
<tr>
<td>(Magnesium)</td>
<td>0.23</td>
<td>0.05</td>
</tr>
<tr>
<td>(Potassium)</td>
<td>0.86</td>
<td>0.36</td>
</tr>
<tr>
<td>(Sodium)</td>
<td>0.26</td>
<td>0.1</td>
</tr>
<tr>
<td>Fiber</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>55</td>
<td>66*</td>
</tr>
<tr>
<td>Calorie (kcal/100 g)</td>
<td>357</td>
<td>390</td>
</tr>
</tbody>
</table>

* 65 out of 66 is fructose

2013, issued in October 1, 1973).

Measurements of body weights and food consumption

The animals of each group were fed their assigned diets for 4 weeks (from 27 weeks of age to 31 weeks of age). Measurements of body weights and food consumption were conducted weekly and total body weight gain and total food consumption during the 4-week period were calculated. The total caloric intake was calculated from the total food consumption.

Clinical chemistry

Blood samples were collected from all the animals twice under non-fasted condition before the allocation (pre-treatment, at 27 weeks of age) and at necropsy (post-treatment, at 31 weeks of age). The blood samples were collected from the subclavian vein without anesthesia at the pre-treatment sampling and from the abdominal aorta under isoflurane anesthesia at the post-treatment sampling. The blood samples were collected into heparinized tubes and cooled on ice immediately after sampling and were centrifuged at 3,000 rev·min⁻¹, 4°C for 30 minutes to obtain the plasma. Plasma glucose (GLU) (Hexokinase-G-6-PDH method), urea nitrogen (UN) (Urease-GLDH method), creatinine (CRN) (CR-SOX-POD-CRN method), total ketone bodies (TKB) (3-HBDH method), non-esterified fatty acid (NEFA) (ACS-ACOD method), triglycerides (TGL) (GPO-HMMPS method and Glycero blanking method), total cholesterol (T-CH) (COD method), phospholipid (PL) (CO-DAOS method) and uric acid (UA) (Uricase-F-DAOS method) were measured at 37°C with an automated analyzer (TBA-120FR, TOSHIBA Corporation, Tochigi, Japan) using standard reagents. Plasma UA level was measured only for the plasma samples collected post-treatment.

Urine chemistry & kidney injury markers

Urine samples were collected from all the animals twice under non-fasted condition, before the allocation (pre-treatment, at 27 weeks of age) and before the necropsy (post-treatment, at 31 weeks of age). The animals were placed in metabolism cages (Tokiwa Kagaku Kikai Co., Ltd., Tokyo, Japan) for approximately 24 hr to collect the urine samples. After measurement of the urine volume, urinary GLU (Hexokinase-G-6-PDH method), total protein (TP) (Pyrogallol red method) and UA (Uricase-F-DAOS method) were measured at 37°C with an automated analyzer (TBA-120FR, TOSHIBA Corporation) using standard reagents. Urinary clusterin, KIM-1, osteopontin, TIMP-1 and VEGF concentrations were determined for each sample using a commercial Milliplex Map Rat Kidney Toxicity Magnetic Bead Panel 1 (Millipore Corporation, Billerica, MA, USA) with standard reagents. These assays were conducted by Luminex® xMAP® technology with the measurement apparatus (Bio-Plex 200 System, BIO-RAD Laboratory Inc.) and analysis software (Bioplex Manager, BIO-RAD Laboratory Inc., Hercules, CA, USA). The urinary excretion per day of the above parameters was calculated individually using each urine sample.

Necropsy & kidney weights

All the animals were euthanized by exsanguination from the abdominal aorta under isoflurane anesthesia and were necropsied at the end of treatment period (31 weeks of age). The animals were examined in detail for gross lesions. The kidneys were weighed and the relative weight to the final body weight was calculated.

