INTRODUCTION

The prevalence of type 2 diabetes mellitus (T2DM) and hypertension has markedly increased worldwide. The risk of cardiovascular disease in diabetic individuals is doubled by concomitant hypertension, which occurs in an estimated 63% of T2DM patients. Genetic and environmental factors play important roles in the pathogenesis of T2DM and hypertension. Evidence from clinical trials and animal studies shows a causal relationship between dietary salt intake and hypertension. Many studies have suggested that blood pressure responses to dietary salt intake vary considerably among people and animals, which is a well-known phenomenon described as salt sensitivity of blood pressure.

Effects of high-sodium intake on systemic blood pressure and vascular responses in spontaneously diabetic WBN/Kob-Lepr\(^{fa/\text{fa}}\) rats

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Summary
The prevalence of type 2 diabetes mellitus (T2DM) and hypertension has markedly increased worldwide. The purpose of the present study was to examine the effects of a high-salt intake on the systolic blood pressure (SBP) and vascular responses in WBN/Kob-Lepr\(^{fa/\text{fa}}\) (WBKDF) rats, a new spontaneous animal model of T2DM. Male WBKDF rats and age-matched Wistar rats at 6 weeks of age were each divided into two groups and fed either a normal-sodium (NS, 0.26%) diet or high-sodium (HS, 8%) diet for 14 weeks: (i) Wistar rats on NS diet (Wistar-NS); (ii) Wistar rats on HS diet (Wistar-HS); (iii) WBKDF rats on NS diet (WBKDF-NS); (iv) WBKDF rats on HS diets (WBKDF-HS). Neither WBKDF-NS nor Wistar-NS rats showed significant changes in SBP throughout the experiment, but both WBKDF-HS and Wistar-HS exhibited significant elevation of SBP, which was more prominent (\(P<.01\)) in WBKDF-HS than in Wistar-HS. Phenylephrine-induced contractions of isolated thoracic aortic rings were significantly (\(P<.01\)) enhanced in WBKDF-HS and Wistar-HS compared with the respective strain of rats on the NS diet. In contrast, acetylcholine- and nitroprusside-induced relaxation were significantly (\(P<.01\)) diminished in both WBKDF-HS and Wistar-HS, and these HS diet-induced changes were more profound (\(P<.01\)) in WBKDF rats than in Wistar rats. Significantly (\(P<.05\)) higher plasma concentrations of 8-iso-prostaglandin F\(_2\alpha\) and sodium ions were observed in WBKDF-HS than in Wistar-HS. The current study demonstrated that WBKDF-HS rats developed salt-sensitive hypertension associated with vascular dysfunction. The WBKDF rat may be a useful model for investigating the etiology of hypertension with T2DM.

KEYWORDS
high-sodium diet, salt-sensitive hypertension, type 2 diabetes mellitus, WBN/Kob-Lepr\(^{fa/\text{fa}}\) rat
blood pressure. Salt sensitivity of blood pressure occurs frequently in individuals with T2DM and is associated with an increased risk for cardiovascular death. The pathophysiological mechanisms that promote salt sensitivity are complicated, and genetic and environmental factors are involved. However, recent studies have indicated a role of vascular dysfunction in driving the development of salt-sensitive hypertension.

The Wistar Bonn Kobori Diabetic Fatty (WBKDF, WBN/Kob-Leprfa/leprfa) rat is a new congenic strain established by introducing the fa allele in Zucker fatty rats into the WBN/Kob (lean) rat genome. Previous studies have shown that the WBKDF rat is a spontaneous model of T2DM that shows obesity, hyperglycaemia, and dyslipidaemia from a young age, resulting in the early onset of diabetic complications. Thus, WBKDF rats are particularly relevant to human T2DM. If WBKDF rats are salt-sensitive, these rats may serve as a useful model for investigating the aetiology of hypertension with T2DM. However, this rat model is not very well characterised in terms of cardiovascular responses.

To further validate WBKDF rats as a T2DM model, in the present study, we examined the effects of high-salt intake on blood pressure and vascular responses in WBKDF rats in comparison with age-matched Wistar rats.

