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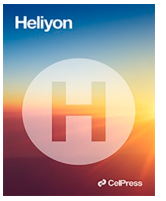

Research article

A traditional Korean fermented food, *Gochujang* exerts anti-hypertensive effects, regardless of its high salt content by regulating renin-angiotensin-aldosterone system in SD rats

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ABSTRACT

The current study aimed to investigate the distinct outcomes of table salt and salt in *Gochujang* on blood pressure (BP). Animals were divided into 3 groups, including normal diet (NS, 0.5 % NaCl), high-salt diet (HS, normal diet with 8 % NaCl), or high-salt *Gochujang* diet (HSG, normal diet with *Gochujang* containing 8 % NaCl). Compared to the NS groups, the HS group showed significantly increased systolic blood pressure (SBP), while the HSG group did not elevate SBP. The HS group had lower serum angiotensin II and aldosterone levels than the NS group, while the HSG group showed higher levels of those parameters than the HS group. The renal mRNA expression related to the renin-angiotensin-aldosterone system (RAAS) was significantly higher in the HS group than the NS group, while the HSG group had markedly lower expression of those markers. The urinary and fecal Na^+/K^+ proportion was higher in both HS and HSG groups relative to the NS group, but the HSG group showed a decreased Na^+/K^+ ratio in urine and feces compared to the HS group. Moreover, the HS group had a significantly upregulated mRNA level of Na^+/HCO_3^- co-transporter (Slc4a4) in the kidney than the NS group, whereas the HSG group showed downregulated mRNA expression of Slc4a4 compared to the HS group. This study demonstrates that *Gochujang* has anti-hypertensive effects regardless of its high salt content and provide the evidence regarding the distinct impacts between salt in *Gochujang* and the table salt.

1. Introduction

Globally, the prevalence of hypertension has steadily increased due to the trend of aging [1]. According to the World Health Organization (WHO), approximately 42 % of people possess hypertension [2,3]. In Korea, the prevalence of hypertension is approximately 30 % and half of this population is the elderly (≥65 years of age) [4,5]. Previous epidemiological research has reported a strong association between a high-salt diet (HSD) and increased blood pressure (BP) [6,7]. Therefore, WHO recommends a lower sodium intake (<2 g of sodium, which is < 5 g of salt) in a day and the "Sodium Reduction" strategy has been actively carried out around the world [8]. The average daily sodium intake range of Koreans was 3.5–3.9 g/day, which is 1.5 times higher than the

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recommendations [9]. It has been studied that the high salt intake of Koreans mainly comes from Korean traditional fermented foods (KTFFs), such as *Kimchi* and seasoning (i.e., *Gochujang*) [9,10].

The renin-angiotensin-aldosterone system (RAAS) is a major homeostatic system for BP by regulating fluid and electrolytic balance. Renin activates angiotensinogen (Agt) to angiotensin I (Ang I), then angiotensin-converting enzyme (Ace) transforms Ang I to angiotensin II (Ang II), causing the release of aldosterone stimulating sodium and water reabsorption in the kidney [11]. Based on the earlier findings, high salt intake causes RAAS dysregulation, resulting in abnormal sodium metabolism, BP elevation, and hypertension, ultimately [11,12]. For instance, HSD increases the levels of plasma and renal renin and aldosterone, also decreases renal Ace and angiotensin receptor type I (Agtr1) mRNA expression, augmenting systolic blood pressure (SBP) [13–15]. Therefore, appropriate sodium intake is essential to prevent and improve hypertension by maintaining normal RAAS functions.

Gochujang, a Korean fermented red pepper paste is one of the traditional sauces of the Korean diet [16]. *Gochujang* is made from the fermentation of a mixture, including fermented soybeans (*Meju*), glutinous rice, salt, and red peppers [16]. To improve preservation and prevent abnormal fermentation, a high level of salt is added during the making process of *Gochujang* (~10 %) [17]. Regardless of its high salt level, diverse health-beneficial effects of *Gochujang* have been studied, such as anti-obesity effects, anti-inflammatory effects, and anti-constipation effects [18–22]. Moreover, the potential hypertension ameliorative effects of *Gochujang* have been also observed [23,24]. For example, *Gochujang* showed an inhibitory effect on Ace [23] and consumption of a *Gochujang*-based diet lowered heart rate in hypertension patients [24]. These paradoxical results have been defined as the "Korean Paradox" [25]; however, the health-beneficial effects of *Gochujang* are still controversial due to the lack of evidence regarding the possible negative effects of its high salt level.

Thus, this study aimed to demonstrate the different effects of the additive salt and salt in *Gochujang* on BP. Moreover, the current study also investigated the distinct outcomes of the table salt and salt in *Gochujang* in RAAS-associated molecular mechanisms. To achieve study aims, the high salt diet (8 % NaCl) and high salt *Gochujang* containing 8 % NaCl were generated, which is the average salt percentage inducing hypertension in Sprague-Dawley (SD) rats [26,27]. The results of this study will provide an understanding of the different health-related outcomes of the additive salt and salt in *Gochujang* and offer concrete scientific evidence supporting the "Korean Paradox".

2. Materials and methods

2.1. The preparation of Gochujang

Two-month matured *Gochujang* was provided by Sunchang Sauce Corporation (Sunchang-gun, Jeollabuk-do, Korea). The detailed process of *Gochujang* production is shown in Supplementary Fig, 1. As the average salinity resulting in the hypertension of SD rats is 8 % [26,27], the contents of salt in *Gochujang* was adjusted with NaCl to 8 % by using Mohr's method [28].