Histopathology

The kidneys and pancreas, collected from all the animals, were fixed in 10% neutral buffered formalin and prepared for histopathological examination by embedding in paraffin wax, sectioning and staining with hematoxylin and eosin (HE). Additional sections of the kidneys stained with periodic acid Schiff (PAS) and Sirius red were also prepared. The slides for the kidneys and pancreas were evaluated microscopically. For immunohistochemistry of the kidneys, primary antibodies for immunostaining included a mouse monoclonal anti-rat vimentin (V9) antibody (Dako, USA), a mouse monoclonal anti-rat desmin (D33) antibody (Dako), a mouse monoclonal anti-rat CD68 (ED-1) antibody (BMA Biomedicals, Augst, Switzerland) and a goat polyclonal anti-rat TIM-1/KIM-1/HAVCR (Kim-1) antibody (R&D systems, Minneapolis, MN, USA). Heat-induced antigen retrieval was achieved in citrate solution (pH 6.0) for all the primary antibodies.
Statistical analysis

The mean values and standard deviations (S.D.) in each group were calculated for body weights, food consumption, clinical chemistry, urine chemistry, kidney injury markers and kidney weights. Statistical analyses were performed to compare the data between the C-SD and C-SDT groups, between the C-SD and HF-SD groups or the C-SDT and HF-SDT groups using an unpaired Student’s t-test. Statistical analyses for comparison between the pre-treatment and post-treatment values were also conducted using a paired t-test. The levels of significance were set at 5% and 1% (two-tailed). Statistical analyses were performed using the Pharmaco Basic (Scientist Press Co., Ltd, Tokyo, Japan).

RESULT

Comparison between the C-SD and C-SDT groups

The data for body weights, food consumptions, total calorie intake, clinical chemistry and urine chemistry are shown in Table 2. Initial and final body weights and total body weight gain were lower in the C-SDT group than in the C-SD group (p < 0.01). Total food consumption and total calorie intake were higher in the C-SDT group than in the C-SD group (p < 0.01). Plasma and urinary GLU levels at pre- and post-treatment were much higher in the C-SDT group than in the C-SD group (p < 0.01). Plasma lipid parameters (TGL, PL, TKB and NEFA levels) except for plasma T-CH levels at the pre- and post-treatment samplings were higher in the C-SDT group than in the C-SD group (p < 0.05 or p < 0.01). Urinary UA levels pre-treatment were higher in the C-SDT group than in the C-SD group (p < 0.05), however, there was no difference in this parameter post-treatment between these groups. There was no difference in plasma UA levels post-treatment between the C-SD and C-SDT groups.

The data for renal function-related parameters in the clinical and urine chemistry are shown in Table 3. Urinary TP and plasma UN levels pre- and post-treatment were higher in the C-SDT group than in the C-SD group (p < 0.05 or p < 0.01). Urinary CRN levels pre-treatment were low-

<table>
<thead>
<tr>
<th>Table 2. The summary of the body weights, food consumptions and clinical and urine chemistry.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals</td>
</tr>
<tr>
<td>Initial body weights (g)</td>
</tr>
<tr>
<td>Final body weights (g)</td>
</tr>
<tr>
<td>Total body weight gain (g/ 4 weeks)</td>
</tr>
<tr>
<td>Total body weight gain (%/ 4 weeks)</td>
</tr>
<tr>
<td>Total food consumption (g/ 4 weeks)</td>
</tr>
<tr>
<td>Total calorie intake (kcal/ 4 weeks)</td>
</tr>
<tr>
<td>Plasma GLU (mg/dL)</td>
</tr>
<tr>
<td>Urinary GLU (mg/day)</td>
</tr>
<tr>
<td>Plasma TGL (mg/dL)</td>
</tr>
<tr>
<td>Plasma T-CHO (mg/dL)</td>
</tr>
<tr>
<td>Plasma PL (mg/dL)</td>
</tr>
<tr>
<td>Plasma TKB (μmol/L)</td>
</tr>
<tr>
<td>Plasma NEFA (μmol/L)</td>
</tr>
<tr>
<td>Plasma UA (mg/dL)</td>
</tr>
<tr>
<td>Urinary UA (mg/day)</td>
</tr>
<tr>
<td>Plasma UA (mg/dL)</td>
</tr>
<tr>
<td>Urinary UA (mg/day)</td>
</tr>
<tr>
<td>Urinary UA (mg/dL)</td>
</tr>
</tbody>
</table>