2 | RESULTS

2.1 | Blood pressure and heart rate

Neither WBN/Kob-Leprfa/leprfa (WBKDF) rats fed a normal sodium (NS) diet (WBKDF-NS) nor Wistar-NS showed significant changes in SBP (120-130 mm Hg) throughout the experiment, and there were no significant differences in systolic blood pressure (SBP) between WBKDF-NS and Wistar-NS (Figure 1A). In contrast, both WBKDF-high sodium (HS) and Wistar-HS exhibited significant elevation of SBP, which was more prominent (180-220 mm Hg, \( P < .01 \)) in WBKDF-HS than in Wistar-HS (Figure 1A,B). Of note, heart rates in WBKDF rats were significantly \( (P < .01) \) lower than those in Wistar rats on the respective diet (Figure 1C). The HS diets significantly \( (P < .01) \) elevated heart rate in only WBKDF rats (Figure 1C).

2.2 | Food and water intake

The NS diet intake in WBKDF rats was significantly \( (P < .01) \) higher than that in Wistar rats. By contrast, there was no significant difference in HS diet intake between WBKDF rats and Wistar rats (Table 1), indicating that both WBKDF-HS and Wistar-HS consumed comparable amounts of sodium. The water intake in WBKDF-NS was significantly \( (P < .01) \) higher than that in Wistar-NS, probably due to the occurrence of DM in WBKDF rats (Table 1). The HS diet increased water intake in both strains of rats, but there was no significant difference in water intake between WBKDF-HS and Wistar-HS.

2.3 | Body weight and organ weight

Body weight in WBKDF at 6 weeks of age was significantly higher than that in Wistar rats at the same age on the respective diets (WBKDF-NS, 194.9±4.3 g; Wistar-NS, 133.3±5.5 g, \( P < .01 \); WBKDF-HS, 193.5±3.6 g; Wistar-HS, 130.7±3.3 g, \( P < .01; n=7 \) in each group). However, there were no significant differences in body weight among the four groups after a 14-weeks feeding period (Table 1). Kidney weight in WBKDF-HS was significantly \( (P < .01) \) lower than that in WBKDF-NS and Wistar-HS (Table 1).
2.4 | Plasma analysis

Plasma glucose level of WBKDF-NS was significantly (P<.01) higher than that in Wistar-NS. The HS diet attenuated the increase in plasma glucose level in WBKDF rats, which was still significantly (P<.01) higher than that in Wistar-HS. There were no significant differences in plasma insulin level among the four groups (Table 1).

The plasma levels of potassium and chloride were comparable among the four groups. In contrast, the HS diet increased the plasma level of sodium slightly but significantly (P<.01) in WBKDF rats (Table 1).

Plasma 8-iso-prostaglandin F$_{2\alpha}$ levels in WBKDF were significantly (P<.05) higher than those in Wistar rats on the respective diet. Additionally, the HS diet slightly increased the plasma 8-iso-prostaglandin F$_{2\alpha}$ levels in both strains of rats (Table 1).

2.5 | Force measurements in aortic rings

There were no significant differences in the pD2 values calculated for the $\alpha_1$-selective agonist phenylephrine hydrochloride (PE; Sigma-Aldrich, St Louis, MO, USA) in aortic rings from WBKDF-NS and Wistar-NS (Figure 2A and Table 2). The HS diet significantly (P<.01) enhanced the PE-induced contraction in WBKDF rats, but not in Wistar rats (Figure 2A).

The relaxation curves to cumulative acetylcholine chloride (ACh; Sigma-Aldrich) and sodium nitroprusside (SNP; Wako Pure Chemicals, Osaka, Japan) were determined in endothelium intact aortic rings precontracted with PE. Both ACh-induced and SNP-induced relaxations were significantly (P<.01) impaired in WBKDF-NS compared with Wistar-NS (Figure 2B,C). Although there was no statistical difference in pD2 values of ACh between WBKDF-NS and Wistar-NS, pD2 values of SNP in WBKDF-NS were significantly lower than Wistar-NS (Table 2). The HS diet significantly (P<.01) impaired vascular relaxations in response to ACh and to SNP in WBKDF rats (Figure 2B,C and Table 2).

2.6 | Histopathological analysis in the kidney

As shown in Figure 3, histopathological analysis demonstrated that diffused clear-cell (glycogenotic) tubules, a key morphological feature of diabetic nephropathy known as Armanni-Ebstein lesions, were evident in the kidneys of WBKDF-NS but not in Wistar-NS. Urinary hyalyn casts with tubular dilation were observed in the kidneys from both WBKDF-HS and Wistar-HS. Additionally, marked infiltration of interstitial inflammatory cells in the renal cortex and a slight mesangial expansion in glomeruli, without Armanni-Ebstein lesions, were evident in the kidneys of WBKDF-HS (Figure 3).