2.2. Animal experiments

The design of the animal study is shown in Fig. 1. All animal protocols were approved by the Institutional Animal Care and Use Committees (IACUC) of Jeonbuk National University (JBNU 2016-0030). Three weeks old male SD rats were purchased from Central Laboratory Animal Co. (Seoul, Korea) and the rats were housed in cages after they arrived, with 12/12-h light and dark cycle at a temperature of 25 ± 2 °C and a humidity of 50 ± 5 %.

During the adaptive period (one week), the animals were maintained on a rodent diet (Research Diets, Inc. New Brunswick, NJ, USA). The rats were randomly divided into three groups, including normal diet (NS, 0.5 % NaCl, n = 10), high-salt diet (HS, normal diet with 8 % NaCl, n = 8), and high-salt *Gochujang* diet (HSG, normal diet with *Gochujang* containing 8 % NaCl, n = 8). Then, the animals were fed the experimental diet for 9 weeks; and the rats freely accessed to diet and water during the study period. *Gochujang* paste was freeze-dried, and its nutritional composition was supplied by Sunchang Traditional Paste Research Institute (Sunchang, Jeollabuk-do, Republic of Korea), which is shown in Table 1. The base of the experimental diet was the AIN-76A diet, and the detail of the experimental diet is described in Table 2. The different diet was standardized based on energy contents (isocaloric diet). The SBP was measured by the tail-cuff method (BP-2000, Visitech Systems, Inc., Apex, NC, USA) every week. During the last 4 weeks, rats were transferred to individual metabolic cages for 24 h per week to measure urine output and water intake. After anesthesia, all tissues and blood were collected and stored at −80 °C until further assays.

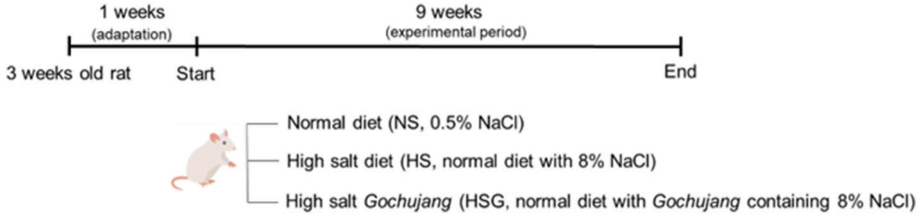

Fig. 1. An experimental design.

Table 1
The nutritional composition of *Gochujang*.

Ingredient (g/100g)	*Gochujang*
Carbohydrate	56.71
Protein	8.41
Fat	4.69
Ash	4.1
Moisture	11.8
NaCl	14.3
kcal/100g	302.69

Table 2
The ingredient composition of the experimental diet.

	NS	HS	HSG
Casein	200.0	200.0	183.18
DL-Methionine	3.0	3.0	3.0
Corn starch	150.0	150.0	150.0
Sucrose	500.0	500.0	500.0
Cellulose	50.0	50.0	50.0
Corn Oil	50.0	50.0	40.62
Mineral Mix	35.0	35.0	35.0
Vitamin Mix	10.0	10.0	10.0
Choline Bitartrate	2.0	2.0	2.0
Gochujang	–	–	200
Extra added Salt	–	87.0	58.4
Total (g)	1000	1087	1118.78
kcal/g	3.85	3.54	3.44

AIN-76 Rodent Diet (Research Diets, Inc., USA). NS, normal diet; HS, normal diet with 8 % NaCl; and HSG, normal diet with *Gochujang* containing 8 % NaCl. The HSG diet used freeze-dried *Gochujang*.

2.3. Biochemical analysis

Serum rennin (Cat No. MBS041519, MyBioSource, San Diego, CA, USA), angiotensin II (Cat No. ADI-900-204, Enzo Life Sciences, Inc., Farmingdale, NY, USA) and aldosterone (Cat No. ADI-900-173, Enzo Life Sciences, Inc., Farmingdale, NY, USA).

2.4. RNA isolation and microarray analysis

Total RNA was isolated from the kidney cortex, and RNA purity and integrity were evaluated by ND-1000 Spectrophotometer (Wilmington, USA). cDNA was synthesized using the GeneChip WT Pico Amplification kit (Thermo Fisher Scientific, Waltham, USA), and the sense cDNA was fragmented and biotin-labeled with TdT (terminal deoxynucleotidyl transferase) using the GeneChip WT Terminal labeling kit (Thermo Fisher Scientific, Waltham, USA). The labeled DNA target was hybridized to the Affymetrix GeneChip Rat 2.0 ST Array (Affymetrix Japan Co., Tokyo, Japan) at 45 °C for 16hr. The signal values were computed using GeneChip Operating Software 1.4 (Affymetrix Japan Co.).

2.5. Microarray data analysis

The data were extracted, summarized, and normalized with the robust multi-average (RMA) method implemented in Affymetrix® Power Tools (APT). The results were exported with gene level RMA analysis and performed the differentially expressed gene (DEG) analysis. The comparative analysis between test and control samples was carried out using one-way ANOVA,. Gene-enrichment and Functional Annotation analysis for a significant probe list was performed using Gene Ontology (GO) (http://geneontology.org). All statistical tests and visualization of differentially expressed genes were conducted using R statistical language 3.3.2 (www.r-project.org).

2.6. Quantitative real-time PCR analysis

Total RNA was isolated using TRIzol solution (Invitrogen Life Technologies, Carlsbad, CA, USA). cDNA was synthesized from 1 μg of RNA using the PrimeScript RT master mix (Takara Bio Inc, Shiga, Japan). Real-time quantitative PCR analysis was performed on the 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA) using SYBR green mix (TOYOBO, Osaka, Japan). Relative mRNA expression was quantified by the cycle threshold (ΔCt) method and β-actin was used as a loading control. The primer sequences were obtained from PrimerBank (http://pga.mgh-.harvard.edu/primerbank) and information on primers used for qPCR is available upon request.