* p < 0.05, ** p < 0.01: statistical significance in comparison between the C-SD and HF-SD groups and between the C-SDT and HF-SDT groups.$ p < 0.05, $$ p < 0.01: statistical significance in comparison between the C-SD and C-SDT groups.± p < 0.05, ++ p < 0.01: statistical significance in comparison between pre- and post-treatment groups.
er in the C-SDT group than in the C-SD group (p < 0.01), however, there was no difference in this parameter post-treatment between these groups. There were no differences in plasma CRN levels pre- or post-treatment between these two groups.

The data for kidney injury markers are shown in Table 4. Urinary KIM-1 levels pre- and post-treatment were higher in the C-SDT group than in the C-SD group (p < 0.05 or p < 0.01). Urinary osteopontin levels pre-treatment were higher in the C-SDT group than in the C-SD group (p < 0.05), however, there was no difference in this parameter post-treatment between these groups. There were no differences in other kidney Injury parameters pre- or post-treatment between these groups.

The results of the histopathology for the pancreas are shown in Table 5. There were no histopathological findings in the C-SD group. In the C-SDT group, atrophy of the islets was noted in all the animals.

The data for kidney weights, and macroscopic and microscopic findings of the kidneys are shown in Table 6. The absolute and relative kidney weights were higher in the C-SDT group than in the C-SD group (p < 0.05 or p < 0.01). The kidney was larger in size in one animal of the C-SDT group, while there were no macroscopic findings in the C-SD group. There are very slight or slight microscopic findings including enlargement of the glomeruli, increased mesangial matrix of the glomeruli, hyaline casts, regeneration of the tubular epithelium, dilatation of the tubules, mineralization of the tubules, accumulation of glycogen in the tubules, interstitial infiltration with inflammatory cells and fibrosis in most of the C-SDT animals, while microscopic findings in the C-SD group was limited to very slight regeneration of the tubular epithelium in one animal.

**Comparison between the C-SD and HF-SD groups**

The data for body weights, food consumptions, total calorie intake, clinical chemistry and urine chemistry are shown in Table 2. The total food consumption tended to be lower in the HF-SD group than that in the C-SD group. There were no differences in the total calorie intake between the C-SD and HF-SD groups. Total body weight gain was lower in the HF-SD group than that in the C-SD group (p < 0.05), although the body weights increased in both the SD groups throughout the treatment period (Fig. 1). There were no differences in any clinical- or urine-chemistry parameter pre-treatment between the C-SD and HF-SD groups. There were no differences in the plasma or urinary GLU levels post-treatment between the C-SD and HF-SD groups. The plasma TGL, T-CH and PL levels were higher in the HF-SD group than those in the C-SD group post-treatment (p < 0.01). The plasma TKB levels were lower in the HF-SD group than that in the C-SD group post-treatment (p < 0.01). There were no differences in plasma NEFA levels or plasma or urinary UA levels post-treatment between these groups.

The data for renal function-related parameters in the clinical and urine chemistry are shown in Table 3. There were no differences in any of the renal function-related parameters pre- or post-treatment between the C-SD and HF-SD groups.

The data for kidney injury markers are shown in Table 4. Urinary clusterin levels were lower in the HF-SD group than in the C-SD group post-treatment (p < 0.05). There were no differences in other kidney injury markers pre- or post-treatment between the C-SD and HF-SD groups.

The results of histopathology for the pancreas are shown in Table 5. There were no histopathological changes in the pancreas in the C-SD or HF-SD groups.
The data for kidney weights, and macroscopic and microscopic findings of the kidneys are shown in Table 6. There were no differences in the kidney weights between the C-SD and HF-SD groups. At necropsy, slight enlargement of the kidney was noted in one animal in the HF-SD group. There were no histopathological findings related to treatment with fructose in the SD rats.

