Title

Salt loading with unilateral nephrectomy accelerates decline in glomerular filtration rate in the hypertensive, obese, type 2 diabetic SDT fatty rat model of diabetic kidney disease

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Short title

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Abstract

For the evaluation of novel therapeutic agents for diabetic kidney disease (DKD), it is desirable to examine their efficacy in animal models by using the glomerular filtration rate (GFR) as an index. For this purpose, animal models that demonstrate a short-term GFR decline due to disease progression are required. Therefore, we aimed to develop such an animal model of DKD by using obese type 2 diabetic Spontaneously Diabetic Torii (SDT) fatty rats treated with salt loading by drinking water containing sodium chloride with or without unilateral nephrectomy. As a result, we have found that 0.3% salt loading with unilateral nephrectomy or 0.8% salt loading alone caused a rapid GFR decline, hypertension and rapid development of tubulointerstitial fibrosis. Moreover, the addition of losartan to a mixed diet suppressed the GFR decline in SDT fatty rats treated with 0.3% salt loading with unilateral nephrectomy. These results suggest that the model of SDT fatty rats treated with 0.3% salt loading and unilateral nephrectomy could be used as a hypertensive DKD model for evaluating therapeutic agents based on suppression of GFR decline.

Keywords: SDT fatty rat, salt loading, unilateral nephrectomy, GFR decline,

1. Introduction

Diabetic kidney disease (DKD) is characterized by a complicated pathology that involves

hyperglycemia, hyperlipidemia, obesity, and hypertension, accompanied by a decline in glomerular filtration rate (GFR). The estimated GFR (eGFR) is frequently used to assess renal function in patients with DKD. That is, an eGFR decline indicates a decrease in renal function. For more than a decade, angiotensin converting enzyme (ACE) inhibitors or angiotensin receptor blockers (ARBs) such as ramipril or losartan have been used for the treatment of diabetic nephropathy[1]. However, the target populations of these agents are limited to patients suffering from diabetic nephropathy with type 2 diabetes, hypertension, elevated plasma creatinine, and proteinuria[2,3]. In recent years, evidence of the efficacy of sodium-glucose co-transporter-2 (SGLT2) inhibitors in patients with DKD has been accumulating[4], and it is likely that the treatment strategy will be changed in the near future so that SGLT2 inhibitors can become the basic medication used for DKD patients[5].

Various animal models of DKD are known, however, the complex etiological complications associated with decreased renal function in patients with DKD, develop in only a few of them. As an example, the widely used streptozotocin (STZ)-induced type 1 diabetes model, unlike humans, shows elevated blood glucose levels but not hyperlipidemia or hypertension. Whereas, endothelial nitric oxide synthase (eNOS) (-/-) db/db mice, SHR/NDmcr-cp (cp/cp) rats, and Zucker diabetic fatty rats, which are hypertensive DKD models with such complications, must be more than 6 months old before nephropathy develops with histopathological changes[6-12]. Furthermore, these animal models do not exhibit a GFR decline

during this period, but rather they show an increase in GFR due to glomerular hyperfiltration[13]. Therefore, there are currently no pharmacologically available animal models of DKD that show a GFR decline, which is a surrogate for renal function in patients with DKD.

The SDT fatty rat is an obese, type 2 diabetic model of DKD characterized by hyperglycemia, hyperlipidemia, and hypertension[14-18]. By utilizing SDT fatty rats, we attempted to establish an accelerated hypertensive DKD model in which GFR decline, and these characteristics develop rapidly. To accelerate the decrease in renal function, salt loading with or without unilateral nephrectomy was performed, and in addition, the efficacy of ARBs (losartan, in particular) was evaluated.

2. Results

2.1 Effect of salt loading with or without unilateral nephrectomy (UNx) on renal function

A significant increase in GFR was observed in untreated SDT fatty rats (SDT fatty rats provided with normal drinking water) compared to Sprague Dawley (SD) rats, and the increase persisted throughout the experiment. On the other hand, a significant decrease in GFR was observed in the rats undergoing 0.6% salt loading without UNx (0.6% salt), 0.8% salt loading without UNx (0.8% salt), or 0.3% salt loading with UNx (0.3% salt/UNx),

compared to the untreated SDT fatty rats. Furthermore, the GFR was significantly lower in the rats with 0.3% salt/UNx at week 13 than in SD rats. In addition, elevated urinary albumin-creatinine ratio (UACR) was observed in the untreated SDT fatty rats, and was further increased by 0.3% salt/UNx, 0.6% salt, or 0.8% salt (Fig. 1).

2.2 Effect on renal related and metabolic parameters

During the experimental period, higher plasma creatinine (pCre) and blood urea nitrogen (BUN) levels were observed in rats treated with 0.3% salt/UNx compared to other treated SDT fatty rats; chronic hyperglycemia was observed in untreated SDT fatty rats compared to SD rats, however, lower plasma glucose levels were observed in rats treated with 0.3% salt/UNx, 0.6% salt, or 0.8% salt, and were accompanied by a decrease in hemoglobin A1c levels at week 12. Plasma total cholesterol and triglyceride levels, which are elevated in SDT fatty rats, were increased by salt loading (Fig. 2).