2.7. The analysis of urinary and fecal Na^+ and K^+

From the metabolic cages, the samples of urine and feces were collected and then analyzed for the levels of Na^+ and K^+, by inductively coupled plasma-mass spectroscopy (ICP-MS; 7500A, Agilent Technologies, Germantown, MD, USA), the center for University-Wide Research Facilities (CURF) at Jeonbuk National University.

2.8. Statistical analysis

Values are expressed as mean ± S.D. Data are analyzed using one-way ANOVA with SPSS 17.0. The differences between groups were determined by Duncan's multiple-range test. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. The effects of Gochujang on body weight, kidney weight, liver weight, and food intake

According to previous studies, a high salt diet is associated with lower body weight [29,30]. However, the current study found that there were no significant differences in body weight among the groups at the end of the experiment (Fig. 2A). Moreover, the kidney weight did not differ among the groups (Fig. 2B), whereas relative kidney weight was significantly elevated in the HS and HSG groups compared to the NS group (Fig. 2C). Food intake also did not differ among the groups (Table 3). Moreover, liver weight was significantly reduced in the HS and HSG groups compared to the NS group (Fig. 2D), however, there was no significant difference in relative liver weight between groups (Fig. 2E). These results confirmed that HS and HSG intake did not affect changes in liver weight.

Compared to the NS group, water intake and urine output were significantly increased in both HS and HSG groups (Table 3). Additionally, the water intake of the HSG group had an increased tendency compared to the HS group without statistical significance, while the urine production of the HSG group was markedly higher than the HS group. In summary, these observations address that *Gochujang* increases urine output compared to the additive salt.

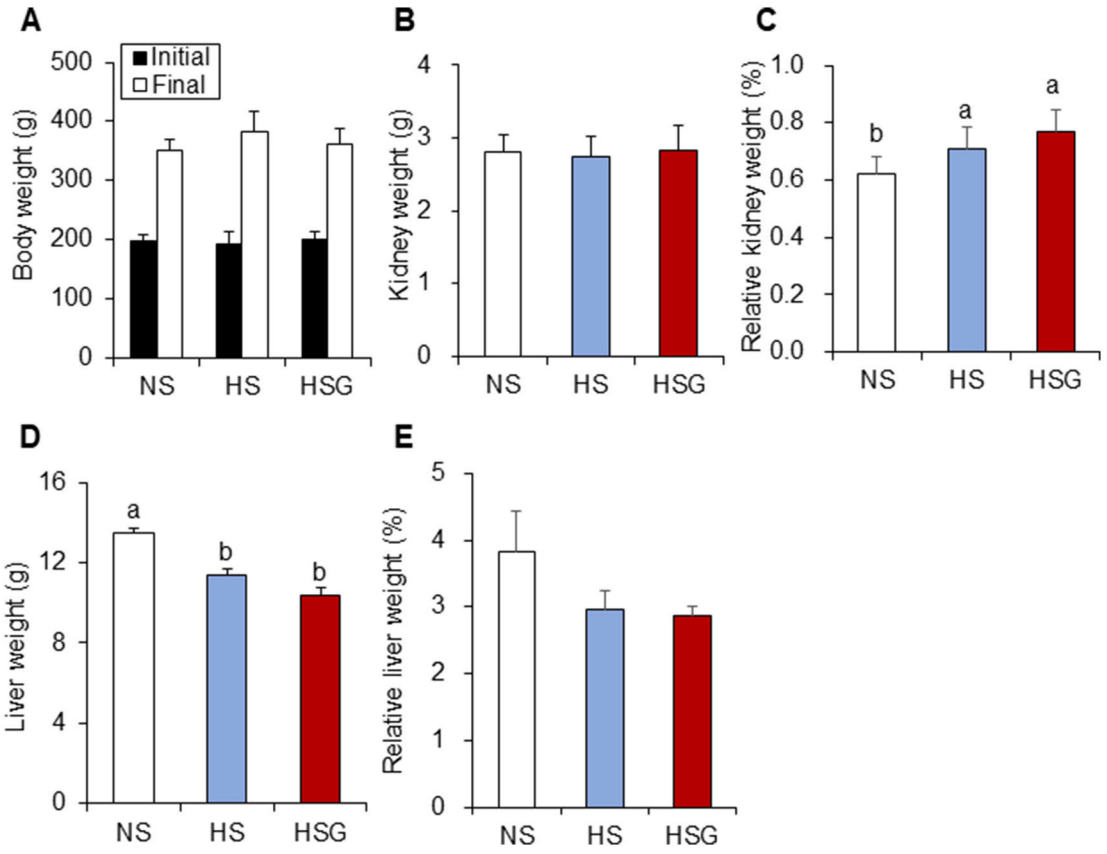

Fig. 2. The effects of *Gochujang* on body, kidney and liver weight (A) The body weight of rats at the beginning and end of the experiments **(B)** The weight of kidney **(C)** The relative kidney weight ratio (g/100g total body weight). **(D)** Liver weight **(E)** The relative liver weight ratio (g/100g total body weight). Data are presented as means ± S.D. (n = 8–10). NS, normal diet; HS, normal diet with 8 % NaCl; and HSG, normal diet with *Gochujang* containing 8 % NaCl.

Table 3
Food intake, water intake, and urine output in the rats.

	NS	HS	HSG
Food intake (g/day)	20.18 ± 3.75	20.21 ± 2.46	19.15 ± 1.49
Water intake (mL/day)	23.00 ± 20.23[b]	85.50 ± 30.13[a]	109.00 ± 31.43[a]
Urine output (mL/day)	17.50 ± 8.66[c]	75.00 ± 19.77[b]	103.00 ± 2.58[a]

Data are presented as means ± S.D. (n = 8–10). a-b-c; Values with different superscripts in the same row are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$. NS, normal diet; HS, normal diet with 8 % NaCl; and HSG, normal diet with *Gochujang* containing 8 % NaCl.