**Comparison between the C-SDT and HF-SDT groups**

The data for body weights, food consumptions, total calorie intake, clinical chemistry and urine chemistry are shown in Table 2. The total food consumption was lower in the HF-SDT group than that in the C-SDT group (p < 0.05). There were no differences in the total calorie intake between the C-SDT and HF-SDT groups. The body weights in the C-SDT group did not change throughout the treatment period while the body weights gradually decreased in the HF-SDT group throughout the treatment period (Fig. 1). The final body weights and total body weight gain were lower in the HF-SDT group than those in the C-SDT group (p < 0.01). Plasma GLU levels in the HF-SDT group were higher post-treatment than those pre-treatment (p < 0.01) and were higher in the HF-SDT group than those in the C-SDT group post-treatment (p < 0.01). Urinary GLU levels in the HF-SDT group were higher post-treatment than those pre-treatment (p < 0.05) and tended to be higher in the HF-SDT group than those in the C-SDT group post-treatment while urinary GLU levels were lower in the HF-SDT group than those in the C-SDT group pre-treatment (p < 0.05). There were no differences in plasma TGL, T-CH or PL levels.
Effects of high fructose diet on nephropathy in SDT rats

Table 5. The summary of the histopathology of the pancreas.

<table>
<thead>
<tr>
<th>Microscopic findings#</th>
<th>C-SD</th>
<th>HF-SD</th>
<th>C-SDT</th>
<th>HF-SDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atrophy, islet</td>
<td>-</td>
<td>-</td>
<td>3+: 3</td>
<td>3+: 3</td>
</tr>
</tbody>
</table>

# Criteria for Grading Microscopic Findings: –: No abnormal changes ±: Very slight +: Slight 2+: Moderate 3+: Severe Number of animals in which the grade was observed.

Table 6. The summary of the organ weights, necropsy and histopathology of the kidneys.

<table>
<thead>
<tr>
<th>Macroscopic findings##</th>
<th>C-SD</th>
<th>HF-SD</th>
<th>C-SDT</th>
<th>HF-SDT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enlargement, glomerulus</td>
<td>-</td>
<td>-</td>
<td>+: 3</td>
<td>+: 3</td>
</tr>
<tr>
<td>Increase, mesangial matrix, glomerulus</td>
<td>-</td>
<td>-</td>
<td>±: 3</td>
<td>±: 2, +: 1</td>
</tr>
<tr>
<td>Cast, hyaline</td>
<td>-</td>
<td>-</td>
<td>±: 1, +: 1</td>
<td>±: 1, +: 1, 2+: 1</td>
</tr>
<tr>
<td>Regeneration, epithelium, tubule</td>
<td>±: 1</td>
<td>±: 1</td>
<td>±: 2, +: 1</td>
<td>2+: 2, 3+: 1</td>
</tr>
<tr>
<td>Dilatation, tubule</td>
<td>-</td>
<td>-</td>
<td>±: 3</td>
<td>2+: 3</td>
</tr>
<tr>
<td>Mineralization, tubule</td>
<td>-</td>
<td>-</td>
<td>±: 2</td>
<td>±: 3</td>
</tr>
<tr>
<td>Accumulation glycogen, tubule</td>
<td>-</td>
<td>-</td>
<td>±: 3</td>
<td>±: 3</td>
</tr>
<tr>
<td>Infiltration, inflammatory cell, interstitial</td>
<td>-</td>
<td>-</td>
<td>±: 2</td>
<td>±: 3</td>
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<tr>
<td>Fibrosis</td>
<td>-</td>
<td>-</td>
<td>±: 1</td>
<td>±: 1, +: 2</td>
</tr>
</tbody>
</table>