3 | DISCUSSION

The main findings of this investigation are: (i) that WBKDF rats and Wistar rats developed salt-sensitive hypertension during the HS diet, which was more prominent in WBKDF-HS than in Wistar-HS; (ii) PE-induced contractions were enhanced and ACh- and SNP-induced vascular relaxations were impaired in aortic rings from WBKDF-HS and Wistar-HS; (iii) plasma 8-iso-prostaglandin F$_{2\alpha}$ levels were higher in WBKDF rats than in Wistar rats.

### TABLE 1

|                      | Wistar-NS (N=7) | Wistar-HS (N=7) | WBKDF-NS (N=7) | WBKDF-HS (N=7) | ANOVA (Strain), P | ANOVA (Treatment), P |
|----------------------|----------------|----------------|----------------|----------------|-------------------|---------------------|
| Food intake (g/d)    | 18.3±0.1       | 23.4±0.1††     | 28.5±0.5**     | 26.9±1.9       | <.0001            | .0948               |
| Water intake (g/d)   | 26±1.4         | 117±3.6††      | 103±5.2**      | 114±6.1†       | <.0001            | <.0001              |
| Body weight (g)      | 381±4.5        | 337±6.2        | 393±9.7        | 363±24.3       | .1854             | .0111               |
| Kidney weight (%)    | 0.32±0.01      | 0.42±0.01††    | 0.36±0.01      | 0.30±0.01††    | .0007             | .0536               |
| Glucose (mg/dL)      | 165±9.7        | 113±17.6       | 545±12.8**     | 210±22.1**     | <.0001            | <.0001              |
| Insulin (ng/dL)      | 6.9±0.7        | 4.2±1.1        | 7.8±1.9        | 4.2±0.9        | .6953             | .0171               |
| Electrolyte          |                |                |                |                |                   |                     |
| Sodium (mEq/L)       | 144±0.4        | 146±0.5        | 145±1.0        | 149±1.4††      | .0366             | .0036               |
| Potassium (mEq/L)    | 4.4±0.1        | 4.8±0.2        | 4.5±0.1        | 4.3±0.3        | .3797             | .4689               |
| Chlorine (mEq/L)     | 105±0.5        | 104±0.9        | 101±0.6        | 102±2.2        | .0242             | .9725               |
| 8-iso-prostaglandin F$_{2\alpha}$ (pg/mL) | 120±13.0       | 261±28.8       | 331±38.1*      | 439±70.0*      | .0001             | .0073               |

Values are mean±SEM (n=7 in each group). The Wistar Bonn Kobori Diabetic Fatty (WBKDF, WBN/Kob-Lepr$_{fa/}$fa) rat and Wistar rats at 6 wk of age were each divided into two groups fed either NS diet or HS diet for 14 wk. Wistar-NS, Wistar rats on NS diet; Wistar-HS, Wistar rats on HS diet; WBKDF-NS, WBKDF rats on NS diet; WBKDF-HS, WBKDF rats on HS diets; NS, normal sodium; HS, high-sodium.

*P<.05 vs Wistar rats on the same diet.

**P<.01 vs Wistar rats on the same diet.

†P<.05 vs the same strain of rats on the same diet.

††P<.01 vs the same strain of rats on the same diet.
In this study, we examined the salt sensitivity of the WBKDF rat, a new model of T2DM, in comparison with Wistar rats. WBKDF rats spontaneously develop T2DM at an early stage. Consistent with previous studies, WBKDF rats developed marked hyperglycaemia, regardless of dietary sodium concentration in the present study, whereas hyperglycaemia was significantly attenuated by HS loading. Neither WBKDF-NS nor Wistar-NS rats showed significant changes in SBP throughout the experiment. Earlier studies reported that T2DM model rats, such as Otsuka Long-Evans Tokushima Fatty rats and Spontaneously Diabetic Torii-Leprfa rats, develop age-related hypertension, but other T2DM model rats, such as Goto-Kakizaki rats or Zucker diabetic fatty rats, do not. Thus, our study provides additional evidence that development of age-related hypertension varies among T2DM model rats.

We selected Wistar rats as the control because WBKDF rats are originally derived from Wistar rats in this study. It is generally believed that standard laboratory rats, such as Sprague-Dawly rats and Wistar rats, are salt-resistant. However, there is contradictory evidence in the literature regarding the presence of salt-sensitive hypertension in Wistar rats. Our present data are consistent with those in earlier studies reporting salt-sensitive hypertension in Wistar rats. Although the mechanism for the conflicts between these data is unclear, differences in experimental conditions, such as the sodium concentration in the diet and duration of HS feeding, may be involved.