3.2. The effects of Gochujang on systolic blood pressure and serum RAAS-associated markers

To compare the different effects of additive salt and salt in *Gochujang* on BP and its regulators, the SBP and serum RAAS-related parameters were analyzed.

Compared with the NS group, the HS group showed a significantly increased level of SBP, while the HSG group had a lower level of SBP relative to the HS group (Fig. 3A). The serum renin level did not differ among the groups (Fig. 3B). Compared to the NS group, the levels of serum angiotensin II and aldosterone in the HS group were significantly decreased, while the HSG group had higher levels of those markers compared to the HS group with or without statistical significance (Fig. 3C and D). These findings imply that the additive salt and salt in *Gochujang* have different effects on SBP and its regulators.

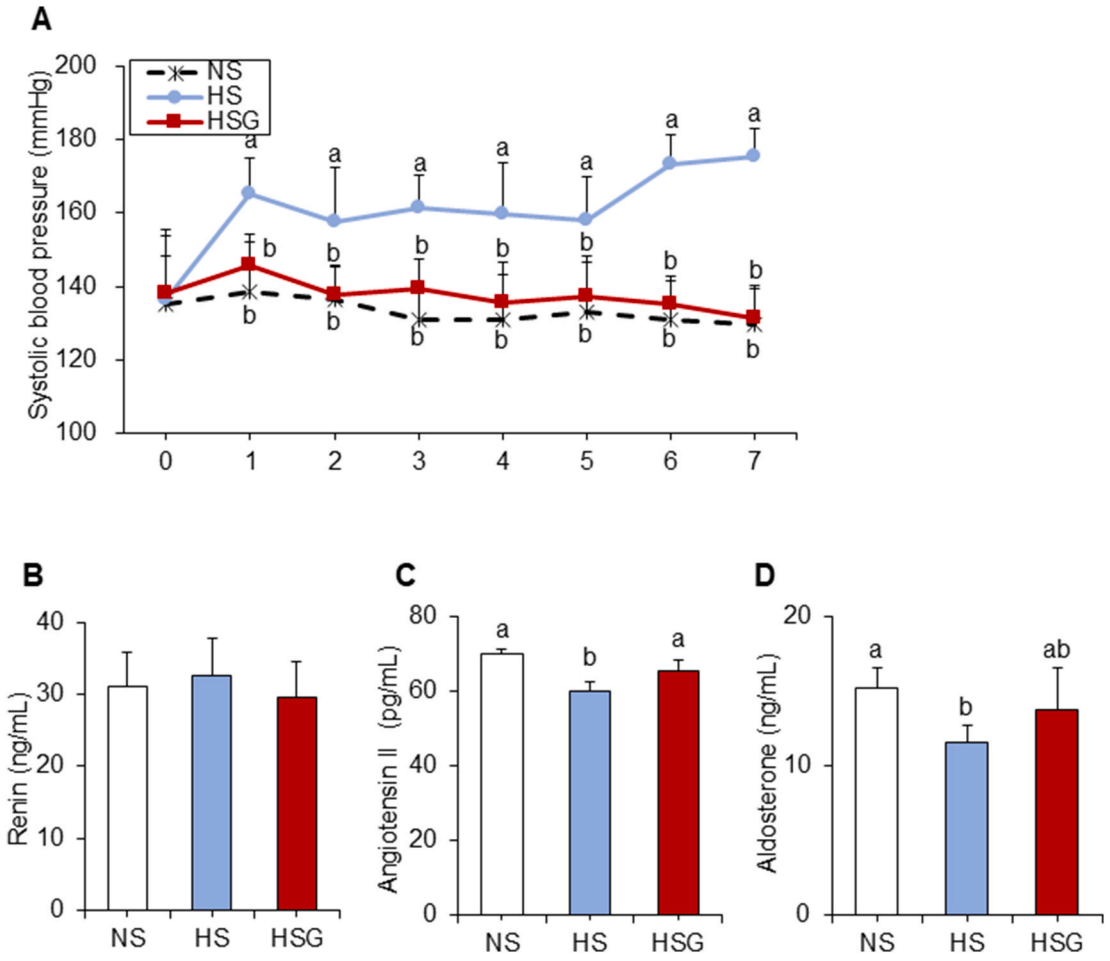

Fig. 3. The effects of *Gochujang* on systolic blood pressure and serum RAAS-related markers (A) Systolic blood pressure, **(B)** serum renin, **(C)** serum Angiotensin II, and **(D)** serum aldosterone Data are presented as means ± S.D. (n = 8–10). a-b-c; Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$. NS, normal diet; HS, normal diet with 8 % NaCl; and HSG, normal diet with *Gochujang* containing 8 % NaCl.

3.3. The effects of Gochujang on renal mRNA expression related to RAAS

As the additive salt and salt in *Gochujang* showed distinct outcomes on the SBP and the serum levels of related markers (Fig. 3), the key RAAS-associated enzyme expressions in the kidney were further evaluated, including mineralocorticoid receptor (MR) acting on aldosterone functions [31].

In the renal cortex, the level of *Agtr1* tended to increase in the HS group compared to the NS group, while the HSG group showed a reduced tendency of *Agtr1* expression relative to the HS group (Fig. 4A). The mRNA expressions of MR and Ace were markedly higher in the HS group compared to the NS group, while MR and Ace mRNA levels in the HSG group were noticeably decreased relative to the HS group with or without statistical significance (Fig. 4B and C). These results present that *Gochujang* has distinct effects on RAAS-involved molecular mechanisms compared to the additive salt.

