Effects of Sleeve Gastrectomy on Blood Pressure and the Renal Renin-angiotensin System in Diet-induced Obese Rats

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What is already known about this subject?

1) Sleeve gastrectomy decreases systolic blood pressure.

2) Sleeve gastrectomy increases cholecystokinin.

3) Cholecystokinin suppresses renal sympathetic nerve system.

What does this study add?

1) Sleeve gastrectomy decreases systolic blood pressure by increasing sodium excretion in urine.

2) Sleeve gastrectomy increases cholecystokinin and supresses intrarenal renin-angiotensin system

3) Cholecystokinin decreases systolic blood pressure by down-regulating intrarenal renin-angiotensin system.

Abstract

Objective

Sleeve gastrectomy (SG) has been reported to decrease blood pressure, although the reason has not been revealed. The present study aimed to establish the reason why SG decreases blood pressure.

Methods

Male Sprague–Dawley rats were subjected to surgical [sham operation (SO) or SG] and dietary interventions [fed a normal diet or high-fat diet (HFD) ad libitum or pair-fed (PF)]. Systolic blood pressure (SBP), urinary sodium excretion, and endocrine parameters were examined 4 weeks after surgery.

Results

Both SG and PF treatments reduced body weight compared with SO. SG exhibited a reduction in SBP compared with PF, which was associated with a reduction in renal renin, Ang-II, and catechol-O-methyltransferase (COMT) levels ($p<0.01$ for each). SG increased plasma CCK levels compared with PF ($p<0.0001$ for each), whereas GLP-1 and PYY were not changed in fasting. Exogenous administration of CCK reduced renal COMT ($p=0.0233$), renin ($p<0.0001$), Ang-II ($p<0.0001$) levels and SBP ($p=0.0053$).

Conclusion

The SG reduced SBP, at least in part, through suppression of the sympathetic nerve action by elevation of CCK which is followed by suppression of the intrarenal renin-angiotensin system.

Key word: angiotensin-II, cholecystokinin, natriuresis, renin, sleeve gastrectomy

Introduction

Obesity has been described as the greatest threat to human health by the World Health Organization because it induces complications such as hypertension, diabetes mellitus, and hyperlipidaemia [1]. The recent rise in obesity parallels the onset of hypertension. Approximately 70% of primary hypertension may be accounted for by obesity [2]. Hypertension complicated by obesity is associated with impaired natriuresis and salt retention [3, 4]. Three mechanisms appear to be especially important in mediating increased sodium reabsorption associated with weight gain: (i) increased renal sympathetic nerve activity (RSNA), (ii) activation of the renin-angiotensin system (RAS), and (iii) altered intrarenal physical forces [5].

Obesity has been shown to induce excessive activation of RSNA and therefore promotes the progression of hypertension [6]. However, the exact mechanism by which obesity stimulates RSNA remains unclear. It may be associated with the activation of the RAS. The RAS is the endocrine system that regulates blood pressure and electrolyte homeostasis. In addition to the classical systemic RAS, the local tissue-specific RAS has also been identified in various organs such as the kidney, heart, and brain. Under normal physiological conditions, angiotensin II (Ang-II) is tightly regulated by renin. In the kidney, renin is regulated by numerous factors, including variations in sympathetic nerve activity and catecholamine (CA) [7]. Intrarenal Ang-II upregulates sodium absorption and raises blood pressure [8]. The renal Ang-II concentration is nearly 1,000 times higher than that in serum, and the renal RAS may be regulated independently of its systemic counterpart [9].

Metabolism by catechol-O-methyltransferase (COMT) is one of the inactivation pathways of CAs [10], which are important hormones for regulating blood pressure both in central and peripheral sympathetic nerves. Several hormones including cholecystokinin (CCK) are also involved in regulating blood pressure via sympathetic nerve activity [11].

Bariatric surgery is regarded as the most effective treatment for obesity [12]. Sleeve gastrectomy (SG) is one of the surgical weight-loss procedures and decreases the amount of food intake because of a reduction in stomach volume. Khorgami et al reported that SG has been increasingly chosen as a primary bariatric procedure since 2013 in the US [13]. Furthermore, SG alters the expression of gastrointestinal hormones such as glucagon-like peptide-1 (GLP-1), CCK, and peptide YY (PYY) as well as leptin and adiponectin and improves glucose and lipid metabolism [14, 15]. Moreover, there are observations that SG also ameliorates obesity-induced hypertension in both rat and human, but the detailed mechanism remains uncertain [16-19].

We hypothesized that the beneficial effects of SG on blood pressure are weight-independent. Our aim is to clarify that SG treatment is more effective for lowering blood pressure than caloric restriction and to analyse the effects of SG and caloric restriction on gastrointestinal hormone responses and the activation of RSNA and the renal RAS.

