Possible Involvement of Up-regulated Salt Dependent Glucose Transporter-5 (SGLT5) in High-fructose Diet-induced Hypertension

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Research Article

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Abstract

Excessive fructose intake causes a variety of adverse conditions (e.g., obesity, hepatic steatosis, insulin resistance and uric acid overproduction). Particularly, high fructose-induced hypertension is the most common and significant pathological setting, however, its underlying mechanisms are not established. We investigated these mechanisms in 7-week-old male SD rats fed a diet containing 60% glucose (GLU) or 60% fructose (FRU) for 3, 6, or 12 weeks. Daily food consumption was measured to avoid between-group discrepancies in caloric/salt intake, adjusting for feeding amounts. The FRU rats' mean blood pressure was significantly higher and fractional sodium excretion (FENa) was significantly lower, indicating that the high-fructose diet caused salt retention. The FRU rats' kidney weight and glomerular surface area were greater, suggesting that the high-fructose diet induced an increase in extracellular fluid volume. The GLUT5 and ketohexokinase expressions, an enzyme required for fructose metabolism, were up-regulated in FRU. Cortical ATP levels were significantly lower in FRU, which might indicate ATP consumption due to fructose metabolism. Unlike previous reports, the high-fructose diet did not affect NHE3 expression. A gene chip analysis conducted to identify susceptible molecules revealed that only Slc5a10 (corresponding to SGLT5) in FRU showed >2-fold up-regulation versus GLU. RT-PCR and in situ hybridization confirmed the SGLT5 up-regulation. Our findings may indicate that the high-fructose diet increased sodium reabsorption principally through up-regulated SGLT5, finally causing salt-sensitive hypertension.

Background

Fructose has strong sweetness compared to glucose[1], and thus a smaller amount of fructose produces sweetness that is comparable to that of glucose. Fructose has therefore become widely used as an inexpensive sweetener in the food industry, and high-fructose corn syrup in particular is used in variety of foods and beverages, with its consumption increasing worldwide[2]. Along with the expansion of the uses of fructose, its various adverse events have also come to be recognized, including obesity[3], hyperuricemia[4], and hypertriglyceridemia[5]. These disorders are also implicated in promoting the development of insulin resistance[6], metabolic syndrome[7,8], and diabetes[6]. Hypertension is considered to be the most important adverse event of excessive fructose intake in terms of frequency and medical impact[4]. Indeed, a randomized cross-over trial of dietary glucose and fructose loads in 15 adults reported a predominant increase in blood pressure in the high-fructose-diet group[9]. Conversely, lifestyle interventions such as limiting the use of high-fructose beverages have also been reported to significantly reduce blood pressure[10]. An excessive intake of fructose was also reported to cause renal damage directly or indirectly[11-13], and it may accelerate the development of chronic kidney disease (CKD), including diabetic kidney disease (DKD)[14]. The progression of CKD was reported to be suppressed by restricting fructose[15]; the mechanism underlying this suppression is not yet clear.

The whole amount of ingested fructose is generally metabolized in the liver, but when fructose is ingested in excess of the liver's metabolic capacity, the blood level of fructose increases, reaches the renal

proximal tubules after glomerular filtration, and is absorbed into the epithelial cells principally via GLUT5 transporter[16,17]. It is consequently metabolized to glyceraldehyde-3-phosphate via fructose-1-phosphate by ketohexokinase (KHK) and utilizes ATP unidirectionally. Gordish et al. created a rat model fed a diet containing 20% fructose for 2 weeks, and they reported that the increase in blood pressure induced by salt loading was significantly higher in the fructose-fed group compared to controls, indicating that a high-fructose diet generates salt-sensitive hypertension[18]. Their findings suggested that the increase in salt reabsorption in the kidneys via unknown mechanisms by the high-fructose diet increases the extracellular fluid (ECF), resulting in an increase in blood pressure; however the underlying mechanisms have not been elucidated.

We conducted the present study to clarify the mechanisms underlying high-fructose-diet (HFD)-induced salt-sensitive hypertension by using a rat model fed a diet containing 60% fructose for 12 weeks. The results showed an increase in blood pressure with an increase in the ECF volume accompanied by a significant up-regulation of salt-dependent glucose transporter-5 (SGLT5). We propose that the increase in the ECF volume through the up-regulation of SGLT5 might be one of the causes of HFD-induced hypertension.

Materials And Methods

Animals and Experimental Design

The study was approved by the Animal Care and Use Committee (approval number: 1695) and conducted according to the Saitama Medical University Animal Experiment Regulations. The study was also conducted in accordance with guideline for Animals in Research: Reporting In vito Experiments (ARRIVE). Seven-week-old male Sprague-Dawley (SD) rats (CLEA Japan, Tokyo) weighing 250–350 g were used. The rats were divided into those fed a diet containing 60% glucose (GLU) and those fed a diet containing 60% fructose (FRU), and the body weights and blood pressure were measured every 3 weeks for up to 12 weeks. Blood pressure was measured by a tail-cuff sphygmomanometer (BP-98A; Softron, Tokyo), and the mean values of three measurements were recorded.

For the rats' diets, specifically adjusted 60% glucose- or 60% fructose-containing food (Oriental Yeast, Tokyo) was used. The contents of the two diets were identical except for carbohydrates (60% wt/wt of fructose or glucose, 21% wt/wt of protein, 5% wt/wt of lipid, 0.1% wt/wt of sodium, and 0.36% wt/wt of potassium). The caloric and salt intakes of the fructose-fed (FRU) and glucose-fed (GLU) groups were equalized by a paired feeding method as described[19]. The rats in the FRU and GLU groups were further classified according to feeding duration (3, 6, and 12 weeks, n=5 in each small group). Blood samples were collected from the abdominal aortas under anesthesia (pentobarbital, 50 mg/kg) after 24-hr urine collection at the end of the feeding period in each group. The kidneys were then perfused with 100 ml of ice-cold phosphate-buffered saline (PBS, pH 7.4) and were removed quickly. The biochemical analyses of blood and urine samples were entrusted to SRL Laboratory (Tokyo) as described[20].

Histology and immunohistochemistry.

Kidney tissue blocks were embedded in paraffin after fixation with PBS containing 4% paraformaldehyde, and microscopic sections (3μm) were made before staining with periodic acid Schif (PAS) and other immunostains. Five microscopic fields at medium power (x200) were randomly selected from the kidney cortical and outer-medullary areas, and multiple glomerular surface areas were measured by using a randomly selected glomerular section including a glomerular tuft area in each section as described[21]. Image J (ver 1. 51a) was used for the area measurement.