3.4. The effects of Gochujang on gene expression related to kidney metabolism

Gochujang alters the renal mRNA expression related to RAAS (Fig. 4); therefore, the effects of *Gochujang* on the overall kidney metabolism were further studied by utilizing microarray and GO term analysis.

Among the groups, 6598 genes were differently expressed, and 2338 genes showed a dramatic upregulation or downregulation with at least two-fold changes. A list of the top 20 regulated pathways is shown in Fig. 5A. Compared to the NS group, the HS group did not show any significant alterations in gene expression related to kidney metabolism, except olfactory transduction (Fig. 5A). HSG group showed significant changes in gene expression related to organismal systems, including aldosterone synthesis and secretion, human disease, environmental information processing (i.e., cell adhesion molecules), genetic information procession (i.e., protein processing in the endoplasmic reticulum), and metabolism (i.e., oxidative phosphorylation) (Fig. 5A). Compared to the HS groups, these significant changes in gene expression patterns of the HSG group were also observed.

Olfactory receptors belong to the G-protein coupled receptor (GPCR) family and they play a critical role in renin release [7–9]. Because *Gochujang* led to significant changes in olfactory transduction and aldosterone synthesis and secretion (Fig. 5A), the detail fold changes were further analyzed and listed in Table 4. The gene expressions of the GPCR family were significantly downregulated in the HSG group, while the HS group showed either up- or down-regulated gene expression without statistical significance, except *Gpr152*. Furthermore, renin secretion-related gene expressions, such as *Calm2* and *Gnαq* were also significantly decreased in the HSG group, whereas the HS group did not show any significant alterations, except *Adcy5*. Most of the genes associated with aldosterone synthesis and secretion and the renin-angiotensin system were noticeably downregulated in the HSG group with statistical significance, while the HS group had either up- or down-regulated gene levels without statistical significance, except *Cacna1h* and *Ctsa*.

Next, the levels of several genes were confirmed by using real-time PCR (Fig. 5B); and consistent with the above findings, aldosterone-associated genes, such as *Cacna1h* and *Calm2*, and renin-angiotensin system-related genes, including *Kik1c9*, *Lnpep*, and *Atp6ap2* were significantly increased in the HS group compared to the NS group, while those gene expressions were markedly decreased in the HSG group relative to HS group (Fig. 5B). In summary, these data suggest that *Gochujang* has anti-hypertensive effects coming from RAAS suppression in the kidney, regardless of its high salt level.

3.5. The effects of Gochujang on electrocytes excretion and its related transporter mRNA levels in the kidney

According to earlier findings, an increased Na^+/K^+ ratio is linked with a higher hypertension risk, which is mainly regulated by RAAS [32]. Since *Gochujang* did not increase SBP and altered serum RAAS-related markers (Fig. 3), the effects of *Gochujang* on

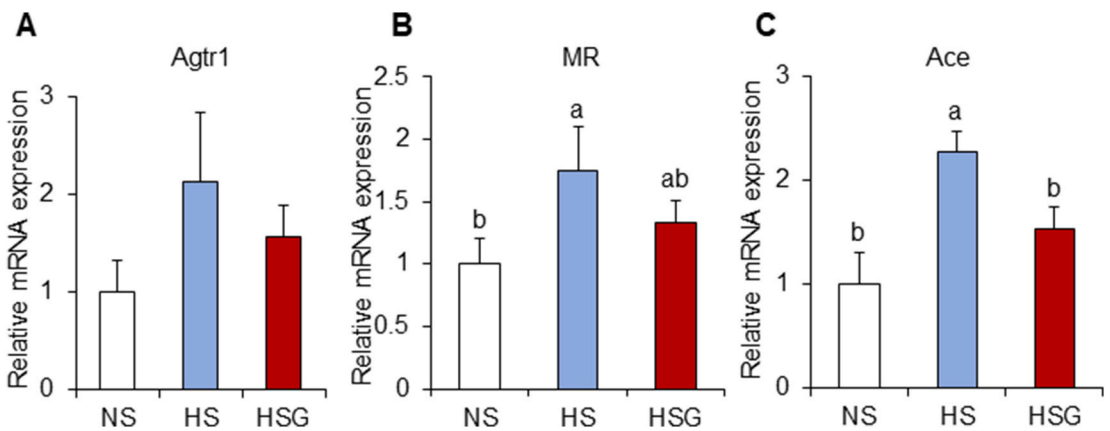

Fig. 4. The effects of *Gochujang* on mRNA expression associated with RAAS in the kidney The mRNA levels of **(A)** Agtr1, **(B)** MR, and **(C)** Ace are shown. Data are means ± S.D. (n = 8–10). a-b-c; Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$. NS, normal diet; HS, normal diet with 8 % NaCl; and HSG, normal diet with *Gochujang* containing 8 % NaCl. *Agrt1*, angiotensin receptor 1; *Ace*, angiotensin-converting enzyme; *MR*, mineralocorticoid receptor; and *Slc4a4*, Na^+/HCO^{3-} co-transporter.