Materials and Methods

Animals

Male Sprague–Dawley rats (250–280 g; Seac Yoshitomi, Fukuoka, Japan) were housed in a room with daily illumination from 07:00 to 19:00 (12-h/12-h light/dark cycle) and were maintained at 21 ± 1 °C with 55 ± 5% humidity. Animals were provided *ad libitum* access to standard chow (Clea chow; Clea, Tokyo, Japan) and tap water. All experiments were performed in accordance with the guidelines established by the National Institutes of Health, USA, regarding the care and use of animals for experimental procedures. Additionally, the ethics committee of the Division of Laboratory Animal Science, Research Promotion Project of Oita University specifically approved this study.

Reagents

On the day of the experiment, CCK (CCK-Octapeptide (26-33) (Sulfated Form; PEPTIDE INSTITUTE, INC., Osaka, Japan) was freshly dissolved in 0.1 M phosphate-buffered saline (PBS) and adjusted to pH 7.0.

Sleeve gastrectomy (SG)

The rats were fasted for 20 h before surgery and anaesthetized with sodium pentobarbital (50 mg/kg, intraperitoneal; i.p.). The methods for performing SG have been described previously [4, 14]. Briefly, an upper abdominal midline incision was made, and then the terminal oesophagus, stomach and initial duodenum were dissected free. The greater curvature from the antrum to the fundus across the forestomach and glandular stomach was incised, and approximately 90% of the forestomach and 70% of the glandular stomach were divided. The divided stomach was closed with 4-0 nylon. For the

sham-operation (SO), the animals were subjected to laparotomy, and the stomachs were elevated and returned to the abdominal cavity.

Experimental protocol

Design 1: Thirty two rats were assigned to one of four groups. All groups were not difference on age, weight, and their environment. In Group 1 (S-sham group, n = 7), rats were fed the standard diet (Standard) for 12 weeks. The SO was performed, and then the rats were fed the Standard for 4 more weeks. In Group 2 (HF-sham group, n = 8), rats were fed a HFD (60% fat, 20% carbohydrate, 20% protein; Diet Research, New Brunswick, NJ, USA) for 12 weeks. The SO was performed, and then the rats were fed the HFD for 4 more weeks. In Group 3 (HF-SG group, n = 8), rats were fed the HFD for 12 weeks. The SG was performed, and then rats were fed the HFD for 4 more weeks. In Group 4 (HFPF-sham, n = 8), rats were fed the HFD for 12 weeks. Pair-feeding was performed after the SO operation. The pair-fed (PF) rats consumed a HFD after the SO. Body weight and food intake were measured (Animal Scale; Clare, Tokyo, Japan) daily in all groups. The quantity of food in the HFPF-sham group was yoked ad lib to the daily intake observed in the HF-SG group. These blood or organs were sampled after 20 h food deprivation.

Design 2: Twelve rats fed the Standard were assigned to one of two groups (n = 6 per group). In Group 1, rats were intraperitoneally administered with saline (0.1 ml) after 20 h food and water deprivation. In Group 2, rats were intraperitoneally administered with CCK (250 ng/kg, i.p.) after 20 h food and water deprivation. Samples were taken 1 h after administrating saline or CCK (Fig. S1).

Measurement of blood pressure

Systolic blood pressure (SBP) was recorded in conscious, resting animals by non-invasive tail-

cuff plethysmography (BP 2000 Visitech Systems, Apex, NC, USA). After a 24-h fast, the tail artery was dilated by placement of the animal into a thermostatically controlled plastic holder heated to 32 °C for 20 min. Tail pulse was detected by passage of the tail through a tail-cuff sensor attached to an amplifier. All measurements were carried out between 4:00 pm and 6:00 pm, and an average of at least three readings was taken for each animal after they became used to the environment.

Blood and Urine Analysis

After all rats were anaesthetized with sodium pentobarbital (50 mg/kg, i.p.), blood was obtained from the portal vein and right ventricle of the heart of each rat. Blood samples were collected and transferred into a polypropylene tube containing EDTA (1 mg/ml of blood, Sigma-Aldrich, USA), aprotinin (500 KIU/ml of blood, Cayman Chemica., Ann Arbor, MI, USA) and DPP4 inhibitor (1 μl/ml of blood, Millipore, Billerica, MA, USA) at 0℃. These blood samples and centrifuged at 4000 × g for 10 min at 4 °C. Plasma was immediately frozen and stored at −80 °C until analysed. Finally, the rats were exsanguinated following transcardial perfusion with 100 ml of saline containing 200 U of heparin. Kidneys were removed, immediately frozen and stored at −80 °C until analysed. Urine was sampled for 24 h by briefly placing the rats in empty cages directly on a plastic surface, which allowed collection of spontaneously voided urine. Urine was stored at −80 °C before analysis.