Some paraffin sections were autoclaved for 15 min and washed with distilled water for 5 min. Endogenous peroxidase was then blocked with 3% hydrogen peroxide for 5 min. Sections were washed three times with tris-buffered saline (TBS, pH 7.4) containing 0.1% Tween 20 (TBST) for 5 min and treated with rabbit anti-rat GLUT5 antibody (1:500; LifeSpan BioSciences, Seattle, WA) for 16 h at 4°C, followed by Dako Cytomation EnVision plus System HRP-Labeled Polymer (Dako, Glostrup, Denmark). A liquid diaminobenzidine substrate chromogen system was used for visualization with counterstaining by hemotoxylin. After the staining was performed, the slides were photographed under a light microscope (BX-61, Olympus) at x200 or x400 magnification and analyzed.

Gene expressions

The cortex and outer stripe of the outer medulla of the kidney were dissected, and total RNA was extracted by using ISOGEN solution (Nippon Gene, Tokyo) and the RNeasy mini kit (Qiagen, Hilden, Germany) as described[20]. We used the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA) to synthesize cDNA, and the gene expressions were assessed by real-time polymerase chain reaction (PCR) with the TaqMan® Gene Expression Assay system. The assay IDs of each target gene were as follows: GLUT5 (assay ID:Rn00582000_m1), KHK (assay ID:Rn00582975_m1), and SGLT5 (assay ID:Rn01773089_m1). The expression of Na-H exchanger-3 (NHE3) was analyzed using the same PCR primers as described20. Rat β-actin (Rat ACTB Endogenous Control VIC®/MGB Probe, Applied Biosystems; Thermo Fisher Scientific) was used for an internal control.

Assessment of ATP level

The rat kidneys were each dissected on ice and immediately frozen. The ATP level in the kidneys was assessed with an ATP Assay Kit (Colorimetric/Fluorometric, Abcam, Cambridge, UK).

Western blot

Homogenization was carried out using T-PER Tissue Protein Extraction Reagent (Pierce/Thermo Fisher Scientific) in a procedure in which the cortex and outer stripe of the outer medulla were added to Halt™ Protease and Phosphatase Inhibitor Cocktail, EDTA-Free (Pierce/Thermo Fisher Scientific). The supernatant obtained after centrifugation was subjected to protein quantitation using an assay kit (Takara Bio, Kusatsu, Japan) to adjust the loading sample.

After sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), anti-NHE3 antibody (MAB3136, Merck, Darmstadt, Germany), anti-pNHE3 antibody (ps-552, Santa Cruz Biotechnology, Dallas, TX) and anti-β actin antibody (cat. #4967, Cell Signaling Technology, Danvers, MA) were used as the primary antibodies, and anti-rabbit IgG, HRP-linked whole antibody (#NA934-100UL, GE Healthcare, Buckinghamshire, UK) was used as the secondary antibody to visualize the NHE3 signal. The signal intensity in SDS-PAGE was quantified by scanning with ChemiDoc XRS Plus (Bio-Rad, Hercules, CA).

DNA microarray

cDNA was synthesized from isolated RNA and analyzed using a DNA microarray (GeneChip Rat Gene 2.0 ST Array, Affymetrix, Santa Clara, CA) on three randomly selected samples per group and evaluated using the mean values.

In situ hybridization

The results of *in situ* hybridization (ISH) were compared between 12w-GLU (n=3) and 12w-FRU (n=3) groups using the QuantiGene ViewRNA system (Affymetrix; Thermo Fisher Scientific). The gene-specific probe set corresponding to mRNA of SGLT1 (accession no. NM_013033, cat.# VC6-3229989-01) and SGLT5 (accession no. NM_001107007, cat.# VC-3067794-01) made by Affymetrix was used in the experiment. RNA probes specific for SGLT1 or SGLT5 visualized using Fast Blue (SGLT1) or Fast Red (SGLT5) as substrates. Fixation and hybridization were entrusted to Tokushima Molecular Pathology Institute, Inc. (Tokushima, Japan).

Statistical analysis

The experimental results are expressed as the mean ± standard error of the mean (SEM) in each group. The FRU and GLU data were compared using Student's t-test, and $p<0.05$ was considered significant. All of the analyses were conducted with SPSS (ver. 25.0, IBM, Armonk, NY).

Results

The results of blood and urinary biochemistry at 3, 6 and 12 weeks in both groups are shown in the Table 1. A statistical analysis of the differences between two groups revealed that only 24-hour urine protein excretion at 12 weeks was significantly elevated in the FRU. Body weight was consistently higher in the FRU than in the GLU after 4 weeks, but there was no statistically significant difference (Fig. 1a). Mean blood pressure was significantly higher in the FRU than in the GLU after 9 weeks (Fig. 1b).

We had equally adjusted the caloric intakes of individual rats by measuring their daily food intake during the entire experimental period (Fig. 2a). Similarly, there was no difference in the daily calculated salt intake between the FRU and GLU groups (Fig. 2b). However, the fractional excretion of sodium (FENa) was significantly lower in the FRU rats after 6 weeks (Fig. 2c). We next investigated the changes in creatinine clearance (Ccr) and the glomerular surface area to examine the presence of glomerular hyperfiltration due to extracellular fluid (ECF) volume expansion. The results demonstrated that the

creatinine clearance using endogenous Cr was not significantly elevated in the FRU rats (Fig 2d), but the renal volume in the FRU rats was significantly elevated throughout the experimental period (Fig 2e). The cross-sectional glomerular area using equatorial sections of glomeruli in the FRU group was significantly greater than that in the GLU group for all three experimental feeding periods (Fig. 2f,g). These results suggested that the HFD caused salt retention, an increase in the ECF volume, and a volume-dependent elevation of blood pressure leading to glomerular hypertrophy.

To confirm the fructose delivery and metabolism in tubular cells in our model, we next examined the expressions of GLUT5 (a major pathway of fructose reabsorption) and keto-hexokinase (KHK; an enzyme required for fructose metabolism). We observed that the GLUT5 mRNA expression was increased significantly after 6 weeks (Fig. 3a), and its immunoreactivity was also clearly increased around the cortico-medullary junction (Fig. 3b). The mRNA of KHK was also significantly up-regulated with the fructose loading (Fig. 3c). It is known that fructose, unlike glucose, unidirectionally consumes ATP during its metabolic process. In our model, the level of ATP in the renal cortex was significantly decreased with the HFD (Fig. 3d).