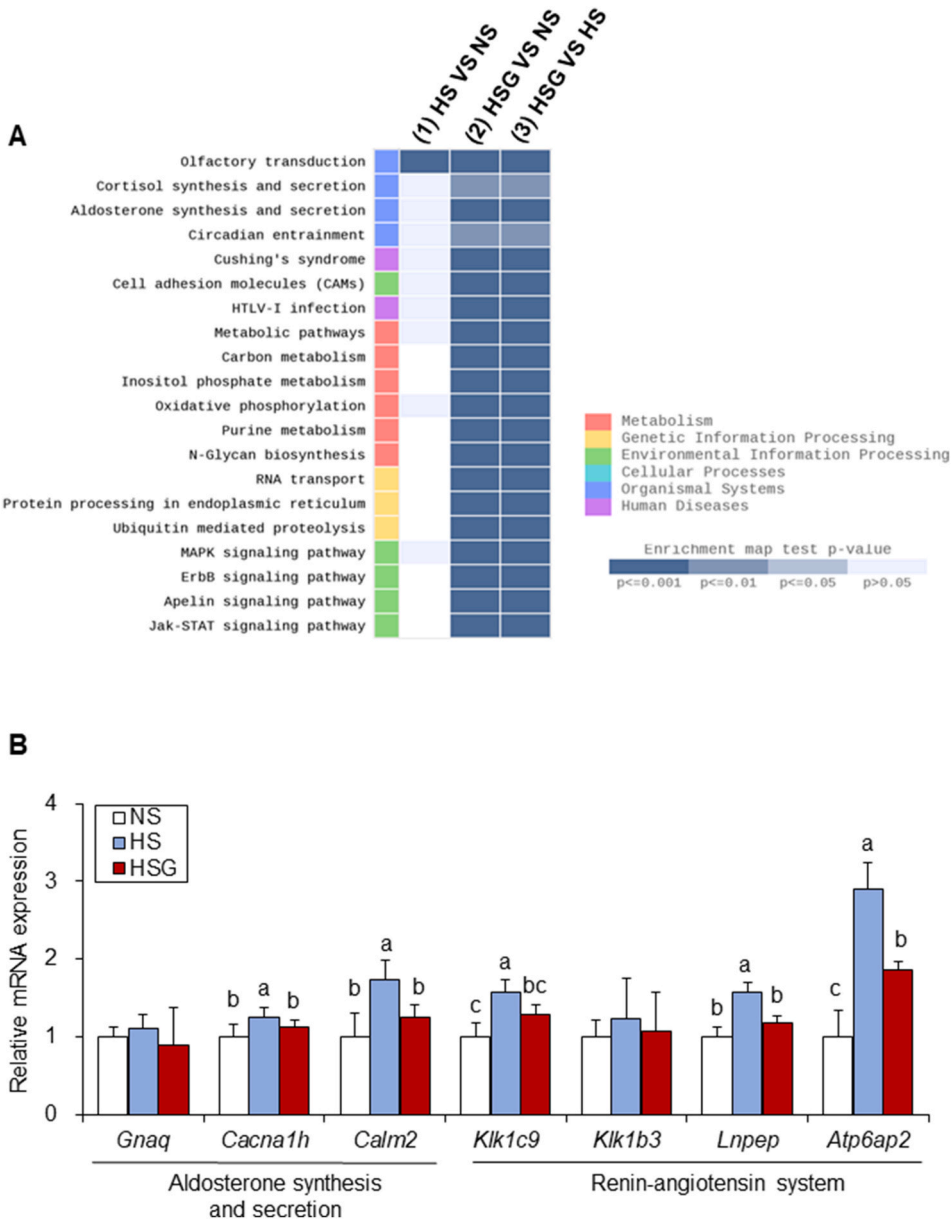

Fig. 5. The effects of *Gochujang* on overall gene expression in the kidney (A) The top 20 pathways in KEGG enrichment analysis are shown (1) HS vs NS, (2) HSG vs NS, and (3) HSG vs HS. The enrichment map is colored by the gradient level of the p-value. **(B)** Confirmation of microarray by qRT-PCR. Relative mRNA expression levels of aldosterone synthesis and renin-angiotensin system related genes in the kidney cortex. Data are means ± S.D. (n = 8–10). a-b-c; Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$. NS, normal diet; HS, normal diet with 8 % NaCl; and HSG, normal diet with *Gochujang* containing 8 % NaCl.). *Gnaq*, guanine nucleotide binding protein (G protein), q polypeptide; *Cacna1h*, calcium channel voltage-dependent T type, α 1H subunit; *Calm2*, calmodulin 2; *Klk1c9*, kallikrein 1-related peptidase C9; *Klk1b3*, kallikrein 1-related peptidase B3; *Lnpep*, leucyl/cystinyl aminopeptidase; *Atp6ap2*, ATPase H^+ transporting, lysosomal accessory protein 2.

electrocyte secretion were further analyzed. Compared to the NS group, both HS and HSG groups showed a significant elevation of urinary Na^+, while the HGS group had a lower tendency of urinary Na^+ than the HS group (Table 5). However, the urinary K^+ level was markedly lowered in both HS and HGS groups compared with the NS group; therefore, the urinary Na^+/K^+ proportion was significantly increased in the HS and HSG groups relative to the NS group, while the Na^+/K^+ ratio of the HSG group showed a reduced tendency compared to HS group without statistical significance (Table 5). Consistent with urinary Na^+ level, fecal Na^+ level was noticeably increased in the HS and HSG groups compared to the NS group; however, the amount of elevation in the HS group was much higher than HSG group. There was no significant difference in fecal K^+ secretion. As a result, the HS group showed the highest fecal Na^+/K^+ ratio among the groups, while the HSG group had a lower fecal Na^+/K^+ ratio relative to the HS group (Table 5).

Table 4
Transcripts expression of enzymes involved in the RAAS in the comparison of HS and HSG.