2.3 Effect on renal histology

Histopathologically, tubulointerstitial fibrosis was prominently observed in the untreated SDT fatty rats at week 13. Moreover, treatment with 0.8% salt and 0.3% salt/UNx resulted in increased interstitial fibrosis. Glomerular hypertrophy, mesangial hyperplasia, and interstitial infiltration of inflammatory cells were induced by treatment with 0.6% salt, 0.8% salt, and

0.3% salt/UNx (Fig. 3). A significant increase in glomerular size was observed in untreated SDT fatty rats compared to SD rats. Furthermore, SDT fatty rats treated with 0.8% salt had significantly larger glomerular size than untreated SDT fatty rats. The average size (μm^2) of glomeruli in the SD rats, untreated SDT fatty rats, 0.6% salt treated rats, 0.8% salt treated rats, and 0.3% salt/UNx treated rats were (7594 ± 484, 12427 ± 1365, 14746 ± 1695, 16030 ± 4192, and 15401 ± 1359), respectively. Disruption of the glomerulus was observed in rats treated with 0.8% salt (data not shown).

2.4 Effect of losartan on GFR decline and renal histology in rats treated with 0.3% salt loading and UNx

Next, we investigated the effect of losartan on GFR decline in rats treated with 0.3% salt/UNx. The results showed that dietary administration of losartan (10 mg/kg/day) significantly suppressed GFR decline in the rats treated with 0.3% salt/UNx (Fig. 4). Histopathologically, it was found that 0.3% salt/UNx caused glomerular lesions (hypertrophy, adhesion, increase in mesangial matrix), tubular lesions (cast formation, regeneration, expansion) and tubulointerstitial lesions (fibrosis, inflammatory cell infiltration). However, no improvement was evident with administration of losartan, and in several cases, limited improvement in tubular dilatation and cast formation was detected (data not shown).

3. Discussion

In SDT fatty rats, 0.3% salt loading by drinking water with unilateral nephrectomy (0.3% salt/UNx), or 0.8% salt loading alone, was able to induce an early decline in GFR as well as an elevated UACR, accompanied by histopathological changes such as kidney fibrosis, glomerular hypertrophy, and inflammatory cell infiltration (Fig. 1A-C, Fig. 3). Furthermore, 0.3% salt/UNx caused renal-related parameters such as pCre and BUN to increase, in contrast to 0.8% salt loading alone (Fig. 2A, 2B). The hallmarks of DKD include GFR decline, albuminuria, and impaired renal morphology (increased glomerular basement membrane thickness, mesangial hyperplasia, interstitial fibrosis, glomerular hypertrophy, glomerulosclerosis, podocyte foot process effacement, and arterial hyalinosis)[19]. The induction of decreased renal function by 0.3% salt/UNx in SDT fatty rats was suggested to be reminiscent of the pathological condition in patients with hypertensive DKD, as indicated above.

Recently, induced renal injury by dietary salt loading in the Dahl salt-sensitive hypertensive rat has been used as an animal model to evaluate the efficacy of agents for antihypertensive treatment[20,21]. Although the Dahl salt-sensitive hypertensive rat is a model of hypertension, it is not a model to study risk factors specific to DKD, such as diabetes mellitus, abnormal lipid metabolism, and obesity. SHR/NDmcr-cp (cp/cp) rats and db/db mice, which are known diabetic models, show signs of DKD, such as abnormal lipid metabolism and obesity,

however, during the lifetime of the animals, renal function is not influenced or in a hyperfiltration state, and infrequently declines as measured by creatinine clearance[7,22,23]. Therefore, the SDT fatty rat treated with salt loading alone exhibits a more rapid GFR decline and this model might mimic the pathogenesis of DKD in patients.

On the other hand, salt loading might not cause hyperfiltration since it would not induce contraction of the efferent arteriole by inhibiting the activation of renin-angiotensin system in the renal glomerulus[24]. This could lead to a more rapid and direct detection of the effects of various risk factors on renal function. However, since salt loading is likely to cause an increase in body fluid volume, renal-related parameters in the blood, such as pCre and BUN, may not be elevated even though these parameters usually increase with a decrease in renal function. Therefore, it is preferable to influence renal function by using a lower salt concentration for loading.

To further accelerate the decline of renal function, we performed unilateral nephrectomy (UNx) in addition to salt loading. By promoting renal dysfunction via reducing the number of nephrons, UNx and 5/6 nephrectomy in rats and mice are widely used as experimental models of chronic kidney disease (CKD)[25-27].

In the present study, we performed UNx as well as 0.3% salt loading in rats, which allowed

us to establish a model in which even loading a low concentration of salt would lead to steady GFR decline, hypertension and worsened renal function parameters, accompanied by renal histopathological changes. It was considered that the decrease in the number of nephrons due to nephrectomy causes an increase in the single nephron GFR, and the sustained hypertension due to salt loading accelerates glomerulosclerosis and intensifies medullary hypoxia, resulting in further reduction in the number of nephrons and hypoxia caused by interstitial fibrosis[28-30]. Furthermore, in this study, plasma total cholesterol (TC) and triglyceride (TG) levels were elevated in rats treated with 0.3% salt/UNx as well as 0.6% salt loading alone or 0.8% salt loading alone (data not shown). In a healthy condition, both TG and TC are known to be absorbed by the renal cells such as tubular epithelial cells and used as energy source or lipid membrane components. However, in CKD condition, the intracellular factors involved in their utilization are decreased, and these could accumulate as lipid droplets and exert cytotoxic effects[31,32]. It has also been reported that triglycerides and free fatty acids (FFAs) bound to albumin accumulated in the proximal tubules could cause damage and induce inflammation[31]. Furthermore, our model is characterized by hypertension. In the preliminary study, systolic blood pressure levels (mmHg) of SD rats and 0.3% salt/UNx treated rats were 105.3 ± 23.8 and 209.6 ± 24.2 after 5 weeks of treatment, and 126.8 ± 12.5 and 191.4 ± 35.4 after 10 weeks of treatment, respectively, measured by the indirect tail cuff method[25].