** p < 0.01; statistical significance in comparison between the C-SD and HF-SD groups and between the C-SDT and HF-SDT groups. $S$ p < 0.01; statistical significance in comparison between the C-SD and C-SDT groups. #: Criteria for Grading Macroscopic Findings: –: No abnormal changes +: Slight 2+: Moderate 3+: Severe. Number of animals in which the grade was observed. ##: Criteria for Grading Microscopic Findings: –: No abnormal changes ±: Very slight +: Slight 2+: Moderate 3+: Severe. Number of animals in which the grade was observed.

post-treatment between the C-SDT and HF-SDT groups. The plasma NEFA and TKB levels were lower in the HF-SDT group than those in the C-SDT group post-treatment (p < 0.01). Urinary UA levels in the HF-SDT group were higher post-treatment than those pre-treatment and while the plasma UA levels were lower in the C-SDT group post-treatment. There was no difference in plasma UN levels the post-treatment between the C-SDT and HF-SDT groups while plasma UN levels was higher post-treatment than those pre-treatment in the C-SDT group (p < 0.05).

The data for kidney injury markers are shown in Table 4. There were no differences in any parameter pre-treatment between the C-SDT and HF-SDT groups. In general, the levels of all the urinary kidney injury markers but VEGF tended to be higher post-treatment than those pre-treatment both in the C-SDT and HF-SDT groups (p < 0.05 for urinary KIM-1 levels in the C-SDT group). Urinary KIM-1 levels were higher in the HF-SDT group than those in the C-SDT group post-treatment (p < 0.05). Urinary levels of clusterin, osteopontin and TIMP-1 also tended to be higher in the HF-SDT group than those in the C-SDT group post-treatment. There was no differ-
ence in urinary VEGF levels post-treatment between the C-SDT and HF-SDT groups.

The results of the histopathology for the pancreas are shown in Table 5. Atrophy of the pancreatic islets was observed in both the SDT rat groups and the severity was comparable between the SDT rat groups.

The data for kidney weights, and macroscopic and microscopic findings of the kidneys are shown in Table 6. The absolute and relative kidney weights were higher in the HF-SDT group than those in the C-SDT group (p < 0.01). At necropsy, slight enlargement of the kidneys was observed in all the animals in the HF-SDT group and in one animal in the C-SDT group. Whitish foci were observed in all the animals in the HF-SDT group while this finding was not noted in the C-SDT group. In both the SDT groups, enlargement of the glomeruli, increased mesangial matrix in the glomeruli, tubular hyaline casts, regeneration of the tubular epithelium, tubular dilatation, tubular mineralization, accumulation of glycogen in the tubules, interstitial inflammatory cell infiltration and fibrosis were observed. Among these, hyaline casts, epithelial regeneration in the tubules, tubular dilatation, mineralization and fibrosis were more prominent in the HF-SDT group than those in the C-SDT group (Fig. 2). Immunohistochemistry revealed that the population of the vimentin (tubular-injury markers) and Kim-1 (tubular-injury markers) positive cells was larger in the HF-SDT group when compared with that in the C-SDT group and the number of the ED-1 (macrophage marker) positive cells was larger in the HF-SDT group than that in the C-SDT group (Fig. 3). There were no differences in desmin (glomerular-injury marker) staining between the C-SDT and HF-SDT groups (data not shown).

**DISCUSSION**

In this study, 27-week-old male SDT rats, which are expected to have already developed diabetes and mild...
nephropathy accompanied by proteinuria, were fed a high fructose diet for 4 weeks and the effects of high fructose diet on the kidneys were examined by measuring renal function parameters and kidney injury markers and by histopathological examinations of the kidneys. The SD rats were also fed basal or high fructose diet for the same period as employed for the SDT rats in order to compare the effects of high fructose intake between normal rats and diabetic rats.