Western blot analysis

Frozen tissue samples were homogenized in TBS-T (Tris-buffered saline - Tween20） buffer, centrifuged, and boiled. Equal amounts of samples were loaded onto 8% sodium dodecyl sulfate-polyacrylamide gels, electrophoresed, and then transferred electrophoretically to a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Richmond, CA). The membranes were blocked with 3%

bovine serum albumin (SIGMA, St. Louis, MO) for 1 h, incubated for 2 h with primary antibodies against COMT, α-tubulin (Santa Cruz Biotechnology, Santa Cruz, CA) in Deign 1, and COMT (abcam, Cambridge, UK), GAPDH (SIGMA) in Design2. Then, incubated for 1 h at room temperature with the secondary antibody. Proteins were detected by enhanced chemiluminescence (Amersham Life Science, Buckinghamshire, UK) and quantitated using imaging software (Bio-Rad Laboratories).

Immunohistochemistry

Kidney samples were fixed in 4% buffered paraformaldehyde, embedded in paraffin, sectioned and deparaffinized in xylene. Tissues were washed three times in PBS and incubated for 1 h in 0.3% H_2O_2 to quench endogenous peroxidase activity. For COMT staining, 5 μm-thick kidney sections were incubated overnight at 4 °C with rabbit anti-rat COMT (1:100, Santa Cruz Biotechnology in Design 1, abcam in Design 2) and then incubated with biotin-conjugated goat anti-rabbit IgG (ABC reagent, Vector Laboratories, Burlingame, CA). Samples were visualized with diaminobenzidine (DAB) substrate. Additionally, as a negative control, normal goat serum was used instead of the aforementioned antibodies with further incubation with a secondary antibody, which resulted in no staining.

Biochemical ELISA Tests

Commercial enzyme-linked immunosorbent assay (ELISA) kits were used to evaluate plasma renin levels (Rat Renin ELISA Kit; CUSABIO, College Park, MD), Ang-II (Rat ANG-II ELISA Kit; CUSABIO, College Park, MD), GLP-1 (Rat GLP-1 ELISA Kit Wako, High Sensitive; Wako Pure Chemical Industries, Ltd., Osaka, Japan), PYY (Rat PYY EIA Kit; Yanaihara Institute Inc., Shizuoka, Japan), leptin (Rat Leptin ELISA Kit; Yanaihara Institute Inc. Shizuoka, Japan), and CCK [Rat

Cholecystokinin Octapeptide (26-33) EIA Kit, Burlingame, CA]. Urine sodium and creatinine levels were also measured using an Enzymatic Sodium Test Kit (Diazyme Laboratories, Inc., Poway, CA) and a Creatinine Assay Kit (QuantiChrom Creatinine Assay Kit, Hayward, CA), respectively. For measurement of renin and Ang-II, renal tissues were homogenized and extracted with 0.1 M PBS. Renal renin and Ang-II concentrations were measured in the supernatant.

Statistical Analysis

All data are expressed as the means ± SEM. Differences in multiple comparisons were analysed using ANOVA post hoc tests appropriately (SAS Institute, Cary, NC). For all tests, the level of significance was set at $p < 0.05$.

Results

Changes in body weight and food intake

Before the beginning of the experiment, we confirmed that there is no difference in body weight, blood pressure and U-Na / U-Cr values among all groups (Table 1). During the 4 weeks after surgery, the body weights were significantly lower in the HF-SG and HFPF-sham groups than in the S-sham and HF-sham groups; however, there were no significant differences in weight between the HF-SG and HFPF-sham groups (Fig. 1a, $p < 0.01$). Daily food intake in the HF-SG and HFPF-sham groups were also decreased compared with that in the S-sham and HF-sham groups during the 1 week after surgery (Fig. 1b, $p < 0.01$). However, there was no significant difference in food intake among all groups in weeks 3 and 4 after surgery (Fig. 1b).

Systolic blood pressure (SBP) and sodium in urine after surgery

Figure 2 shows SBP and sodium in urine at 4 weeks after surgery. SBP was increased in the HF-sham and HFPF-sham groups compared with the S-sham group, and these elevations were not present in the HF-SG group (Fig. 2a, $p < 0.05$). In addition, the U-Na / U-Cr values were increased in the HF-SG group compared with the HFPF-sham group (Fig. 2b, $p = 0.0190$).

Changes in metabolic parameters and gastric hormones

The plasma levels of leptin, CCK, GLP-1 and PYY are shown in Figure 3. Peripheral leptin levels were significantly higher in the HF-sham group than in other groups (Fig. 3a, $p < 0.05$). In addition, we examined portal levels of GLP-1 and PYY because peripheral plasma levels of GLP-1 and PYY were not detected with ELISA kits for the parameters we used. However, there was no significant

alteration in portal GLP-1 (Fig. 3b) or PYY (Fig. 3c) levels among all groups. The peripheral level of CCK was dramatically increased in the HF-SG group compared with that in the S-sham, HF-sham and HFPF-sham groups (Fig. 3d, $p < 0.0001$).