Some prior studies have reported that an HFD induces the up-regulation of NHE3 as a cause of salt retention[22-24]. We therefore next investigated the effect of the HFD on the changes in NHE3 expression. The NHE3 mRNA expression was not different between the GLU and FRU groups (Fig. 4a). The results of the protein expression analysis also showed no significant difference between the GLU and FRU groups in NHE3 or phosphorylated NHE3 (Fig 4b,c). Based on our findings, the HFD seemed to cause the up-regulation of NHE3.

The gene chip analysis was conducted to explore the molecules involved in the increased sodium reabsorption induced by the HFD. As shown in Table 2, SGLT5 showed the most significant increase in expression, and we therefore selected SGLT5 as a candidate molecule which might function to increase the sodium reabsorption in response to the HFD. Based on the results of the gene chip analysis, we examined the expression of SGLT5 mRNA. As shown in Figure 5a, significant increases in mRNA expression were revealed by RT-PCR. In the in situ hybridization, the expression of SGLT5 mRNA was increased in the tubules of the outer medullary layer of the FRU rat kidneys (Fig. 5d,e) compared to those of the GLU rats (Fig. 5b,c).

We also performed a dual in situ hybridization of SGLT5 and SGLT1 to clarify the tubular distribution of SGLT5 mRNA. As shown in Figure 5f–h, SGLT5 mRNA was co-expressed with SGLT1, indicating that SGLT5 was principally localized in the S3 segment of the proximal tubules.

Discussion

In the present rat model, the feeding of the high-fructose diet was accompanied by a significant decrease in sodium excretion and a significant increase in the blood pressure. On the other hand, there seemed to be no difference in salt intake between the two diet groups, and we suspect that sodium reabsorption was

increased by the HFD, resulting in sodium retention. This sodium retention due to the HFD resulted in an increased ECF volume, which was confirmed by the increased kidney volume and glomerular hypertrophy. Contrary to the results of several prior investigations, our study did not obtain positive results for an up-regulation of NHE3 associated with the HFD. We then investigated the molecules that caused the increase in sodium reabsorption with the HFD, and er concluded that SGLT5 was a candidate as a susceptible molecule.

Through clinical observations, multiple studies have shown that an HFD induces salt-sensitive hypertension and that fructose restriction improves hypertension[4,8]. The detailed mechanisms of these observations are not yet known, but several animal studies have shown that salt retention and salt-sensitive hypertension could be provoked by an HFD[18,25,26]. This enhanced salt sensitivity induced by an HFD has been reported in more detail by Oudot et al. They demonstrated fructose-induced glomerular hypertrophy, macrophage infiltration into the renal interstitium, and elevations of inflammatory cytokines and oxidative stress-related molecular markers, which are prevented by salt restriction[27]. Another study also reported that fructose loading caused increases in sodium reabsorption and blood pressure[28], which is in agreement with our results. Increased sodium reabsorption may lead to an increase in the ECF volume. Our gene chip analysis showed a relative increase in 11β-HSD expression. The expression of 11β-HSD is known to be induced by an increase in ECF volume[29], indicating that the up-regulation of 11β-HSD might indirectly indicate an increase in the ECF volume, which might lead to glomerular hyperfiltration. Indeed, glomerular hyperfiltration associated with fructose loading has been reported[30]. These prior studies agree with our present findings in terms of the salt-sensitive hypertension and ECF volume expansion caused by the high-fructose diet.

Nakayama et al. reported that renal interstitial fibrosis was induced in SD rats fed a diet containing 60% fructose for 6 weeks[12]. Hu et al. also reported that a 12-week diet containing 60% fructose resulted in mild interstitial fibrosis and glomerular damage[31]. However, we did not observe any obvious histological abnormalities in the glomeruli or interstitium by 12 weeks of the fructose-enriched diet. There was also no significant increase in the gene expressions of molecules associated with fibrosis, such as alpha-SMA (see Suppl. Fig. S1). Despite no major differences in the species used in the experiments and no differences in the quantity or duration of the fructose diet, it is unknown at present why major differences in terms of interstitial damage have been observed. It might be possible that the rats' salt intake and the degree of blood pressure increase associated with the present HFD differed from those in the prior studies. Oudot et al. conduct experiments with special attention to ensure that the actual calories consumed by fructose and glucose groups were identical, and they reported that interstitial damage was present but only mildly impaired[27]. Together with our present results, such findings might indicate that the interstitial damage associated with an HFD may differ depending on the caloric intake, salt intake, and degree of blood pressure elevation.

Ingested fructose is absorbed in the small intestine and metabolized in the liver. When fructose is loaded in excess of the liver's processing capacity, the blood level of fructose rises, and the fructose reaches the

kidneys[16]. Filtrated fructose in the glomerulus is principally reabsorbed via GLUT5 on the apical side of proximal tubules[12], and due to the localization of GLUT5, the principal site of fructose reabsorption is thought to be S3 segment[32]. Fructose that is reabsorbed into tubular cells is metabolized by KHK, a fructose-degrading enzyme[33], which is up-regulated by fructose loading[12]. Both glucose and fructose consume ATP in their metabolization, but glycolysis is regulated by an increase or decrease in the intracellular ATP level, whereas the metabolization of fructose proceeds regardless of the ATP level, which has been speculated to result in a persistent consumption of intracellular ATP as long as fructose is continuously loaded[34]. These cellular events in terms of fructose metabolism are fully consistent with the results of our present study.

As a cause of increased sodium reabsorption associated with fructose loading, multiple studies have reported the involvement of NHE3 up-regulation[22-24]. We therefore first investigated whether changes in NHE3 expression occurred in our model. No change was revealed by the analyses of mRNA or protein expression and immunohistochemistry (see Suppl. Fig. S2), suggesting that NHE3 might not be involved in fructose-induced hypertension, at least in our model. We do not know the reason for this discrepancy at present. Studies reporting the involvement of NHE3 in HFD-induced hypertension include those using cell lines rather than animals and studies using mammals such as swine rather than rodents, which may account in part for the difference in results.

The gene chip analysis performed to identify genes involved in the tubular salt reabsorption affected by fructose loading in our model revealed that only SGLT5 showed a significant up-regulation, which was confirmed by the RT-PCR analysis. Based on the outcome of our in situ hybridization, the principal tubular expression site of SGLT5 is considered to be the outer medullary layer, and SGLT5 is also co-expressed with SGLT1. Since the proximal tubules' S3 segment is the major site of SGLT1 localization[35], we speculate that SGLT5 induced by the HFD is also expressed in the S3 segment. SGLT5 is highly expressed in the kidney[36] and transports fructose and mannose efficiently[37] and has been shown to be a fructose entry pathway in renal tubules[38].