In comparison between the SD rats and SDT rats fed
basal diet (CRF-1), the body weight gain of the SDT rats was lower than that in the SD rats, although total food consumption and total caloric intake during the experiment period were higher in the SDT rats than in the SD rats. Plasma and urinary glucose levels and plasma lipid parameters were higher in the SDT rats than in the SD rats. Alteration of renal function-related parameters, including urinary protein levels, and kidney injury markers (Kim-1 and osteopontin), renal macroscopic and histopathological changes and histopathological changes in the pancreas were also noted in the SDT rats. Changes in plasma and urinary glucose levels, renal function-related parameters and kidney injury markers were noted at the initiation of the experiment period, indicating that the basal diet-fed SDT rats had developed diabetes and mild nephropathy by the initiation of the experiment period as expected. The renal histopathological changes noted in the basal diet-fed SDT rats included glomerular, tubular and interstitial lesions. In diabetic patients with DN, various glomerular lesions accompanied by microalbuminuria, including thickened glomerular basement membrane, mesangial expansion and nodular and/or advanced glomerulosclerosis, and tubulointerstitial lesions including tubular atrophy, interstitial fibrosis and inflammatory cell infiltration have been reported (Tervaert et al., 2010). The characteristics of the kidney lesions in the basal diet-fed SDT rats obtained in the present study were similar to those in diabetic patients with DN.

In this study, the high fructose diet given for 4 weeks caused alteration of the plasma lipid parameters, including increased plasma triglyceride, total cholesterol and phospholipid levels and decreased plasma total ketone body levels in the SD rats. It's known that high fructose intake leads to hypertriglyceridemia and hypercholesterolemia through increased synthesis of fatty acids and esterification of fatty acids in the liver and increased secretion of VLDL from the liver (Basciano et al., 2005). The alteration of the plasma lipid parameters noted in the high fructose-fed SD rats in this study was considered to be related to enhanced synthesis of fatty acids in the liver due to the high fructose intake. In our study, there were no biologically significant findings in the renal function-related parameters, kidney injury markers or histopathology of the kidney in the high fructose-fed SD rats, although enlargement of the kidney was noted in one SD rat fed the high fructose diet. It's known that a high fructose diet induces renal failure in normal rats (Sanchez-Lozada et al., 2007; Nakayama et al., 2010). In these studies, fructose was given by a diet containing 60% fructose for 6 or 8 weeks or as a 10% solution in drinking water for 8 weeks. Although the reason for the absence of renal failure in the high fructose-fed SD rats is not clear, the difference in the concentration of fructose in the diet, treatment period, the animals' age at the initiation of treatment and/or the methods of treatment with fructose between our study and other studies may contribute to the absence of the renal failure in the high fructose-fed SD rats in our study.