Protein expression of COMT in the kidney

Western blot analyses were performed to confirm protein expression of COMT in the kidney. The levels of COMT in the HF-SG group were decreased compared with those in the HF-sham and HFPF-sham groups (Fig. 4a, $p < 0.01$). This finding was confirmed by immunohistochemistry of COMT protein expression in the cortex and medulla of kidney with immunohistochemical staining (Fig. 4b).

Tissue Contents of renin and Ang-II in the kidney

The levels of both renin and ANG-II in the kidney were dramatically decreased in the HF-SG group compared with those in the HF-sham and HFPF-sham groups (Fig. 5a and 5b, $p < 0.0001$). The aldosterone level in the kidney did not change in any of the groups (date not shown).

The effects of CCK on intrarenal COMT, renin, Ang-II levels and systolic blood pressure

We investigated whether acute administration of CCK affects renal levels of renin and Ang-II. Both food and water deprivation increased renin and Ang-II levels in the kidney compared to only food deprivation. The acute CCK treatment reduced COMT level in kidney (Fig. 6a, $p < 0.05$ and 6b). Moreover, CCK administration also decreased intrarenal renin and Ang-II levels (Fig. 6c and 6d, $p < 0.05$) as well as SBP (Fig. 6e, $p < 0.05$).

Discussion

The intake of HF is implicated in the development and maintenance of obesity, which promotes hypertension in humans and rodents [20]. SG is one of the effective treatments for morbid obesity and leads to appetite and weight loss and significant improvements in comorbidities such as hypertension [16-18]. In the present study, ad libitum consumption of a HF diet led to obesity and elevated SBP to a greater extent than standard feeding, and SG treatment decreased body weight and SBP values. Moreover, we found that HF-SG group rats showed lower SBP values than HFPF-sham rats, although there was no difference in body weight between the HF-SG and HFPF-sham groups, supporting the hypothesis that the beneficial effects of SG on blood pressure are body weight-independent. In this regard, both human and animal models of obesity-related hypertension have been explained by retention of sodium, which may contribute to the development of hypertension [3, 21]. We observed that HF-SG group promoted natriuresis compared with HFPF-sham, which appears to be involved in the hypotensive effect of SG. Therefore, weight loss alone after SG treatment cannot explain the improvement in obesity-related hypertension.

The pathophysiology of obesity-related hypertension is complex, and multiple potential mechanisms include RSNA and the activation of the RAS. There is considerable evidence for increased activation of RSNA throughout the evolution of obesity-related hypertension [22]. In the present research, SG treatment abolished the obesity-induced elevation in SBP values, whereas this alteration was not found with caloric restriction. Moreover, our study demonstrated that kidney levels of COMT were decreased in the HF-SG group compared with the HF-sham group and HFPF-sham group. This finding was also confirmed by immunohistochemical staining of COMT in the kidney. Although COMT is a catecholamine-metabolizing enzyme, expression levels and enzyme activities of COMT alone do not necessarily account for systemic catecholamine levels in the blood or blood

pressure levels in vivo. Previous studies reported that COMT was one of the markers which are related with hypertension and sympathetic nerve activity. Intrarenal COMT levels of normotensive rats were lower than those of spontaneously hypertensive rats [23]. Intracellular COMT-mRNA level was increased by administration of epinephrine and decreased by administration of morphine that suppressed sympathetic nerve activity [24] These results were considered by negative feedback against sympathetic nerve activity. We therefore presume that expression of intrarenal COMT may correlate sympathetic nerve activity positively. In fact, since CCK has already been shown to suppress the sympathetic nerve activity in vivo [11], we understood that reduction of COMT expression by CCK administration are compatible with suppression of the sympathetic nerve activity in our animal models. That way, reduction of intrarenal renin and Ang-II was, at least in part, attributable to suppression of the renal sympathetic nerve activity by CCK in HF-SG animals (Fig. 7).

There is a great deal of evidence supporting the idea that RAS activation is associated with obesity. The RAS is a critical regulator of blood pressure, in addition to modulating fluid and sodium homeostasis [25]. The intrarenal RAS is unique because all the components necessary to generate intrarenal Ang-II are present along the nephron in both the interstitial and intratubular components [,26-29]. In general, hypertension exhibits a progressive increase in intrarenal Ang-II levels that is greater than can be explained by the circulating Ang-II level alone, and these elevated Ang-II levels are closely associated with progressive increases in arterial pressure [30]. Our data suggest that SG down-regulates RSNA and the intrarenal RAS as well as SBP values, and weight loss alone cannot explain the improvement in SBP values after SG treatment in obesity. Interaction between the RAS and RSNA has been traditionally regarded as bidirectional. We observed that the alteration in COMT levels in the kidney was in concordance with that of RAS activation in the kidney. Considering the results of previous research showing that the contribution of RSNA to intrarenal renin release is clear,

it is indicated that the increase in RSNA by obesity promotes RAS activation in the kidney, although the exact mechanism underlying the intrarenal interaction between RSNA and the RAS has been fully explained [22].