The up-regulation of SGLT5 by fructose loading has been reported, but its physiological and pathophysiological significance has not been elucidated[38]. SGLT5 has been reported to localize principally in the S2 proximal tubule segment[38], and the findings of our in situ hybridization indicate that the principal parts of SGLT5 expression are segments from S2 to S3, which were generally considered compatible with previous studies. Our findings suggest that SGLT5 may play a pivotal role in the pathway causing the increased sodium reabsorption associated with the HFD rather than the previously estimated NHE3. The mRNA of SGLT5, which is normally only minimally expressed, was significantly increased by the HFD, and it is thought to be a potent molecular pathway for increased sodium reabsorption, leading to ECF volume expansion.

Figure 6 provides a summary and hypothesis of this study. In the absence of fructose loading, sodium reabsorption occurs in the proximal tubules through the sodium-dependent transporters such as NHE3,

sodium-phosphate cotransporter, SGLT1 and SGLT2. However, when excessive fructose is filtered through the glomeruli, GLUT5 and SGLT5 are up-regulated, resulting in the excessive reabsorption of sodium. The sodium retention leads to an increase in the ECF volume, leading to an increase in blood pressure. Our findings may indicate that the high-fructose diet increased the sodium reabsorption principally through the up-regulated SGLT5, and finally caused salt-sensitive hypertension.

Limitations

Since this study did not set a normal-diet group, the results of the high-fructose diet were compared with those of a high-glucose diet and not with a normal diet. The primary reason for the lack of a normal-diet group was that the caloric intake between the experimental groups needed to be identical. We thus equalized the number of calories per unit weight of the diets, and the rats' daily food intake was measured, with adjustments in the feeding quantity on the next day when necessary. Corn starch (which is normally used as a sugar in animal diets) also contains fructose, and thus eliminating this effect was another reason to not include a normal-diet group.

Abbreviations

GLUT5: glucose transporter-5, SGLT5: sodium glucose cotransporter-5, NHE3: sodium hydrogen exchanger-3

Declarations

Conflicts of interest

The authors state that they have no conflicts of interest to disclose.

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Author contribution

H. Hara and K. Takayanagi equally contributed to this work including the animal care, experiments, sampling, and immunohistochemistry and molecular biology. They also contributed to the data analysis and draft writing. T. Shimizu and T. Iwashita were also equally contributed to this work (data analysis and valuable advice). A. Ikari contributed to the design of the research strategy and data analysis. H. Hasegawa was responsible for this work as a corresponding author and contributed to the study's general strategy, the data analysis, the draft writing, and finishing the final version of this manuscript.

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Tables

Table 1. Blood and urine biochemistry data of the glucose and fructose groups

		3 wks	6 wks	12 wks
		n=6	n=6	n=6
Blood:				
Total protein, g/dL	glucose	5.62±0.16	5.94±0.17	6.42±0.36
	fructose	6.54±0.41	7.06±0.48	6.88±0.24
BUN, mg/dL	glucose	22.5±1.97	23.5±2.16	24.5±1.02
	fructose	21.6±1.74	23.0±1.51	20.6±0.71
Cr, mg/dL	glucose	0.26±0.03	0.30±0.02	0.31±0.04
	fructose	0.30±0.01	0.22±0.03	0.26±0.02
Na^+, mEq/L	glucose	145±0.32	148±1.36	149±1.24
	fructose	144±0.97	147±0.60	148±1.18
K^+, mEq/L	glucose	3.44±0.09	3.28±0.26	4.06±0.50
	fructose	4.28±0.43	3.82±0.35	3.50±0.09
Glucose, mg/dL	glucose	154±13.9	193±11.9	166±44.0
	fructose	191±33.5	211±10.8	126±19.5
Urine:				
Urine protein, mg/day	glucose	20.7±3.0	11.1±1.6	10.3±1.0
	fructose	20.9±3.0	14.9±1.6	16.3±2.0*
NAG, IU/L	glucose	5.50±2.63	0.40±0.00	2.98±2.58
	fructose	0.40±0.00	1.68±1.28	1.32±0.92
Cr, mg/dL	glucose	66.5±24.8	50.2±5.06	125±41.6
	fructose	33.8±2.28	58.1±10.6	96.4±13.5
Na^+, mEq/L	glucose	21.0±2.9	24.4±3.0	46.6±12.7
	fructose	14.8±1.5	15.8±2.6	30.8±3.2
K^+, mEq/L	glucose	66.4±24.2	39.4±4.7	80.1±25.4
	fructose	44.0±3.8	58.7±10.4	73.2±8.4
Food intake:				
Calorie intake, Kcal	glucose	83.6±1.3	93.1±1.8	87.2±2.4
	fructose	80.5±1.1	92.5±2.3	88.1±4.5

		3 wks	6 wks	12 wks
		n=6	n=6	n=6
Sodium intake, mg	glucose	24.3±0.4	27.0±0.5	25.3±0.7
	fructose	23.4±0.3	26.9±0.7	25.6±1.3

*p<0.05.

Table 2. Analysis of gene chip expression of salt transporter in the rat kidney

	Gene symbol	Gene expression ratio 12w FRU/GLU
SGLT1	SLC5A1	1.2572
SGLT2	SLC5A2	1.0132
SGLT3	SLC5A4	undetected
SGLT4	SLC5A9	undetected
SGLT5	SLC5A10	2.2554
SGLT6	SLC5A11	1.1205
NHE3	SLC9A3	0.8720
NaPi-2a	SLC34a1	1.0358
NKCC2	SLC12A1	0.8222
NCC	SLC12A3	0.7883
ENAC β-subunit	Scnn1b	0.7239
ENAC γ-subunit	Scnn1g	0.7296
MR	Nr3c2	0.8033
11β-HSD	Hsd11b1	1.790
Nedd4	Nedd4	0.9524
Wnk4	Wnk4	0.8162

The gene expression ratio was calculated by using relative expression against the internal control in the FRU and GLU groups. 11β-HSD: 11β hydroxysteroid dehydrogenase, ENAC: epithelial Na channel, MR: mineralocorticoid receptor, NaPi-2a: Na-phosphate cotransporter , NCC: Na-Cl cotransporter, Nedd4:

developmentally downregulated gene 4 isoform, NHE3: Na-H exchanger 3, NKCC2: Na-K-Cl cotransporter, SGLT: Na-glucose cotransporter, Wnk4: with-no-lysine kinase 4.