Unfortunately, it was not possible to identify the lesion as a prominent change due to the

depth of the renal area observed. In addition, because the changes appeared in each nephron, we considered it difficult to observe pathological shift from a cortical layer to a deeper layer. Additionally, decrease in blood glucose levels were observed in these rats (Fig. 2C). The reason for the decrease in the levels might be related to the downward trend in food intake of SDF Fatty rats treated with salt loading[33]. On the other hand, there is also a possibility that sodium loading might impair the function of SGLT1 in the intestine, however, these should be the themes for our future study.

Next, we confirmed the effect of losartan, which is widely used for the treatment of diabetic nephropathy[4]. The results showed that dietary administration of losartan attenuated the GFR decline induced by 0.3% salt loading and UNx in rats (Fig. 4). However, the results of histopathological evaluation showed that the efficacy of losartan was partial. The average size (μm^2) of glomeruli was comparable to the normal diet, showing no efficacy (normal diet : 14475 ± 3016, losartan diet : 17173 ± 3539). Previous reports suggest that it could be that the ARBs acted as neuroprotective agents to prevent GFR decline as another function of losartan other than reducing efferent artery constriction[34,35]. These results suggest that this model might mimic the later stages of DKD, where ACE inhibitors and ARBs are less effective in clinical care. Whereas, the GFR of SD rats appeared spontaneously decreased in Fig.4, it was considered that there was a possibility of detecting the aging-related decrease to some degree[36].

In preclinical studies, the lack of animal models that mimic the pathophysiological characteristics of patients with DKD has been an obstacle to address the clinical needs precisely[37]. Animal models might provide new-insights into the development and progression of nephropathy in patients with DKD, and help us better understand the etiology of the disease. In addition, animal models could be used to explain how novel therapies might function, identify alternative pathways for these therapies, and even help to validate the onset of potential side effects.

In patients with DKD, risk factors such as hypertension, dyslipidemia, and hyperuricemia, in addition to diabetes mellitus, are considered to be complex pathological determinants. Thus, we considered that our model is also characterized by including these risk factors in addition to diabetes. On the other hand, it is unknown which of these factors, including hypertension, contributes to the pathogenesis of the patients. In this study, plasma TG and TC levels were elevated in our hypertensive DKD model (data not shown). The dyslipidemia in CKD patients is characterized by elevated TG and TC, therefore, in our model, we intended to mention these elevations. For the mechanism of TG and TC elevation in our model, the article by M Lee et al. that studied the response of Adipocytes to salt loading[38] might be informative. This study reported that high salt increased the expression of adipogenic/lipogenic genes and, inversely, decreased the gene of lipolysis.

In the past, ACE inhibitors and ARBs, two groups of anti-hypertensive medications that slow the progression of diabetic nephropathy (DN), have been extensively studied in various experimental DN models. However, not all the typical DN features develop in many of these models. For example, the mouse model has several limitations, and only the early stages of diabetic kidney disease develop in this model. In fact, the classical model of DN only exhibits the features of early stage DKD: moderate albuminuria, glomerular hypertrophy, and slight expansion of the mesangial matrix[39]. Glomerulosclerosis, tubular atrophy, or interstitial fibrosis is rarely presented in these animals. The model presented herein, which is produced by the combination of UNx and salt loading, is a novel animal model that exhibits many of the features observed in patients with DKD and therefore might be helpful in characterizing the mechanisms involved in this disease. Recently, it was reported that, in hypertensive DKD mouse model, under conditions in which spironolactone and esaxerenone showed similar reduction in blood pressure, esaxerenone elicited a greater attenuation of albuminuria, glomerular injury, tubulointerstitial fibrosis, and renal inflammation than spironolactone[40].

In the future, we expect to use our model to evaluate the effects of DPP-IV inhibitor/SGLT2 inhibitor alone or in combination with ACE inhibitors or ARBs and to understand the characteristics and limitations of this model by comparing this model to patients with DKD, which will allow us to further characterize the pathogenic factors other than hypertension in our model. It was reported that ARB reduced proteinuria even though it was not effective for

blood pressure[41]. In addition, ARBs are neuroprotective agents, therefore, it is possible that ARBs have a renoprotective effect through this action[35]. As mentioned above, we would like to establish the position of our model by evaluating the efficacy of agents with other mechanisms of action other than the antihypertensive effect of ARBs on our model in the future.

4. Materials and Methods

4.1 Animals

Male SDT fa/fa (fatty) rats and age-matched Sprague-Dawley (SD) rats were purchased from CLEA Japan, Inc. (Tokyo, Japan), and maintained in a specific pathogen-free room at a temperature of 23 ± 3°C and air humidity of 55 ± 15%, on a 12-h/12-h light/dark cycle. This animal study was conducted in accordance with the Japanese Law for the Humane Treatment and Management of Animals (Law No. 105, October 1, 1973).

4.2 Chemicals

Losartan (≥98% purity) was purchased from LKT Laboratories, Inc. (St. Paul, Minnesota, USA). The losartan diet was prepared every week by mixing losartan with standard powdered chow (CE-2, CLEA Japan Inc.), adjusting the mixture ratio based on the body weight and

average dietary intake of the rats. The mixed diet containing approximately 0.015% losartan (10 mg/kg/day) was fed for 10 weeks.