Here, essential questions can be raised as to why SG treatment down-regulates RSNA in obesity. Previous studies indicate that SG treatment alters serum leptin levels and the expression of gastrointestinal hormones such as GLP-1 and PYY in obesity [31]. Regarding gastrointestinal hormones, we focused on the SG-induced alterations in plasma CCK, PYY and GLP-1 levels in addition to the leptin level. The present study shows that HF-SG group increased the plasma CCK level but not PYY, GLP-1 and leptin levels, but this elevation in the CCK level was not induced by HFPF-sham group, suggesting that the alteration in the plasma CCK level might be independent of meal size. We have to admit that these data surprised us because previous studies describe that SG increases plasma GLP-1, PYY and CCK levels [32]. One possible explanation for this discrepancy is that the fasting period in this research is longer than that in other studies. Postprandial elevation in plasma gastrointestinal hormone levels such as GLP-1 and PYY has been reported to be proportional to meal size, and these concentrations were restored to their original condition within 4 h [33]. In this study, blood sampling was performed after 20 h of fasting, whereas other reports examine in the postprandial state or short fasting term. Another explanation is that intestinal releasing factors secreted into the lumen of the upper small intestine stimulate CCK release, and it is believed that CCK release is mediated by these releasing factors. For example, CCK release is promoted by “intestinal luminal CCK-releasing factor (LCRF)”, which is active within the lumen of the intestine and does not affect GLP-1 or PYY secretion [34]. Thus, there is a possibility that LCRF might be responsible for the increased levels of CCK following SG treatment, although we did not evaluate LCRF secretion.

Here, we documented markedly increased CCK by SG treatment. CCK is secreted by I cells in

the duodenum and proximal jejunum in response to food intake. [35]. Plasma CCK concentrations tend to remain low in obese people, and rats maintained on a HF diet demonstrate reduced sensitivity to the satiating effects of CCK [36]. Furthermore, the effect of bariatric surgery on CCK homeostasis remains unclear. Moreover, other studies reported that CCK also inhibits RSNA via a vagal afferent mechanism and that the acute administration of a CCK antagonist in rats undergoing bariatric surgery failed to reveal any effect of the surgery on endogenous CCK satiation [37, 38]. Considering this evidence together, we speculated that the SG-induced increase of CCK secretion down-regulates RSNA through the central nervous system and reduces obesity-related high BP values by decreasing renin and Ang-II levels in the kidney.

In this study, we observed that SG decreased COMT as well as renin and Ang-II levels in the kidney compared with PF. There are conflicting observations given that caloric restriction is a first-line therapy recommendation for obese individuals, and several clinical studies have shown specific renal benefits of caloric restriction on blood pressure, proteinuria, and renal function. However, these findings are not necessarily contradictory to our results. The studies described above are usually associated with lifespan research, and the period of caloric restriction lasts for months or several years, which is different from short-term and strict caloric restriction ranging from days to weeks such as that in our experimental procedure. Theoretically, caloric restriction may lead to reduced sodium intake, therefore elevating RAS activity and then adjusting the balance of sodium and water, which is competitive with our results. Considering our observations that the alteration in plasma CCK, renal COMT levels, and renal RAS activity, the increase in CCK secretion by SG may affect RSNA and renal RAS activity, although we cannot explain why renin and Ang-II levels in the kidney alone were increased compared with PF.

Finally, we examined whether CCK administration also decreases intrarenal COMT, renin,

Ang-II levels and SBP. We clarified that CCK administration reversed the imbalance of renal RAS components, supporting the finding that administration of CCK inhibited renal sympathetic nerve discharge [39]. In line with our findings, SG treatment increases CCK secretion from the duodenum, and this increase promotes natriuresis by down-regulating RSNA and RAS activity and then lowers BP (Fig. 7).

There are several limitations in this study. First, we need to verify that SG suppresses renal RAS in obese patients, even though SG decreased SBP in obese rats by inhibition of the renal RAS. However, alternative approaches for assessment of the renal RAS must be developed, because sampling the renal tissue from patients by needle biopsy is too invasive to evaluate the renal RAS. There is a previous study indicating that urinary angiotensinogen is clinically useful for evaluating the renal RAS, indicating the possibility that it is important to examine alterations in urinary angiotensinogen in obese patients during the perioperative period [40]. Second, it is uncertain how the elevation in CCK release regulates RSNA and RAS activity. Furthermore, the possibility that gastrointestinal hormones other than CCK might contribute to the requirement of SG to reduce BP as well as down-regulate RSNA and RAS activity cannot be excluded, although we could examine only PYY and GLP-1 as gastrointestinal hormones other than CCK. Third, it is known that SG alters microbiota [41, 42], paticuraly increased *Lactbacillus*. Another previous study reported that *L. murinus* reduced salt-sensitive hypertension in mice [43]. There is the possibility that SG might decrease BP by changing microbiota, however the alteration of microbiota by SG was not evaluated in the present study. Fourth, body composition, for example, the amount of fat or muscle, might differ between the SG and PF groups, although the body weight was equivalent in the SG and PF groups. It is possible that a change in body composition affects RSNA or the intrarenal RAS; however, we could not examine the body components in the present study because a previous study reported that