Figures

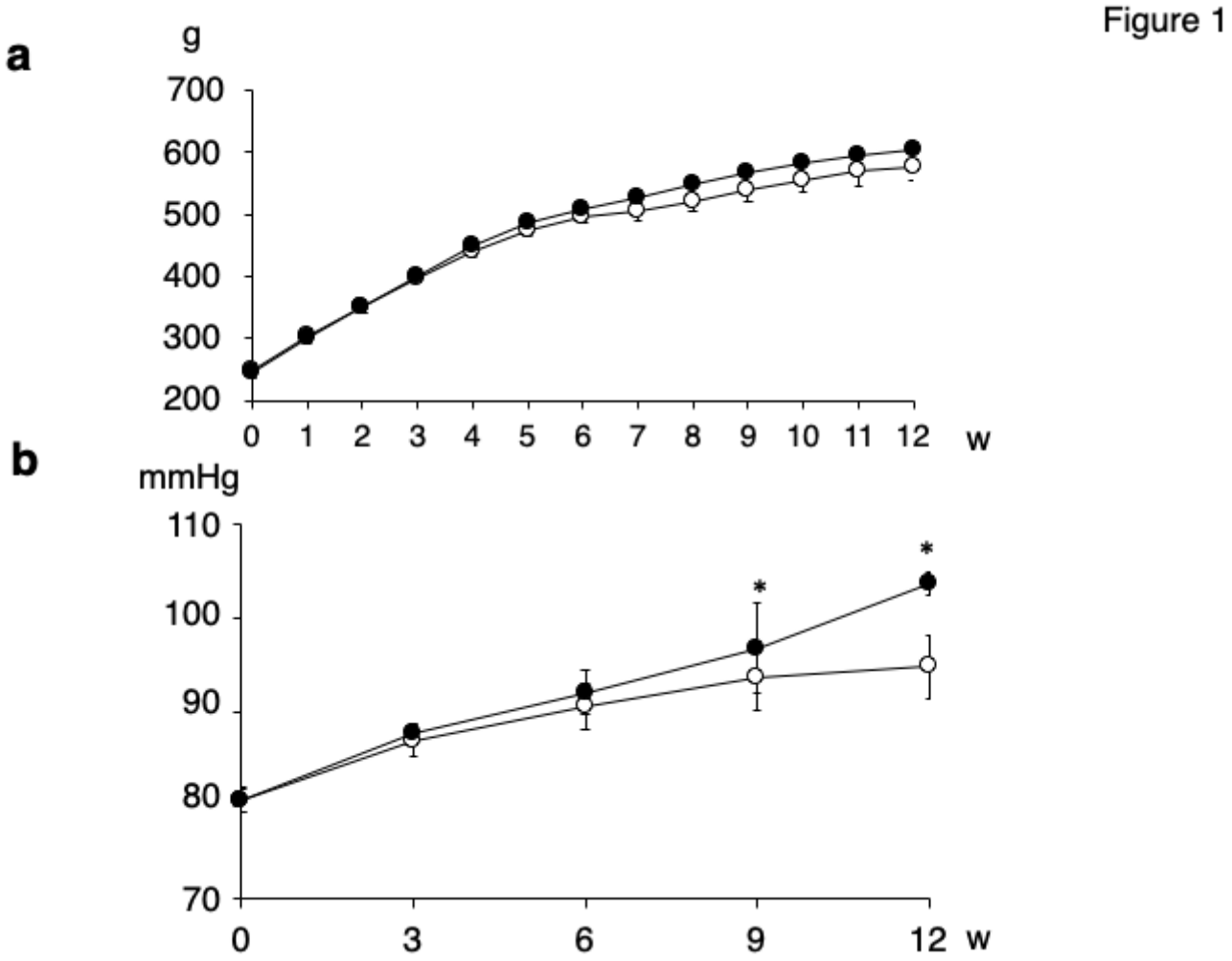

Figure 1

Changes in the rats' (a) body weight and (b) mean blood pressure during the experimental period. Open circles: High-glucose diet (GLU). Closed circles: High-fructose diet (FRU). *p<0.05 vs. glucose.