4.3 Effects of salt loading with or without unilateral nephrectomy (UNx) on glomerular filtration rate (GFR)

The study design is shown in Figure 5A. Animals at 9 weeks old were divided into 5 treatment groups based on the measurements of GFR: 1) SDT fatty rats drinking normal water and not nephrectomized (without UNx), 2) SDT fatty rats salt loaded by drinking 0.3% salt water and nephrectomized (with UNx), 3) SDT fatty rats salt loaded by drinking 0.6% salt water without UNx, 4) SDT fatty rats salt loaded by drinking 0.8% salt water without UNx, or 5) SD rats not salt loaded by drinking normal water without UNx. Unilateral nephrectomy (left kidney) for the rats in the 0.3% salt loaded group was performed as previously described[25]. In order to establish a model with a decrease in GFR, the salt concentration used for salt loading was investigated. Our previous study showed that 0.3% salt loading alone nor UNx treatment alone did not decrease creatinine clearance[16], so the combination of the 0.3% salt water with UNx group was set up. In the following week, these groups started to the salt-loading treatment by drinking water containing 0, 0.3, 0.6, or 0.8% salt for 13 weeks. During the experimental period, body weight was measured sequentially, and blood and urine samples were collected from the tail vein and using metabolic cages,

respectively. As a parameter of renal function, GFR was measured before group assignment and at 2, 6, 10, and 13 weeks after salt loading. GFR was determined by measuring the plasma clearance of fluorescein isothiocyanate (FITC)-labeled inulin after a single bolus injection as previously described[42]. After the last sampling at week 13, the rats were euthanized and their right kidneys were excised and processed for histological evaluation. Plasma glucose, pCre, BUN, and urine creatinine levels were measured using an automatic biochemical analyzer (Model 7180, Hitachi High-Technologies Corporation, Tokyo, Japan). Plasma insulin levels were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science, Inc., Kanagawa, Japan). Urine albumin levels were measured using a commercially available ELISA kit (Shibayagi Co., Ltd., Gunma, Japan). For histological analysis, the 10% neutral formalin-fixed right kidneys were sectioned and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Sirius red. PAS staining was used to evaluate the degree of glomerular alteration; HE staining and PAS staining were used to evaluate the degree of tubulointerstitial alteration, and Sirius red staining was used to evaluate the degree of interstitial fibrosis. The histological evaluation was assessed by the following four parameters defined in the preliminary examination: interstitial fibrosis, glomerular hypertrophy, mesangial hyperplasia, and interstitial infiltration of inflammatory cells. Preliminary observation of the entire cortical to medullary regions was performed by using renal

specimens and 4 parameters described above that showed prominent changes were examined in this study. The severity of each histological change was scored on a 5-point scale ranging from 0 to 4 (Score 0: within normal limits; Score 1: minimal, solitary [very small] lesion; Score 2: slight, focal [small] lesion; Score 3: moderate, scattered lesion; and Score 4: severe, marked, extensive lesion) based on the severity and extent of the change. For evaluation of glomerular change, glomerular sizes in the cortical area were analyzed. To measure glomerular size, one section per rat was photographed under a light microscope (BX51, Olympus Corporation, Tokyo, Japan) using the 4× objective lens and analyzed using ImageJ software (Rasband WS, ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, https://imagej.nih.gov/ij/, 1997-2018) as previously described[43]. Six animals per group were used for measurement of glomerular size. The glomerular area measurement was performed by randomly divided into sections of the entire kidney from the cortical surface to the juxtamedullar, and all glomeruli within the area and overlapping the upper and right edges were counted. Approximately 50 to 100 glomeruli were counted per animal.

4.4 Effects of losartan on GFR decline in SDT fatty rats undergoing 0.3% salt loading with unilateral nephrectomy (UNx)

The study design is shown in Figure 5B. SDT fatty rats were salt-loaded by drinking 0.3% salt water and subjected to UNx based on the results of the study showing the fastest GFR

decline (Fig. 1A).

UNx was done in the SDT fatty rats at 9 weeks old. In the following week, group assignment was performed based on the GFR, and then 0.3% salt loading was started in all rats except SD rats (control group). The groups were: 1) SDT fatty rats fed a normal diet, salt-loaded by drinking 0.3% salt water, and nephrectomized (with UNx); 2) SDT fatty rats fed the 10 mg/kg losartan diet, salt-loaded by drinking 0.3% salt water, and with UNx; 3) SD rats drinking normal water and not nephrectomized (without UNx). Furthermore, a standard powder chow or mixed diet containing losartan (10 mg/kg/day, approximately 0.015%) was fed for 10 weeks. GFR was measured before and at 2, 5, and 10 weeks after salt loading by intravenous injection of FITC-sinistrin as previously described[44]. After the last measurement of GFR, the rats were euthanized, and their right kidneys were excised and processed for histological evaluation as described above.

4.5 Statistical analysis

Data are expressed as the mean and standard deviation (S.D.) of the indicated numbers of animals or samples. All statistical analyses were performed using Statlight 2000 (Yukms Co., Ltd, Kanagawa, Japan). In a two-group comparison, the statistical significance was assessed using Student's t-test (for homoscedastic data) or Aspin-Welch's t-test (for heteroscedastic data) after homoscedasticity analysis by an F-test. In a multi-group comparison, the statistical

significance was assessed using Dunnett's test (for homoscedastic data) or Steel's test (for heteroscedastic data) after homoscedasticity analysis by Bartlett's test. For histological scores, the statistical significance was assessed by Wilcoxon rank sum test for two-group comparison and Steel's test for a multi-group comparison. All statistical analyses were two-sided, and statistically significant level was set at $p < 0.05$.

Disclosure

The authors declare that there are no conflicts of interest regarding this article.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Figure Legends

Figure 1. Effects of salt loading with or without UNx on GFR and urinary albumin level.