In this study, high fructose diet given for 4 weeks decreased body weights, total body weight gain and total food consumption when compared with the feeding of the basal diet in the SD rats, however, there was no difference in total calorie intake between the basal diet-fed SDT rats and high fructose-fed SDT rats. Plasma and urinary glucose levels and urinary uric acid levels were higher after feeding with high fructose diet when compared with the basal diet-fed animals in the SDT rats. Plasma total ketone bodies and NEFA levels were lower after feeding with the high fructose diet when compared with the basal diet-fed rats. Feeding with the high fructose diet also led to increases in urinary total protein levels and urinary levels of kidney injury markers including clusterin, KIM-1, osteopontin and TIMP1, all of which are tubular-injury markers, but not in urinary VEGF levels (a podocyte-injury marker) in the SDT rats. The kidney weights were higher in the SDT rats fed high fructose diet than those in the SDT rats fed basal diet. Gross and microscopic renal lesions in the basal diet-fed SDT rats were exaggerated by feeding with high fructose diet. Slight enlargement of the kidneys was observed in all the SDT rats fed high fructose diet but was limited to one animal in the basal diet-fed group. Whitish foci were observed in all the SDT rats fed high fructose diet while this finding was not noted in the SDT rats fed the basal diet. In the histopathological evaluation, the severity of the tubular lesions (hyaline casts, epithelial regeneration, dilatation and mineralization) and interstitial lesions (fibrosis) were clearly enhanced in the high fructose diet-fed rats when compared with the basal diet-fed SDT rats while there were no differences in the severity of the glomerular lesions (enlargement and an increase in mesangial matrix) between the basal diet-fed and high fructose-fed SDT rats. Immunohistochemistry for vimentin (a marker of tubular injury and epithelial-mesenchymal transition) and Kim-1 (a marker of tubular injury) also revealed that the SDT rats fed high fructose diet had more prominent tubular injury when compared with the SDT rats fed basal diet. On the other hand, immunohistochemistry of desmin (a marker of glomerular injury) revealed that there was no difference in the severity of the glomerular injury between the basal diet-fed and high fructose-fed SDT rats. These results indicated that high fructose diet mainly affected the tubules
rather than the glomeruli in the SDT rats, and that fructose affected the tubules directly and progression of the tubular lesions was independent of any glomerular injury. This discussion is supported by the selective increase in the tubular-injury markers (KIM-1, Clusterin, osteopontin and TIMP-1) after feeding with high fructose diet in the SDT rats. The slight increase in the urinary protein levels in the high fructose-fed SDT rats was considered to be secondary to the tubular damage, because tubular dysfunction also leads to reduction of protein reabsorption from the tubules and increased urinary protein excretion (Christensen and Gburek, 2004). In humans with diabetes, glomerular changes are considered primarily more important than tubulointerstitial changes and it is a common recognition that tubular dysfunction occurs late stage in DN and develops following the glomerular injury (Kashgarian et al., 1977; Benigni and Remuzzi, 1996). Generally, the earliest sign of DN in human is microalbuminuria as the result of glomerular dysfunction and podocyte loss (Pagtalunan et al., 1997; Reidy et al., 2014; Gross et al., 2005). On the other hand, DN is also characterized by tubulointerstitial injury as well as by glomerular disease (Tervaert et al., 2010). Some studies reported that the direct effects of oxidative stress and inflammatory cytokines on tubules and interstitium might contribute to the progression of tubulointerstitial injury of DN in humans and experimental animals (Kanauchi et al., 2002; Nakayama et al., 2010). Furthermore, recent studies suggested that tubular damage was induced earlier than glomerular injury in the course of DN in humans and experimental animals (Hasegawa et al., 2013). Some studies reported that future renal function was more closely associated with severity of tubulointerstitial lesions than that of glomerular lesions in DN patients (Gilbert and Cooper, 1999; Thomas et al., 2005). Taking the histopathological profile obtained in the present study for the high fructose diet-fed SDT rats and the profile of DN according to recent studies, in which tubular lesions are considered to be primary, together, the effects of fructose on the tubules and interstitium can be critical for the progression of DN.

The results of our study also revealed that diabetic animals or animals with nephropathy are more sensitive to fructose-induced nephropathy than normal animals in comparison of the effects of high fructose diet between the SDT rats and SD rats and indicates that the effect of fructose on renal function is greater in diabetes patients and DN patients than in healthy individuals.

The relationships between excessive fructose intake and DN are not clear; however, there are some mechanisms which could explain the adverse effects of fructose on the kidneys. One of the mechanisms is the contribution of uric acid to renal failure. Fructose is phosphorylated to fructose-1-phosphate by fructokinase, which uses ATP in the phosphorylation leading to generation of AMP and activation of AMP deaminase (Mäenpää PH et al., 1968). The activation of AMP deaminase leads to enhancement of the production of uric acid (Perheentupa and Raivio, 1967; Morris et al., 1978). This is evidenced by the results of a study, in which serum uric acid levels were increased rapidly after ingestion of fructose in humans (Perheentupa and Raivio, 1967). Hyperuricemia is one of the risk factors of renal failure (Iseki et al., 2001; Siu et al., 2006) and there are some studies reporting that microalbuminuria in type 2 diabetes mellitus patients is associated with high serum uric acid levels (Fukui et al., 2008; Kim et al., 2011). Hyperuricemia also leads to renal injury in normal animals and lowering blood uric acid ameliorates renal lesions in diabetic model animals (Kosugi et al., 2009; Kang et al., 2002). A recent study demonstrated that blocking fructose metabolism in a diabetic mouse model mitigated the development of tubulointerstitial injury through lowering tubular uric acid production (Bjornstad et al., 2015). In our study, urinary uric acid excretion in the SDT rats fed high fructose diet was higher than that in the SDT rats fed basal diet and was much higher than that in the high fructose-fed SD rats. It’s known that AMP deaminase activity in the liver is higher in diabetic animals (Cicerchi et al., 2014). The differences in the response of urinary uric acid levels between the high fructose-fed SDT rats and the high fructose-fed SD rats were considered to be related to higher AMP deaminase activity in the SDT rats. Urinary uric acid levels were clearly different between the high fructose-fed SDT rats, the basal diet-fed SDT rats and the high fructose-fed SD rats, however, there were no differences in the plasma uric acid levels between these conditions. It is difficult to detect the change of plasma uric acid levels in rats because rats have uricase, which degrades uric acid to allantoin, and plasma uric acid levels are maintained at very low levels in rats. Therefore, the urinary uric acid level is a good marker for estimating the amount of uric acid synthesized in the body for rats. The increase in urinary uric acid excretion detected in the high fructose diet-fed SDT rats indicates that high uric acid levels due to high fructose intake contribute to the progression of kidney injury in the SDT rats.