Gene name	Gene symbol	Fold change		p-value		Genebank ACC
		HS	HSG	HS	HSG	
G-protein coupled receptor activity						
G protein-coupled receptor 152	*Gpr152*	1.37	−1.01	0.032	0.983	XM_001068698
G protein-coupled receptor class C group 5	*Gprc5c*	1.01	−1.30	0.954	0.025	NM_001191591
G protein regulated inducer of neurite outgrowth 2	*Gprin2*	1.01	−1.37	0.982	0.020	XM_001059778
G protein-coupled receptor 137B	*Gpr137b*	−1.01	−2.51	0.998	0.007	NM_001105978
G protein-coupled receptor 89B	*Gpr89b*	1.07	−2.80	0.959	0.043	NM_001139486
G protein-coupled receptor 108	*Gpr108*	−1.11	−2.13	0.629	0.012	NM_199399
Renin secretion						
Adenylate cyclase 5	*Adcy5*	1.24	−1.07	0.016	0.233	NM_022600
Calmodulin 2	*Calm2*	1.03	−2.58	0.984	0.023	NM_017326
G protein subunit αq	*Gnaq*	1.18	−2.69	0.579	0.014	NM_031036
Guanylate cyclase 1 soluble subunit β2	*Gucy1b2*	1.07	−1.89	0.516	0.003	NM_001270711
Aldosterone synthesis and secretion						
Guanine nucleotide binding protein (G protein), q polypeptide	*Gnaq*	1.18	−2.67	0.579	0.014	NM_031036
Calcium channel, voltage-dependent, T type, α1H subunit	*Cacna1h*	1.68	−1.03	0.016	0.950	NM_153814
ATPase, Ca^{++} transporting, plasma membrane 4	*Atp2b4*	−1.02	−3.50	0.996	0.018	NM_001005871
Adenylate cyclase 1	*Adcy1*	−1.27	−2.14	0.339	0.025	NM_001107239
Calmodulin 2	*Calm2*	1.03	−2.58	0.984	0.023	NM_017326
Inositol 1,4,5-trisphosphate receptor, type 1	*Itpr1*	−1.02	−1.53	0.726	0.001	NM_001007235
cAMP responsive element binding protein 3-like 2	*Creb3l2*	1.01	−3.45	0.999	0.040	NM_001012188
Calcium/calmodulin-dependent protein kinase I	*Camk1*	−1.35	−2.85	0.503	0.043	NM_134468
Inositol 1,4,5-trisphosphate receptor, type 2	*Itpr2*	1.02	−2.27	0.990	0.020	NM_031046
Protein kinase D3	*Prkd3*	1.05	−1.76	0.921	0.042	NM_001024263
Guanine nucleotide binding protein, α11	*Gna11*	−1.12	−2.23	0.492	0.006	NM_031033
Adenylate cyclase 6	*Adcy6*	−1.09	−2.99	0.827	0.010	NM_001270785
Renin-angiotensin system						
Kallikrein 1-related peptidase C9	*Klk1c9*	1.92	−8.54	0.270	0.016	NM_175759
Kallikrein 1-related peptidase B3	*Klk1b3*	1.08	−17.65	0.987	0.024	NM_031523
Leucyl/cystinyl aminopeptidase	*Lnpep*	1.13	−3.02	0.483	0.003	NM_001113403
Cathepsin A	*Ctsa*	−1.17	−1.56	0.037	0.002	NM_001011959
ATPase, H^+ transporting, lysosomal accessory protein 2	*Atp6ap2*	1.04	−2.16	0.854	0.003	NM_001007091

Fold change of HS in NS versus HS, Fold change of HSG in NS versus HSG animals. NS, normal diet; HS, normal diet with 8 % NaCl; and HSG, normal diet with *Gochujang* containing 8 % NaCl.

Table 5
The levels of Na^+ and K^+ in urine and feces.

	Urine (ppm)			Feces (ppm)		
	Na^+	K^+	Na^+/K^+	Na^+	K^+	Na^+/K^+
NS	2019.04 ± 1107.11^{b}	4811.00 ± 2571.69^{a}	0.42 ± 0.04^{b}	240.37 ± 73.47^{c}	602.54 ± 225.90^{ns}	0.33 ± 0.03^{c}
HS	8607.03 ± 1098.53^{a}	645.16 ± 141.78^{b}	13.34 ± 0.77^{a}	3242.66 ± 1516.79^{a}	1311.92 ± 858.72^{ns}	1.77 ± 0.18^{a}
HGS	6910.60 ± 2556.99^{a}	668.73 ± 331.65^{b}	10.33 ± 0.77^{a}	599.82 ± 349.28^{bc}	966.17 ± 219.35^{ns}	1.59 ± 0.16^{bc}

Data are presented as means ± S.D. (n = 8–10). a-b-c; Values with different superscripts in the same row are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$. NS, normal diet; HS, normal diet with 8 % NaCl; and HSG, normal diet with *Gochujang* containing 8 % NaCl.

Na^+/HCO_3^- co-transporter (Slc4a4) and Na^+/H^+ exchanger 3 (Slc9a3) play critical roles in the sodium reabsorption in proximal tubule [33,34]. As *Gochujang* improved both urinary and fecal Na^+/K^+ ratio (Table 5), the effects of *Gochujang* on the levels of transporter related Na^+ reabsorption in the kidney was further analyzed. The mRNA expression of Slc4a4 in HS groups was markedly increased relative to the NS group, while the HSG group showed lower levels of Slc4a4 mRNA compared with the HS group without statistical significance (Fig. 6A). There were no statistical differences in Slc9a3 mRNA expression among the groups (Fig. 6B). These outcomes present that *Gochujang* has anti-hypertensive effects by lowering both urinary and fecal Na^+/K^+ ratio via regulation in transporter expression, despite of its high salt.