A: GFR changes during the experiment period. B: GFR at week 13. C: UACR at week 13

Data points and bars represent the mean and S.D. (n = 6).

$P < 0.01$ vs. SD-water group (Student's *t*-test)

** $P < 0.01$ vs. SDTF-water group (Dunnett's test)

ᵇ $P < 0.05$ vs. SD-water group (Steel's test)

‡ $P<0.01$ vs. SD-water group (Welch's test)

§ $P<0.05$ vs. SDTF-water group (Steel's test)

UNx, unilateral nephrectomy; GFR, glomerular filtration rate; UACR, urinary albumin-creatinine ratio; SDTF, SDT Fatty

Figure 2. Effect on renal-related and metabolic parameters

A: Plasma creatinine (pCre). B: Blood urea nitrogen (BUN). C: Plasma glucose. D: HbA1c at week 12. E: Plasma triglyceride. F: Plasma total cholesterol. G: Body weight.

Data points and bars represent the mean and S.D. (n = 6).

$P < 0.01$ vs. SD-water group (Student's *t*-test)

† , ‡ $P<0.05$, $P<0.01$ vs. SD-water group (Welch's test)

** $P<0.01$ vs. SDTF-water group (Dunnett's test)

§ $P<0.05$ vs. SDTF-water group (Steel's test)

SDTF, SDT Fatty

Figure 3. Effects of salt loading with or without UNx on renal histopathology.

A: Representative images of Sirius red staining.

B: Interstitial fibrosis. C: Glomerular hypertrophy. D: Mesangial hyperplasia. E: Interstitial infiltration of inflammatory cells

Data points and bars represent the mean and S.D. (n = 6).

$P < 0.01$ vs. SD-water group (Student's *t*-test)

*, ** $P<0.05$, $P<0.01$ vs. SDTF-water group (Dunnett's test)

$$, $P < 0.01$ vs. SD-water group (Wilcoxon rank sum test)

§, §§ $P<0.05$, $P<0.01$ vs. SDTF-water group (Steel's test)

UNx, unilateral nephrectomy; SDTF, SDT Fatty

Figure 4. Effect of losartan diet on GFR decline in rats salt-loaded with 0.3% salt water and subjected to UNx.

Data points and bars represent the mean and S.D. (n = 6).

+ $P<0.05$ vs. SDTF-0.3% salt/UNx group (Dunnett's test)

GFR, glomerular filtration rate; UNx, unilateral nephrectomy; SDTF, SDT Fatty

Figure 5. Experimental design.

A. Effect of salt loading with or without unilateral nephrectomy (UNx) on renal function

B. Effects of losartan on GFR decline

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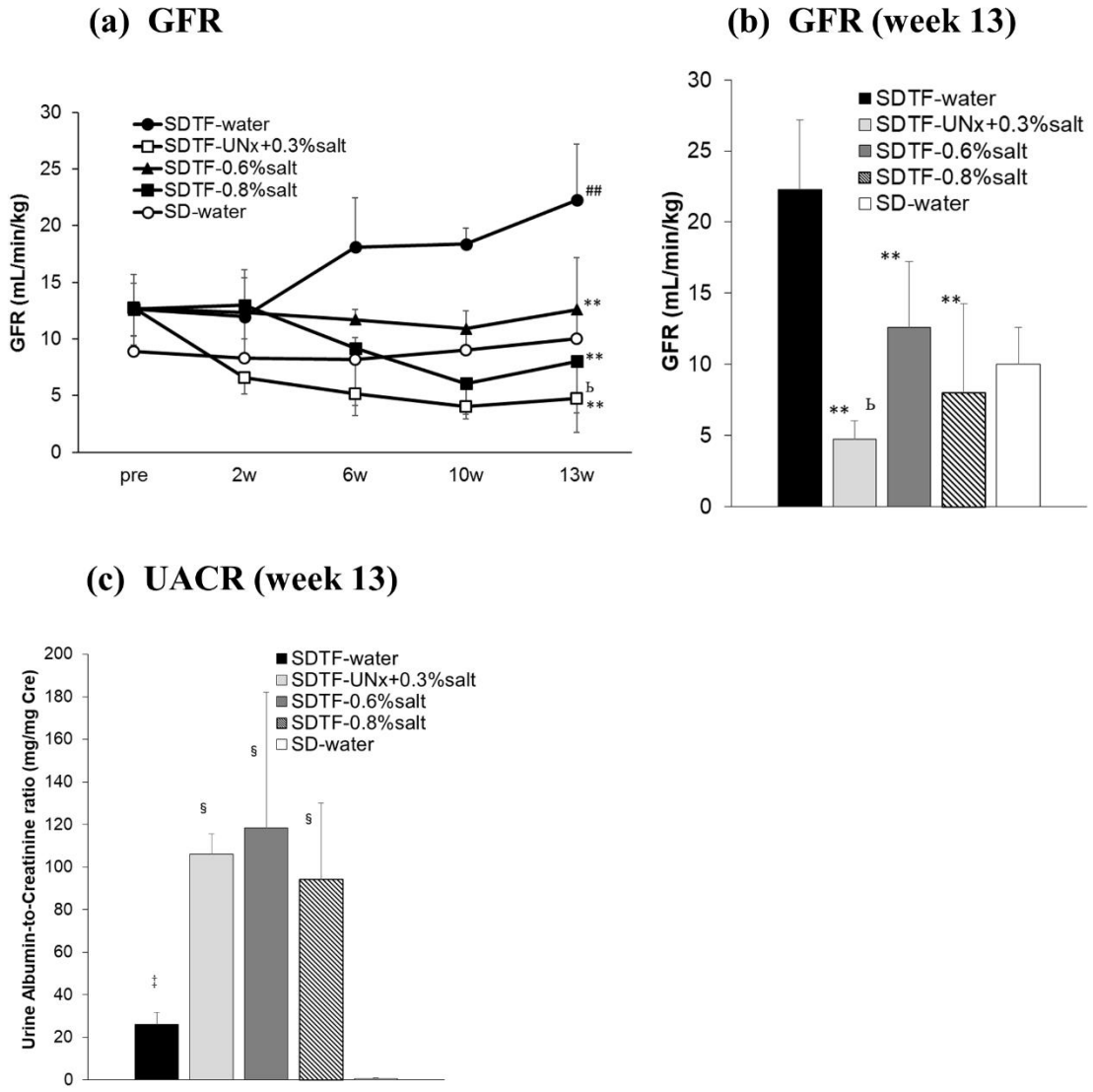
(a) GFR
GFR (mL/min/kg)
SDTF-water
SDTF-UNx+0.3%salt
SDTF-0.6%salt
SDTF-0.8%salt
SD-water
pre
2w
6w
10w
13w
##
**
**
**
Þ
(b) GFR (week 13)
GFR (mL/min/kg)
SDTF-water
SDTF-UNx+0.3%salt
SDTF-0.6%salt
SDTF-0.8%salt
SD-water
**
**
Þ
**
(c) UACR (week 13)
Urine Albumin-to-Creatinine ratio (mg/mg Cre)
SDTF-water
SDTF-UNx+0.3%salt
SDTF-0.6%salt
SDTF-0.8%salt
SD-water
‡
§
§
§