Figure 2

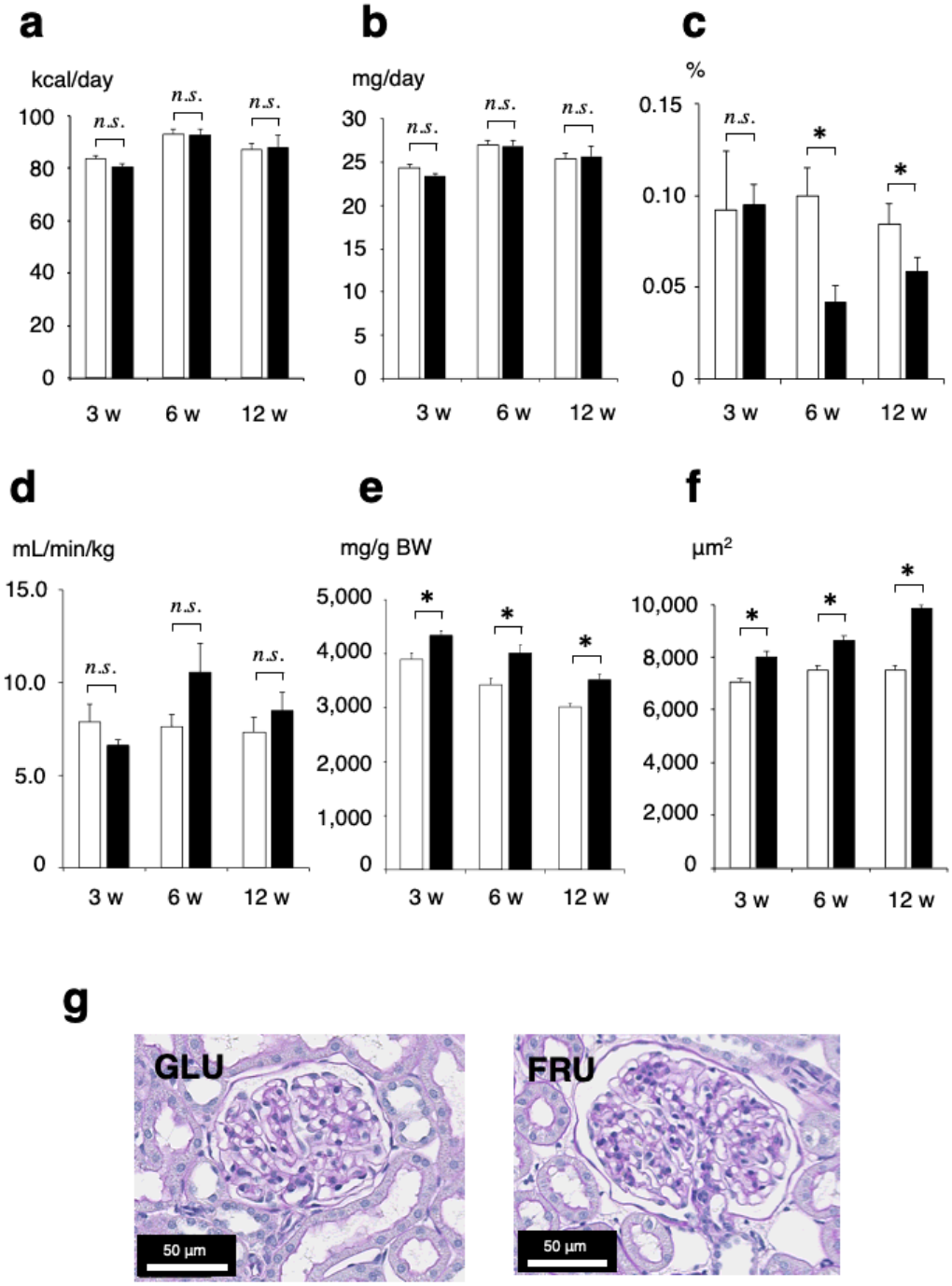

Figure 2

Changes in renal salt handling and responses to the increased extracellular fluid (ECF). Changes in (a) daily calorie intake, (b) salt intake, (c) fractional excretion of sodium, (d) creatinine clearance, and (e) kidney weight corrected by body weight are demonstrated. Changes in (f) the glomerular surface area are shown. For the measurement of the area, maximal transverse sections containing the vascular pole were randomly selected from the superficial and juxtamedullary cortex. The surface area of 10 glomeruli in six

animals each was measured using the NIH Image-J 1.36b program, and the obtained number of pixels was converted to the surface area. (g) Representative micrographs (400x) of measured glomeruli in the FRU and GLU groups. Open bars: High-glucose diet (GLU). Closed bars: High-fructose diet (FRU). Results are mean ± SEM (n=50). *p<0.05 vs. glucose by Student's t-test. n.s.: not significant.

Figure 3

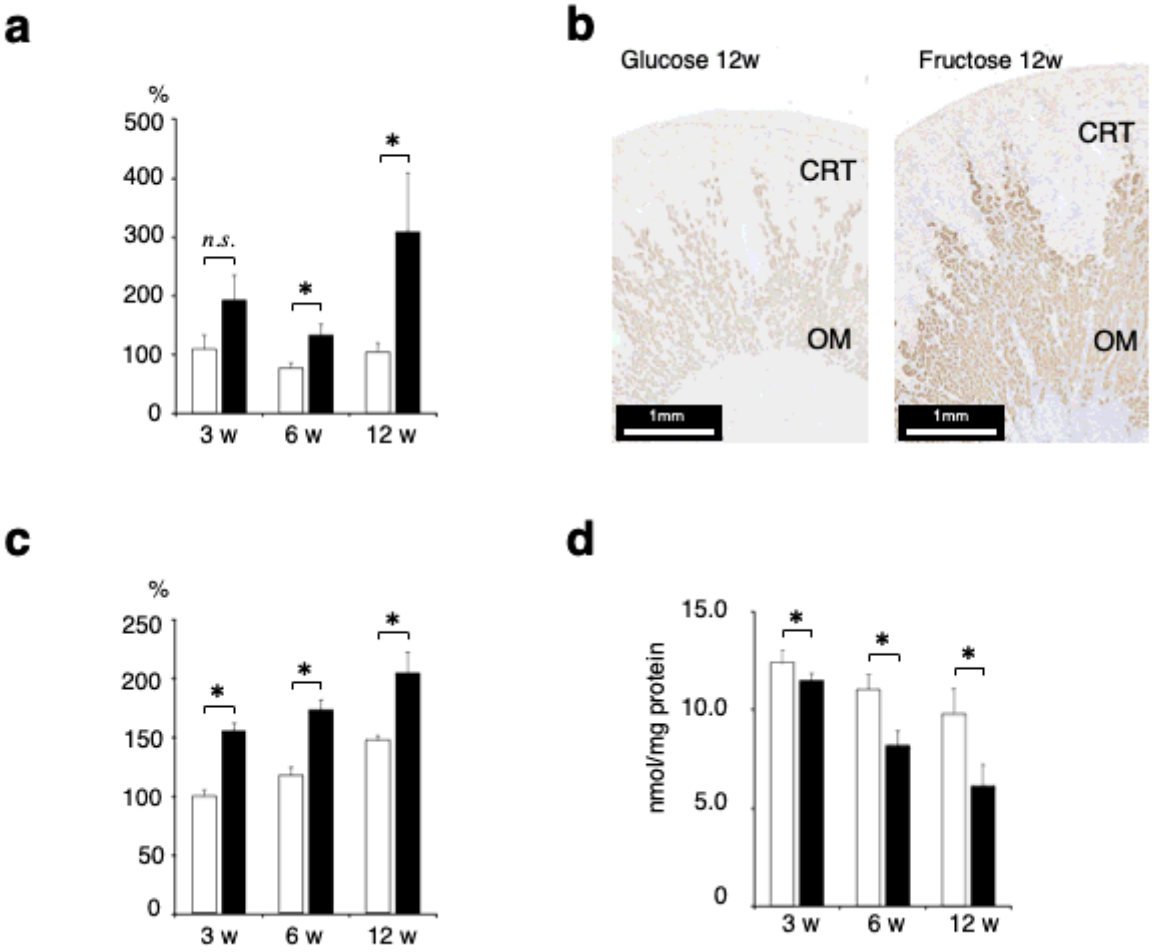

Figure 3

Changes in fructose delivery and metabolism in tubular cells. As a fructose entry pathway, (a) the mRNA expression of GLUT5 in kidney cortex and (b) the immunohistochemistry of GLUT5 are shown. Related to fructose metabolism, (c) the mRNA expression of ketohexokinase in the kidney cortex and the (d) ATP levels are shown. Open bars: High-glucose diet (GLU). Closed bars: High-fructose diet (FRU). Results are mean ± SEM (n=50). *p<0.05 vs. glucose by Student's t-test.