alterations in body composition was not observed between the SG and PF groups [44].

In conclusion, our current study provides convincing and substantial evidence that the SG-induced elevation in plasma CCK levels improves obesity-related hypertension through an RSNA-RAS interaction in obesity and that the improvement in hypertension by SG might exceed a reduction in body weight by caloric restriction. The effect of SG on CCK secretion is an interesting and under-researched phenomenon that may lead to new opportunities for obesity therapy, although further investigation is required to determine whether these findings are attributable to the CCK-related network that involves the duodenum, brain, and kidney.

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Competing Interests

No potential conflicts of interest relevant to this article were reported.

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Figure legends

Fig. 1 Changes in body weight and food intake.

Changes in body weight (a) and daily food intake (b) after surgery.

S-sham : normal-chaw, sham operated, HF-sham : high fat-chaw, sham operated, HF-SG: high fat-chaw, sleeve gastrectomy, HFPF-sham: high fat-chaw pair fed groups. * $p < 0.05$ versus S-sham, ** $p < 0.01$ versus S-sham, ## $p < 0.01$ versus HF-sham. Bars show the mean value ± SEM.

Fig. 2 Effects of SG on SBP and sodium in urine 4 weeks after surgery.

(a) SBP; systolic blood pressure with tail cuff. (b) Sodium level in urine collected for 24 h. S-sham: normal chow, sham-operated, HF-sham: high-fat chow, sham-operated, HF-SG: high-fat chow, sleeve gastrectomy, HFPF-sham: high-fat chow, pair-fed, sham-operated groups. Bars show the mean value ± SEM.

Fig. 3 Effects of SG on several hormones 4 weeks after surgery.

Leptin levels (a) in peripheral vein, GLP-1 (b) and PYY levels (c) in portal vein, and CCK levels (d) in peripheral vein. S-sham: normal chow, sham-operated, HF-sham: high-fat chow, sham-operated, HF-SG: high-fat chow, sleeve gastrectomy, HFPF-sham: high-fat chow, pair-fed, sham-operated groups. Bars show the mean value ± SEM.

Fig. 4 Tissue COMT expression and histological alterations in the kidney.

(a) Tissue COMT contents were measured in the cortex and medulla of the kidney. (b) Representative COMT staining in the kidney. S-sham: normal chow, sham-operated, HF-sham: high-fat chow, sham-

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Fig. 5 Tissue renin and angiotensin II concentrations.

Renal renin (a) and Ang-II (b) levels of each group. S-sham: normal chow, sham-operated, HF-sham: high-fat chow, sham-operated, HF-SG: high-fat chow, sleeve gastrectomy, HFPF-sham: high-fat chow, pair-fed, sham-operated groups. Bars show the mean value ± SEM.

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Fig. 7 The mechanism of SG on obesity-induced hypertension.

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47

48 **Table.1 Baseline data of each group before the beginning of experiment**

49 S-sham: normal chow, sham-operated, HF-sham: high-fat chow, sham-operated, HF-SG: high-fat

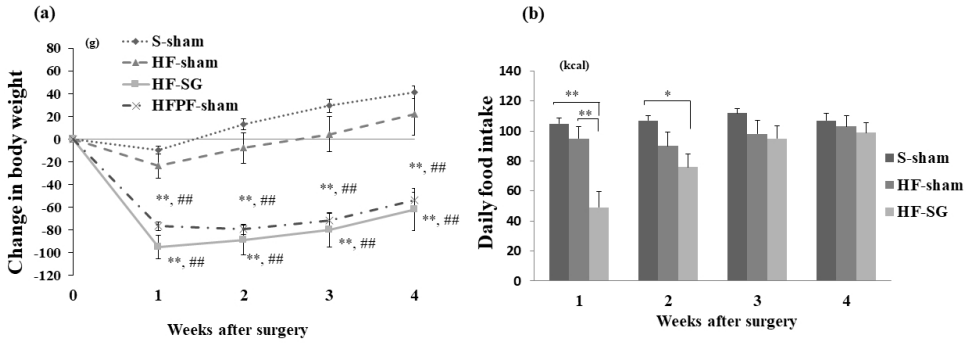
50 chow, sleeve gastrectomy, HFPF-sham: high-fat chow, pair-fed, sham-operated groups. The data are

51 expressed the mean ±SD. N.S.; no significance.