Figure 1

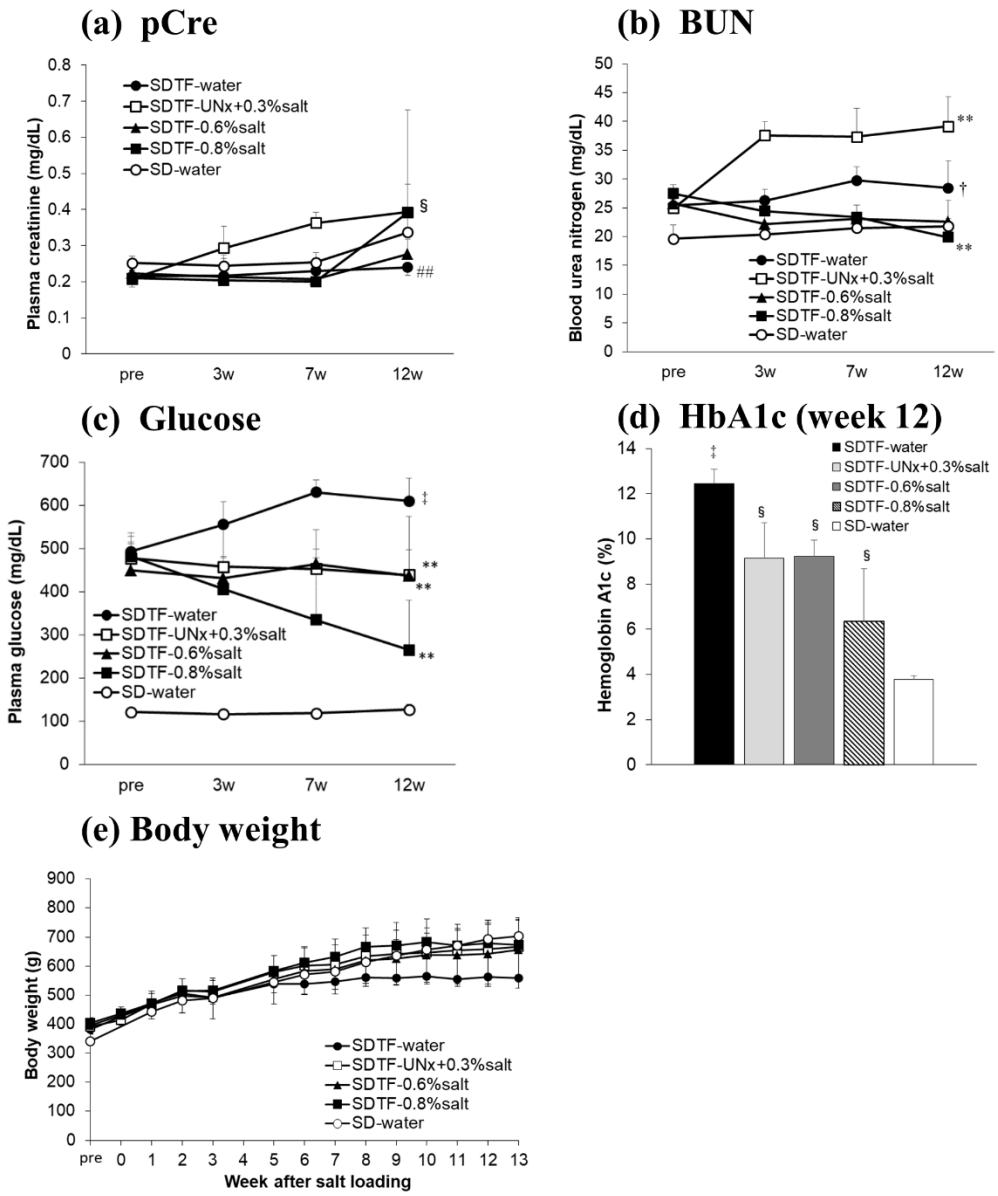
(a) pCre
Plasma creatinine (mg/dL)
SDTF-water
SDTF-UNx+0.3%salt
SDTF-0.6%salt
SDTF-0.8%salt
SD-water
§
##
pre
3w
7w
12w
(b) BUN
Blood urea nitrogen (mg/dL)
**
†
**
(c) Glucose
Plasma glucose (mg/dL)
‡
**
**
(d) HbA1c (week 12)
Hemoglobin A1c (%)
‡
§
§
§
(e) Body weight
Body weight (g)
Week after salt loading