Earlier studies reported salt-sensitive hypertension in a DM model of rats including streptozotocin-induced T1DM rats, T2DM models of Goto-Kakizaki rats and Spontaneously Diabetic Torii-Leprfa rats. In the current study, HS loading led to the development of salt-sensitive hypertension in both WBKDF rats and Wistar rats, but the elevation of SBP was greater in WBKDF-HS than in Wistar-HS. Our findings suggest that WBKDF rats have a noticeably high salt-sensitivity.

It has been suggested that many factors are involved in the development of salt-sensitive hypertension, and that changes in vascular reactivity may be one of these factors. The current study showed that HS intake caused vascular dysfunction characterised by an exacerbated response to vasoconstrictions or impaired vascular dilators. Several studies have demonstrated that vascular dilations by ACh were impaired in different diabetic vessels, suggesting that T2DM is associated with endothelial dysfunction. HS loading caused more significant impairment of SNP-induced relaxations compared with ACh-induced relaxations in both strains of rats. This result agrees with the hypothesis that HS intake causes vascular dysfunction characterised by an exacerbated response to vasoconstrictions or impaired vascular dilators.

It is likely that production as well as bioavailability of nitric oxide (NO) within the vascular endothelium may be markedly reduced in WBKDF-HS rats. A greater degree of impairment of NO-induced vasodilation in WBKDF-HS than in Wistar-HS may be associated with a greater degree of salt-sensitive

**FIGURE 2** Comparison of (A) PE-induced contractions and (B) ACh- and (C) SNP-induced relaxations in thoracic aorta rings with intact endothelium. Contractions induced by 60 mmol/L KCl and relaxations induced by papaverine (100 μmol/L) were taken as 100%. The Wistar Bonn Kobori Diabetic Fatty (WBKDF, WBN/Kob-Leprfa) rat and Wistar rats at 6 wk of age were each divided into two groups fed either NS diet or HS diet for 14 wk. Values are mean±SEM (n=7 in each group). *P<.05 vs Wistar rats on the same diet; **P<.01 vs Wistar rats on the same diet; ***P<.001 vs the same strain of rats on the NS diet. Data on thoracic aorta rings were from seven animals. PE, phenylephrine; ACh, acetylcholine; SNP, nitroprusside; (→) Wistar-NS, Wistar rats on NS diet; (→) Wistar-HS, Wistar rats on HS diet; (→) WBKDF-NS, WBKDF rats on NS diet; (→) WBKDF-HS, WBKDF rats on HS diets; NS, normal sodium; HS, high sodium.
### TABLE 2 pD2 values of PE, ACh and SNP in thoracic aorta rings

|                | Wistar-NS (N=7) | Wistar-HS (N=7) | WBKDF-NS (N=7) | WBKDF-HS (N=7) | ANOVA (Strain), P | ANOVA (Treatment), P |
|----------------|-----------------|-----------------|----------------|----------------|-------------------|-----------------------|
| **Contraction**|                 |                 |                |                |                   |                       |
| PE             | 5.76±0.71       | 6.26±0.31       | 5.93±0.41      | 7.64±0.20      | .0964             | .0213                 |
| **Relaxation**|                 |                 |                |                |                   |                       |
| ACh            | 8.12±0.26       | 7.04±0.99       | 6.31±0.47      | 3.23±0.46**††  | .0001             | .0023                 |
| SNP            | 9.93±0.27       | 7.85±0.32††     | 8.32±0.24**    | 6.73±0.20††    | <.0001            | <.0001                |

Values are mean±SEM (n=7 in each group). The Wistar Bonn Kobori Diabetic Fatty (WBKDF, WBN/Kob-Lep[fa/fa]) rat and Wistar rats at 6 wk of age were each divided into two groups fed either NS diet or HS diet for 14 wk.

PE, phenylephrine; ACh, acetylcholine; SNP, nitroprusside; Wistar-NS, Wistar rats on NS diet; Wistar-HS, Wistar rats on HS diet; WBKDF-NS, WBKDF rats on NS diet; WBKDF-HS, WBKDF rats on HS diets; NS, normal sodium; HS, high sodium.

*P<.05 vs Wistar rats on the same diet.
**P<.01 vs Wistar rats on the same diet.
††P<.01 vs the same strain of rats on the NS diet.