Baseline data of each group before the beginning of experiment

	S-Sham	HF-Sham	HF-SG	HFPF-Sham	P value
Body weight (g)	356 ± 2.4	361 ± 4.3	359 ± 5.1	353 ± 2.3	N.S.
Systolic blood pressure (mmHg)	127.6 ± 4.2	124.2 ± 4.3	126 ± 5.7	127 ± 4.6	N.S.
U-Na/U-Cr (mEq/gCr)	147 ± 32.1	171 ± 9.3	161 ± 10.0	131 ± 35.2	N.S.

S-sham: normal chow, sham-operated, HF-sham: high-fat chow, sham-operated, HF-SG: high-fat chow, sleeve gastrectomy, HFPF-sham: high-fat chow, pair-fed, sham-operated groups. The data are expressed the mean ±SD. N.S.; no significance



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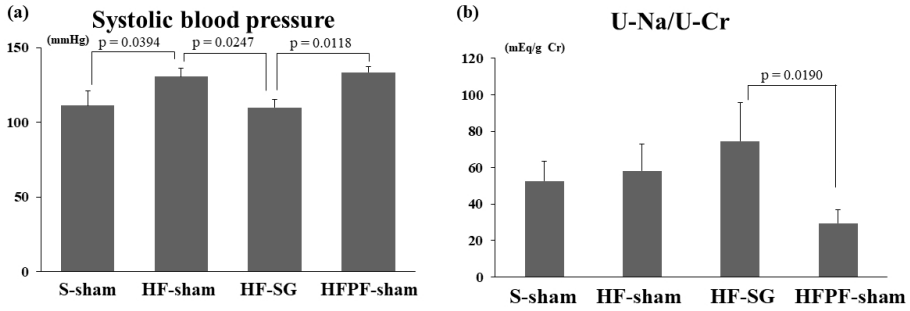

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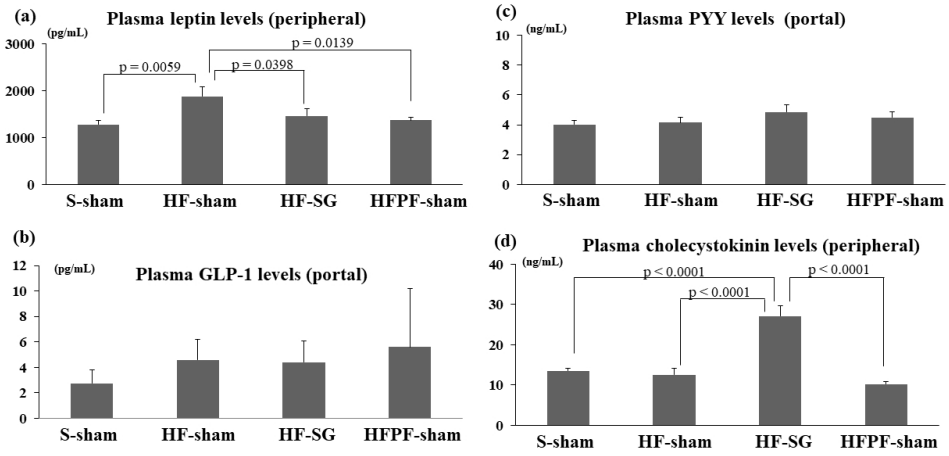

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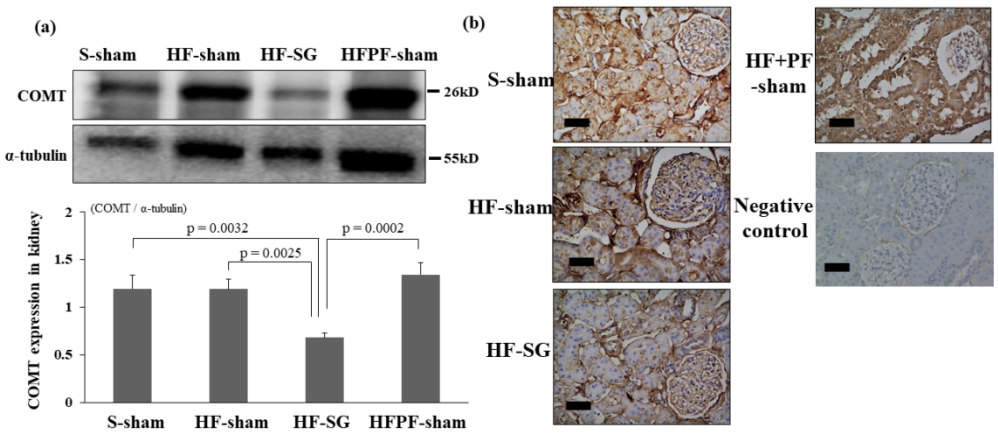