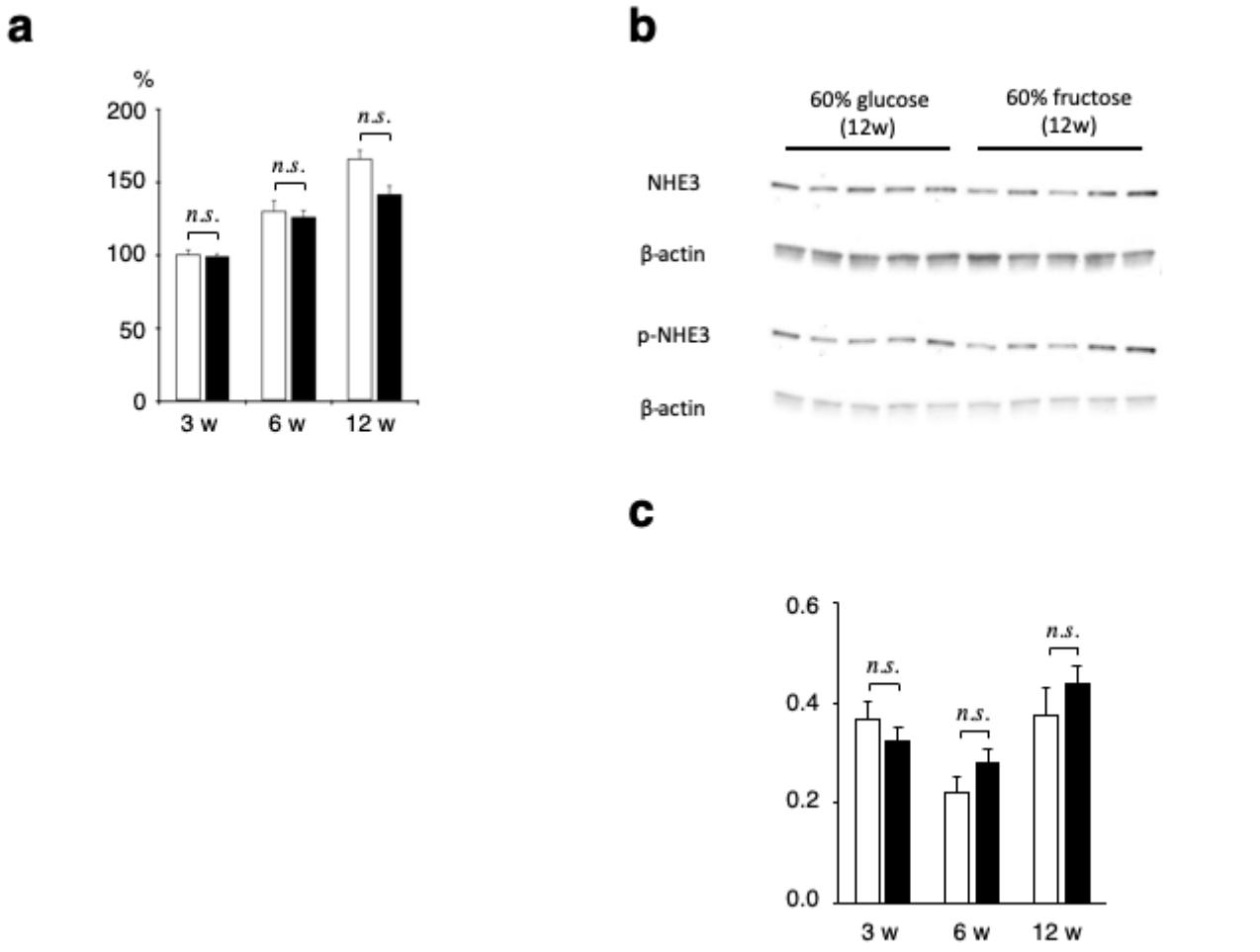

Figure 4

Changes in Na-H exchanger-3 (NHE3) expression in response to fructose load. (a) mRNA expressions of NHE3 in the kidney cortex. (b) Protein expressions of NHE3 and phosphorylated NHE3 by Western blotting at 12 wks and (c) results of intensity analysis of NHE3 signals. Open and closed bars indicate high glucose diet and high fructose diet groups, respectively. Open bars: High-glucose diet (GLU). Closed bars: High-fructose diet (FRU). Results are mean ± SEM (n=50). *p<0.05 vs. glucose by Student's t-test. The original image of the western blot is presented in Suppl. Fig. S3.