Figure 2

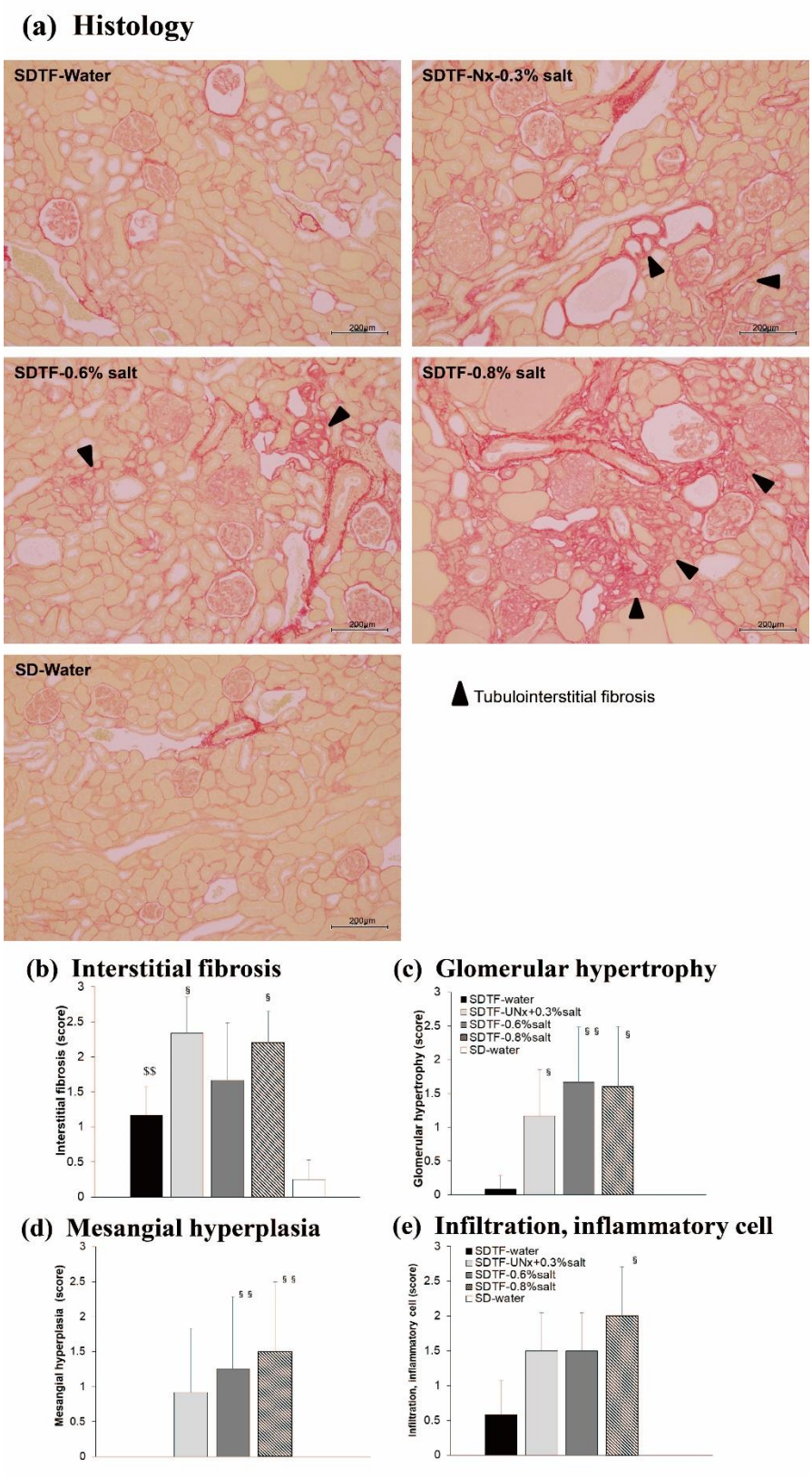
(a) Histology
SDTF-Water
SDTF-Nx-0.3% salt
SDTF-0.6% salt
SDTF-0.8% salt
SD-Water
200μm
Tubulointerstitial fibrosis
(b) Interstitial fibrosis
Interstitial fibrosis (score)
$$
§
§
(c) Glomerular hypertrophy
Glomerular hypertrophy (score)
SDTF-water
SDTF-UNx+0.3%salt
SDTF-0.6%salt
SDTF-0.8%salt
SD-water
§
§ §
§
(d) Mesangial hyperplasia
Mesangial hyperplasia (score)
§ §
§ §
(e) Infiltration, inflammatory cell
Infiltration, inflammatory cell (score)
SDTF-water
SDTF-UNx+0.3%salt
SDTF-0.6%salt
SDTF-0.8%salt
SD-water
§