In this study, plasma and urinary glucose levels were increased in the SDT rats fed high fructose diet while high fructose diet feeding did not elevate glucose levels in the SD rat. The difference in the response of glucose to high fructose diet between the SD and SDT rats

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might be related to spontaneous abnormalities in glucose metabolism in the SDT rats. It has been reported that the SDT rats exhibit glucose intolerance with impaired insulin secretion (Masuyama et al., 2003). In this study, the plasma insulin levels in the SDT rats are supposed to be decreased because plasma and urinary glucose levels were increased and severe atrophy of the pancreatic islets was observed in all the SDT rats. Fructose has also been reported to induce insulin resistance via de novo lipogenesis although fructose itself does not increase blood glucose directly and signal insulin release (Elliot et al., 2002; Johnson et al., 2009). The induction of insulin resistance by high fructose diet was considered to enhance impaired glucose tolerance in the high fructose-fed SDT rats. In humans, high blood glucose level is one of the risk factors for DN of diabetes patients (Delahanty and Halford, 1993; Kim, 2014). Therefore, elevation of plasma glucose levels in the SDT rats fed a high fructose diet was considered to contribute to progression of the kidney injury.

Fructose is also known as an inducer of obesity and dyslipidemia due to stimulation of triglyceride synthesis and increases fat deposition in the liver (Basciano et al., 2005). Human epidemiological studies revealed that dyslipidemia is a risk factor for development and progression of DN (Ravid et al., 1998) and animal studies also revealed that dyslipidemia leads to renal injury (Kasiske et al., 1990). In the present study, high fructose diet did not increase body weights or did not enhance dyslipidemia in the SDT rats when compared with the SDT rats fed the basal diet. Therefore, the contribution of lipid-related factor was considered to be low in the progression of the kidney lesions in the SDT rats fed high fructose diet.

In addition to the above mechanisms, it has been reported that fructose can induce renal injury through mechanisms including high blood pressure, depletion of ATP and production of inflammatory molecules and proinflammatory mediators (Sánchez-Lozada et al., 2007; Glushakova et al., 2008; Cirillo et al., 2009; Gersch et al., 2007). Taking all the above mechanisms together, the complicated mechanisms including hyperglycemia, uric acid production and other factors contributed to the progression of the renal lesions in the SDT rats fed high fructose diet.

In conclusion, high fructose diet enhanced renal lesions, especially tubulointerstitial lesions, in the SDT rats in this study. Based on the results obtained in this study, excessive fructose intake can be a risk factor of the development and progression of DN in diabetic patients.

**Conflict of interest**— The authors declare that there is no conflict of interest.

**REFERENCES**


Gilbert, R.E. and Cooper, M.E. (1999): The tubulointerstitium in progressive diabetic kidney disease: more than an aftermath of...
Effects of high fructose diet on nephropathy in SDT rats