Figure 5

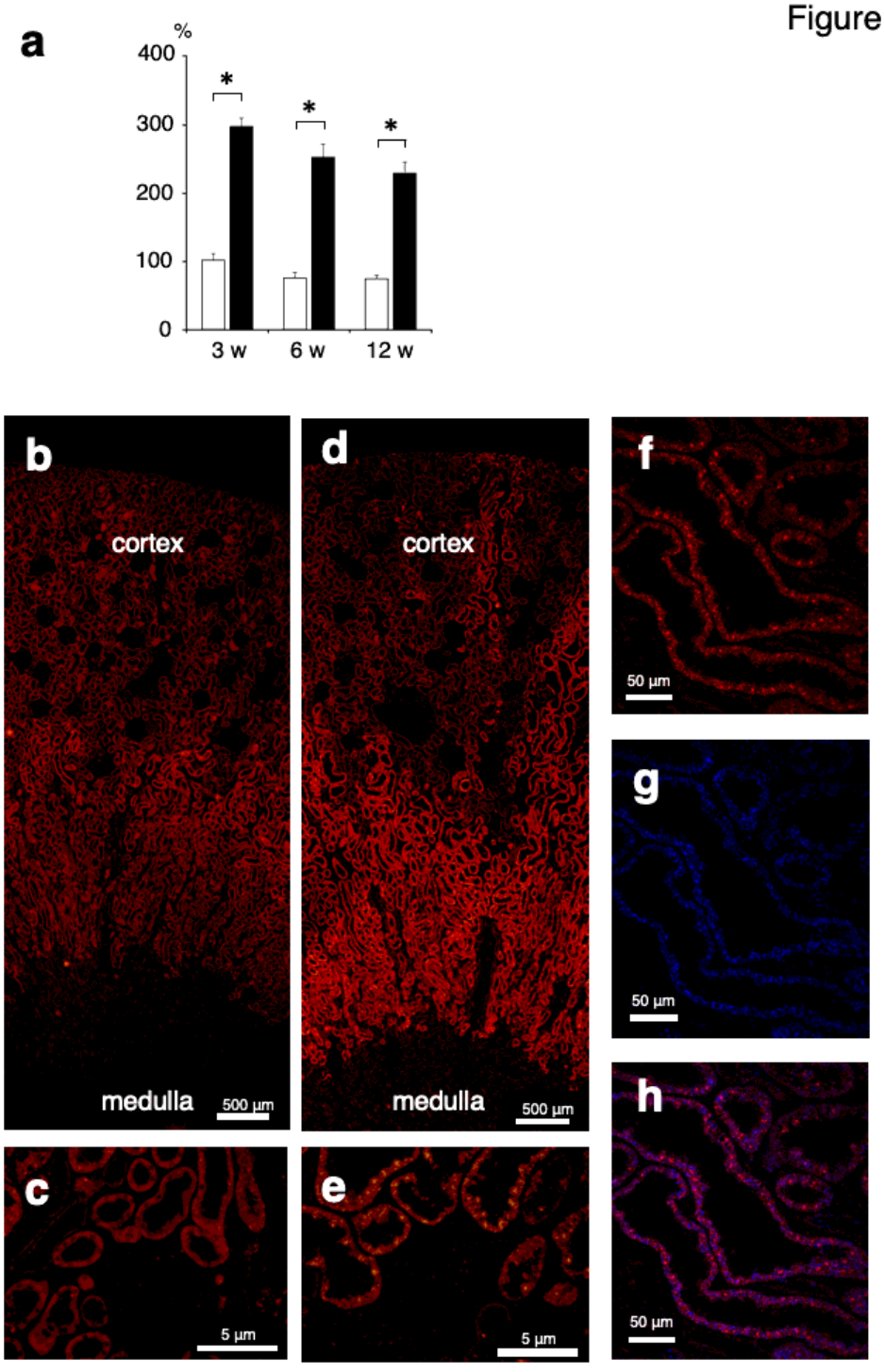

Figure 5

Changes in sodium-dependent glucose transporter expression. (a) The mRNA expression of SGLT5 in the kidney cortex. Open bars: High-glucose diet (GLU). Closed bars: High-fructose diet (FRU). Results are mean ± SEM (n=50). *p<0.05 vs. glucose by Student's t-test. Tissue distribution and intensity are shown by in situ hybridization. Each picture shows low magnification of (b) the GLU group and (d) FRU group and a high magnification of (c) the GLU group and (e) FRU group. Dual in situ hybridization of SGLT5 (f)

and SGLT1 (g) mRNA was conducted in the same tissue sections in the outer medullary layer. Merged picture (h). *p<0.05 vs. GLU.

Figure 6

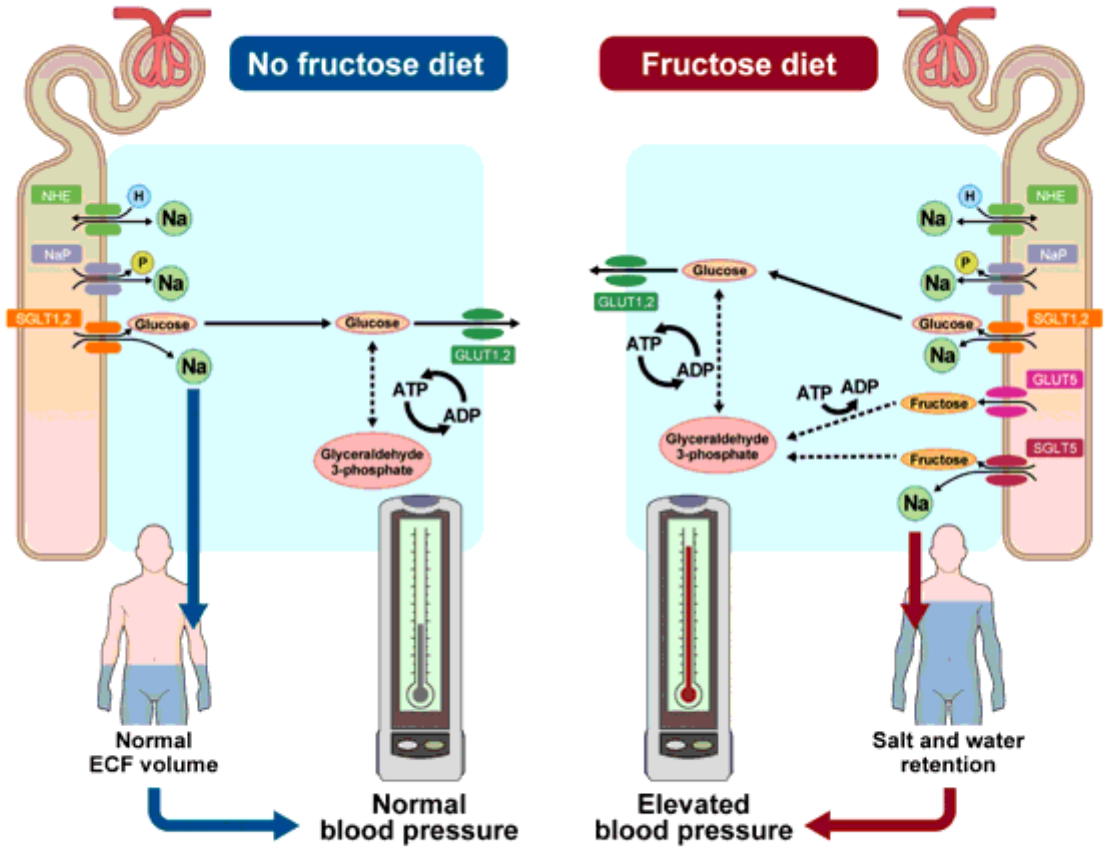

Figure 6

The summary and hypothesis of this study. The hypothesized development of hypertension associated with a high-fructose diet obtained herein is presented. In the absence of fructose loading, sodium is reabsorbed in the proximal tubules via multiple sodium-dependent co-transporters. Glucose reabsorbed via SGLT is metabolized in an ATP level-dependent manner. When excessive fructose is loaded, SGLT5 is up-regulated and sodium reabsorption is persistently increased, leading to salt retention and finally developing salt-sensitive (volume dependent) hypertension with a unidirectional consumption of intracellular ATP.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- S001.tiff

- S002.tiff
- S003.tiff