Figure 3

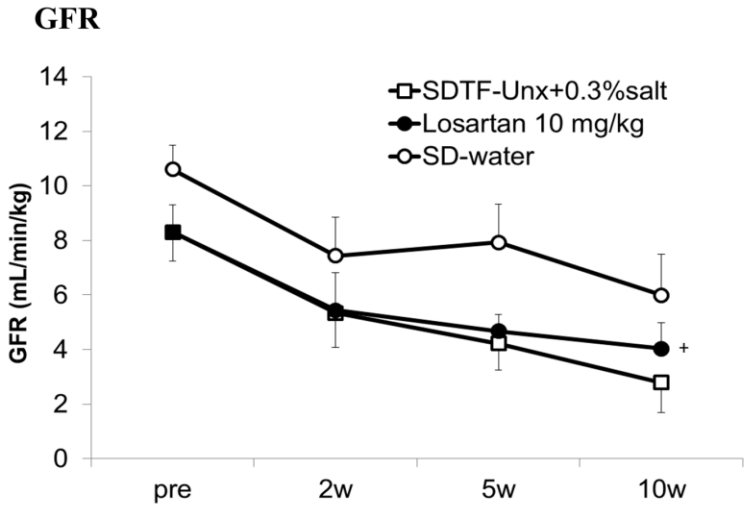
GFR
SDTF-Unx+0.3%salt
Losartan 10 mg/kg
SD-water
GFR (mL/min/kg)
14
12
10
8
6
4
2
0
pre
2w
5w
10w
+

Figure 4

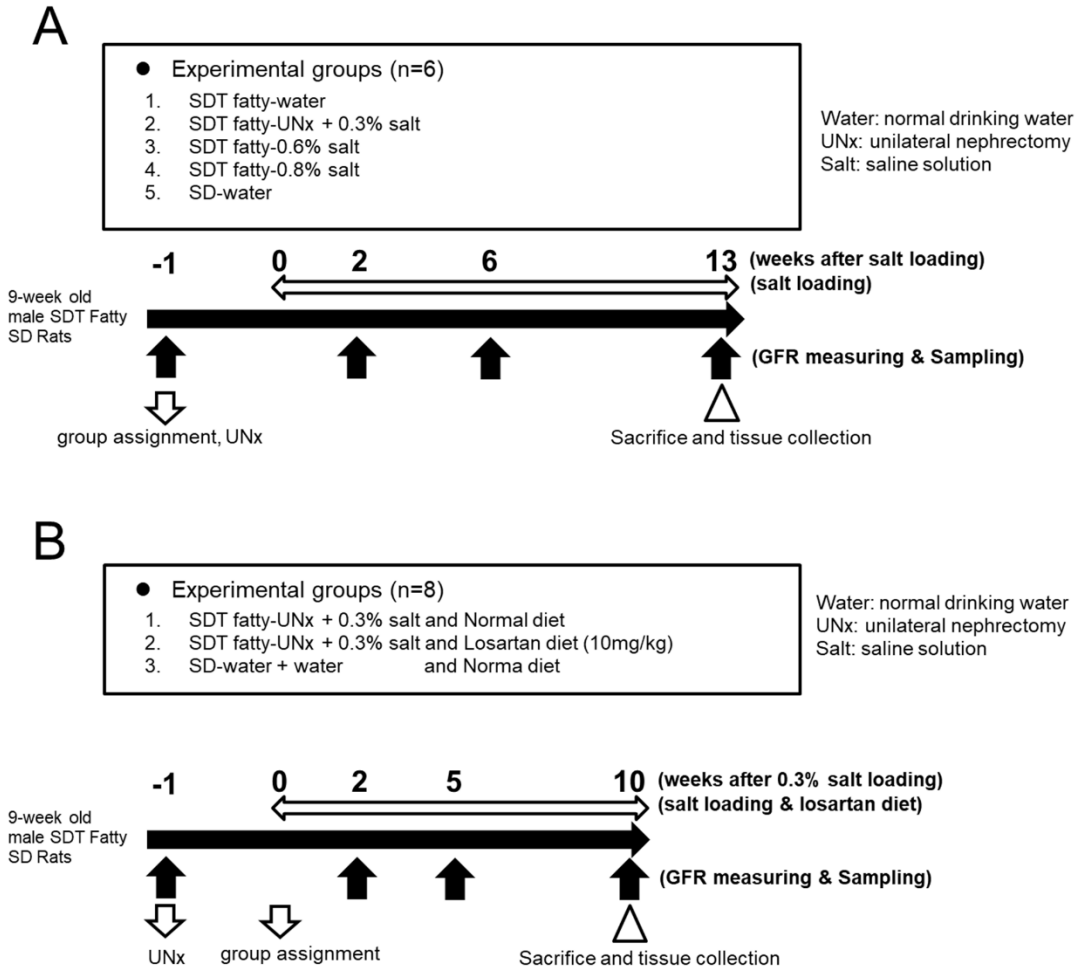
A
Experimental groups (n=6)
1. SDT fatty-water
2. SDT fatty-UNx + 0.3% salt
3. SDT fatty-0.6% salt
4. SDT fatty-0.8% salt
5. SD-water
Water: normal drinking water
UNx: unilateral nephrectomy
Salt: saline solution
-1
0
2
6
13
(weeks after salt loading)
(salt loading)
9-week old
male SDT Fatty
SD Rats
(GFR measuring & Sampling)
group assignment, UNx
Sacrifice and tissue collection
B
Experimental groups (n=8)
1. SDT fatty-UNx + 0.3% salt and Normal diet
2. SDT fatty-UNx + 0.3% salt and Losartan diet (10mg/kg)
3. SD-water + water and Norma diet
Water: normal drinking water
UNx: unilateral nephrectomy
Salt: saline solution
-1
0
2
5
10
(weeks after 0.3% salt loading)
(salt loading & losartan diet)
9-week old
male SDT Fatty
SD Rats
(GFR measuring & Sampling)
UNx
group assignment
Sacrifice and tissue collection

Figure 5