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**FIGURE 3** Comparison of histopathological appearance of the kidneys from the Wistar Bonn Kobori Diabetic Fatty (WBKDF, WBN/Kob-Lep[fa/fa]) rat and Wistar. Hematoxylin and eosin staining (A, B). (A) Wistar-NS normal tubule and glomerulus of the renal cortex; (B) Wistar-HS Hyalin casts (arrow); (C) WBKDF-NS Armanni-Ebstein changes (arrow); (D) WBKDF-HS Predominantly small mononuclear cell infiltration of the interstitium (arrows) and hyaline casts with tubular dilation, and mesangial expansion in glomeruli (arrowhead). Scale bars=50 μm. Wistar-NS, Wistar rats on NS diet; Wistar-HS, Wistar rats on HS diet; WBKDF-NS, WBKDF rats on NS diet; WBKDF-HS, WBKDF rats on HS diets; NS, normal sodium; HS, high sodium.
hypertension in WBKDF-HS compared with Wistar-HS. It is conceivable, therefore, that the vascular dysfunction observed in our study plays a causative role in salt-sensitive hypertension in WBKDF rats. However, the possibility that altered vascular responsiveness in WBKDF rats was due to consequences of long-lasting salt-induced hypertension cannot be ruled out. Thus, to determine whether altered vascular responsiveness plays a causative role in salt-sensitive hypertension in WBKDF rats, additional study with an earlier treatment period may be necessary.

Oxidative stress is defined as an increased bioavailability of reactive oxygen species. In the present study, the plasma concentrations of 8-iso-prostaglandin F$_{2\alpha}$, an oxidative stress marker, in WBKDF rats were higher than those in Wistar rats on the NS and HS diet, respectively. In addition, HS loading resulted in moderate increases in plasma 8-iso-prostaglandin F$_{2\alpha}$ levels in WBKDF rats and Wistar rats. These findings are in agreement with experimental and clinical observations that implicate oxidative stress as an important mechanism for vascular dysfunction in diabetes and in hypertension. It is generally accepted that both reduced NO synthesis from endothelial NO synthase and NO bioavailability within vascular wall by oxidative stress lead to vascular dysfunction. Collectively, it is suggested that the increased oxidative stress generated during HS feeding might be associated with salt-sensitive hypertension by affecting the vascular contractile and relaxation responses in WBKDF rats and Wistar rats.

It is noteworthy that HS loading resulted in a significant elevation of plasma sodium concentrations and the renal infiltration of inflammatory cells in WBKDF rats but not in Wistar rats. This elevation of plasma sodium in WBKDF-HS may be due to disturbed sodium homeostasis, in which the kidney plays a critical role. Several lines of evidence suggest that a small increase in plasma sodium concentration may cause an increase in vascular stiffness and reduction in NO levels, ultimately leading to endothelial dysfunction. In the current study, histopathological analysis showed that kidney injury associated with diabetes was evident in WBKDF-NS. Thus, it is possible that elevated plasma sodium may contribute to salt-sensitive hypertension in WBKDF-HS.

There were some limitations to the present study. First, previous studies suggested that the physiological mechanisms underlying salt-sensitive hypertension involve alterations in renal function, the heart, sympathetic outflow and vasculature. However, our study mainly focused on the vasculature in WBKDF rats. Secondly, significantly higher heart rates in WBKDF-HS than WBKDF-NS rats may have been due to impaired compensation of the baroreceptor reflex by HS supplementation. The contribution of the baroreceptor reflex to salt-induced hypertension in WBKDF rats should be examined in future studies. Thirdly, the present study used pre-diabetic, but not overtly diabetic, WBKDF rats to investigate the association between salt-sensitive hypertension and T2DM. Furthermore, it is particularly noteworthy that the hyperglycaemia that spontaneously developed in WBKDF rats was significantly attenuated by HS loading. This finding might be clinically relevant to human T2DM patients, because the association of dietary salt intake with insulin sensitivity has been demonstrated in human and animal studies. Thus, additional studies to elucidate the underlying mechanisms for this attenuation by HS loading in WBKDF rats are clearly needed.

In summary, we demonstrated in the present study that WBKDF-HS rats develop salt-sensitive hypertension associated with vascular dysfunction. The WBKDF rat may be a useful model for investigating the aetiology of hypertension with T2DM.