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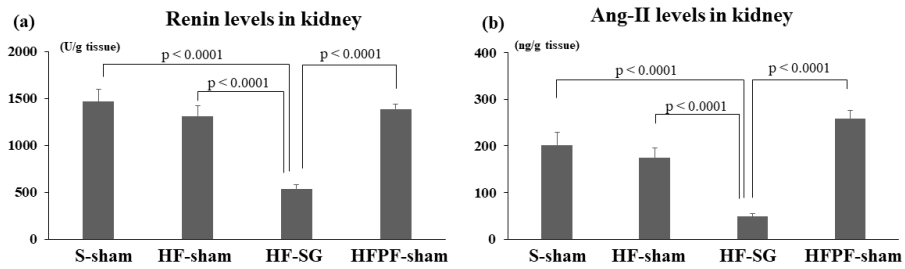

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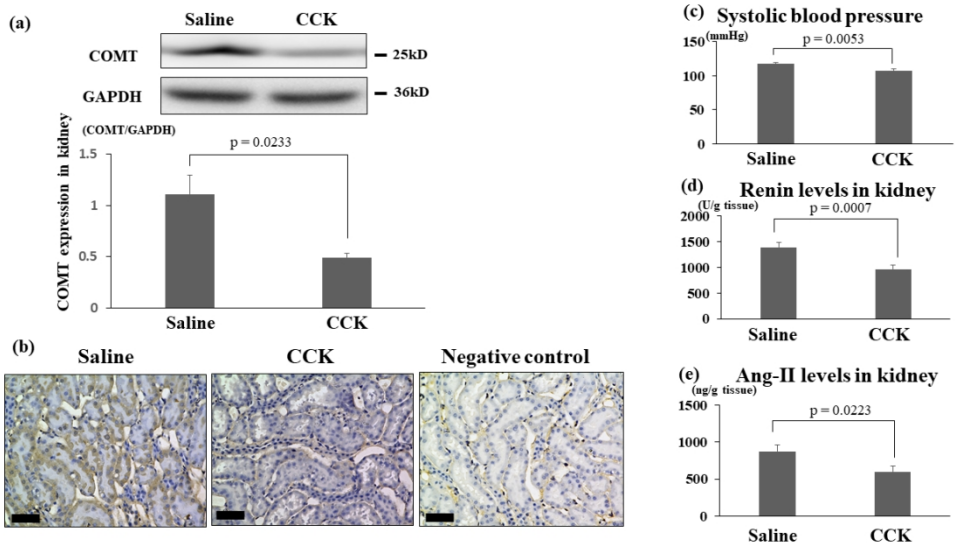

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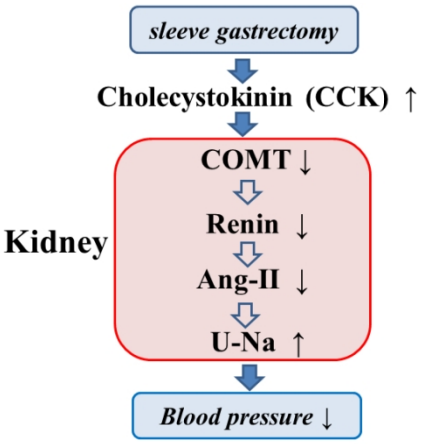

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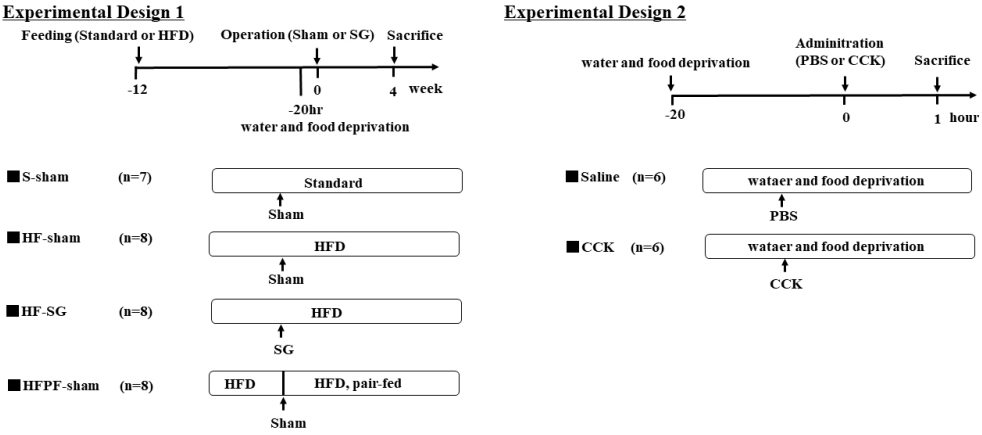

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