4 | METHODS

4.1 | Experimental design

Male WBKDF rats and age-matched male Wistar rats (Japan SLC, Inc., Shizuoka, Japan) were housed in plastic cages and allowed ad libitum access to either a normal sodium (0.26%, NS) diet or a high sodium (8%, HS) diet and fresh tap water. Room temperature was controlled at 21±1°C with humidity of 50%-60%. The room was lit between 7 AM and 7 PM daily. This protocol was approved by the Azabu University Committee on the Ethics of Animal Experiments.

Six-weeks-old WBKDF rats (n=14) and age-matched Wistar rats (n=14) were randomized into one of two groups (n=7 in each group) to either remain on the NS diet or to be switched to the HS diet. Groups were as follows: (i) Wistar rats on the NS diet (Wistar-NS); (ii) Wistar rats on the HS diet (Wistar-HS); (iii) WBKDF rats on the NS diet (WBKDF-NS); (iv) WBKDF rats on the HS diets (WBKDF-HS).

Body weight, food intake and water consumption were measured between 10.00 and 14.00 hours. Systolic blood pressure and heart rate were monitored using a tail cuff blood pressure analyzer (BP98A-L, Softron, Tokyo, Japan). The rats were prewarmed for 15-20 minutes at 32°C to improve the detection of pulsation of the tail artery. SBP and heart rate were determined with the mean value after three successive measurements without signal disturbances. The area under the curve (AUC) of SBP was derived according to the trapezoidal rule.

After 14 weeks of feeding, when the rats were 20 weeks old, blood samples were collected under pentobarbital sodium anaesthesia (50 mg/kg IP; Kyoritsu Seiyaku, Tokyo, Japan). Rats were then killed with an overdose of pentobarbital sodium, and the thoracic aorta was isolated for in vitro force measurements. Finally, the kidneys were harvested and weighed.

4.2 | Force measurements in aortic rings

The thoracic aortic rings (3 mm in length) were prepared for force measurements as described in our previous paper. Briefly, the aortic ring with endothelium was mounted on a wire-myograph (Kishimoto Medical, Kyoto, Japan), and suspended in an organ bath containing 5 mL Tyrode solution having the following composition (mmol/L): 136.8 NaCl; 5.4 KCl; 2.5 CaCl$_2$; 1.0 MgCl$_2$; 11.9 NaHCO$_3$; and 5.5 glucose (pH 7.3-7.5 when gassed with 95% O$_2$ and 5% CO$_2$) at 37°C. Two tungsten wires that were connected to a force-displacement transducer (Nihon-Kohden, Tokyo, Japan), were inserted into the lumen of the rings. Changes in tension were recorded under constant resting tension (0.8 g) with a force-displacement transducer.
After a 60 minutes equilibration period, the preparations were repeatedly contracted with 60 mmol/L KCl until the response became reproducible. The contraction induced by 60 mmol/L KCl and the relaxation induced by papaverine (100 μmol/L) were taken as 100%. Then, the contractile response to PE was observed to determine the concentration to induce 50%-60% of the maximum contraction. When the relaxation response to ACh or sodium nitroprusside (SNP; Wako Pure Chemicals) was measured, the preparation was precontracted with PE at a sufficient concentration to induce 50%-60% of the maximum contraction, and ACh and SNP were then cumulatively added. The negative logarithm of the concentration to cause a 50% response (pD2) was calculated from the individual dose-response relationship.

4.3 Plasma analysis

Heparinized plasma was separated from the collected blood by centrifugation at 3000×g for 10 minutes. Plasma levels of glucose, as well as electrolytes including sodium, potassium and chloride in heparinized plasma, were measured using an automatic analyzer (JCA-BM 2250; JEOL, Tokyo, Japan). Plasma insulin was measured using a rat insulin ELISA kit (Morinaga Institute of Biological Science, Yokohama, Japan). The plasma 8-iso-prostaglandin F2α concentration was determined by a Plasma 8-iso-prostaglandin F2α ELISA kit (Cayman Chemical, Ann Arbor, MI, USA).

4.4 Histopathological analysis of the kidneys

Isolated kidneys were fixed in 10% phosphate-buffered formalin for 2 days and embedded in paraffin. Transverse sections (5 μm) were stained with hematoxylin and eosin.

4.5 Statistical analysis

The data are presented as means±SEM. Statistical analysis was carried out by 2-way ANOVA supported with post hoc. Data for multiple observations over time were analyzed by 2-way ANOVA with repeated measures. Differences between means with P<.05 were considered significant. The data were analyzed using PRISM statistical software (Graphpad, San Diego, CA, USA).

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CONFLICTS OF INTERESTS

The authors have no conflicts of interests to disclose.

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