4. Discussion

Previously, the multiple health-advantageous effects of *Gochujang*, a Korean traditional fermented red pepper seasoning have been well demonstrated; however, it is still unclear the possible negative effects of high salt levels in *Gochujang*. This study found that high salt in *Gochujang* did not elevate SBP, unlike the high table salt. Moreover, the high additive salt increased renal RAAS-associated enzyme mRNA levels, whereas high salt in *Gochujang* did not alter those parameters. Furthermore, high salt in *Gochujang* significantly altered the gene expression trend related to RAAS. Lastly, the additive salt and high salt in *Gochujang* showed different outcomes

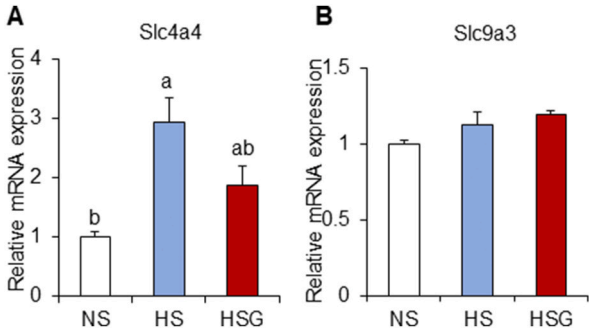

Fig. 6. The effects of *Gochujang* on mRNA levels related to Na $^{+}$ transport in the kidneyThe mRNA levels of **(A)** Slc4a4 and **(B)** Slc93 are shown. Data are means ± S.D. (n = 8–10). a-b-c; Values with different superscripts are significantly different by ANOVA with Duncan's multiple range test at $p < 0.05$. NS, normal diet; HS, normal diet with 8 % NaCl; and HSG, normal diet with *Gochujang* containing 8 % NaCl. *Slc4a4,* Na^{+}/HCO^{3-} co-transporter; and *Slc9a3*, Na^{+}/H^{+} exchanger 3.

in electrocyte excretion and Na $^{+}$ transporter expression.

Generally, fermented foods have high salt contents to improve fermentative quality and extend the preservation period [17,35]; therefore, their high salt contents have been problematic, when the effects of fermented foods are discussed. Regardless of high salt concentration, diverse KTFFs, including *Gochujang* showed multiple health-beneficial effects [18–21,23,24]. Consistent with previous findings, this study observed that high salt in *Gochujang* did not increase SBP differently from the high additive salt. Interestingly, another representative KTFF, *Kimchi* consumption was not associated with the risk of hypertension, although it contains high salt [36,37]; moreover, *Doenjang* (Korean traditional fermented soybean paste) and *Ganjang* (Korean traditional fermented soy sauce) also showed anti-hypertensive effects, regardless of their high salt content (*Doenjang*, ~12 % and *Ganjang*, 16–18 %) [38–41]. These observations strongly suggest that high salt in *Gochujang* and other KTFFs does not have negative effects and the possibility of the different metabolism between the additive salt and salt in fermented foods, requires future studies.

HSD leads to the abnormal release and expressions of RAAS-associated parameters, elevating SBP and hypertension risk [11–15]. As one of the critical members of RAAS, Ace activates Ang II and increases BP by regulating vasoconstriction [11]; therefore, inhibitions of Ace and Agtr1 have been utilized as therapeutic approaches to control hypertension [42]. The current study found that high salt in *Gochujang* decreases renal mRNA expression related to RAAS, while the high additive salt increases those markers; moreover, *Gochujang* also led to the downregulated gene expression related to RAAS, including renin secretion, aldosterone synthesis, and secretion, and the renin-angiotensin system. Due to its base ingredient (soybean and red chili pepper), *Gochujang* contains many functional bioactive compounds, such as bioactive peptides and capsaicin [17,43]; moreover, fermented soybean-based foods, including G*ochujang* contain higher aglycones, which are known to be more bioavailable compared with glycoside [44]. According to the previous findings, soybean-derived bioactive peptides and functional compounds (i.e., genistein) exert hypertension-inhibitory effects by blocking the vasoconstrictor system, also elevating kidney blood flow and sodium excretion [45]. Additionally, capsaicin improves hypertension by ameliorating vasorelaxation [46,47]. For example, a low amount of capsaicin improves kidney dysfunction and high salt-derived hypertension by ameliorating renal inflammation and oxidative stress [46]. Therefore, future studies are required to scrutinize the involvement of diverse compounds in *Gochujang* in its hypertension-inhibitory effects by establishing positive control in the study design. Also, the effects of *Gochujang* on impaired kidney function need to be studied by investigating kidney toxicity parameters and tissue structural changes in future studies.

There is a strong positive association between the Na^{+}/K^{+} ratio and hypertension [32]. Therefore, a lower Na^{+}/K^{+} ratio diet has been recommended to prevent the prevalence of hypertension [48]. In the present study, the consumption of high salt *Gochujang* lowered urinary and fecal Na^{+}/K^{+} proportion compared to the additive salt intake, even though both diets contain the same amount of high salt. Moreover, *Gochujang* improves Na $^{+}$ reabsorption-associated transporter expression in the kidney. The significant roles of gut microbiota in Na^{+} absorption in the intestine have been reported; furthermore, gut microbiota plays a role in BP regulation [49].

Gochujang shows anti-inflammatory effects and anti-constipation effects by improving gut microbiota dysbiosis [19,20], suggesting the possible positive roles of *Gochujang* in hypertension-related gut microbiota dysbiosis. Thus, future research regarding the roles of *Gochujang* in gut microbiota alterations related to Na $^{+}$ absorption and reabsorption, and BP regulation will provide another critical underlying mechanism of the anti-hypertensive effects of *Gochujang*. Furthermore, to conclude *Gochujang*'s blood pressure regulation efficacy, the effects of *Gochujang* on cardiac hypertrophy, heart fibrosis, and vascular malfunction are required to be investigated in the future.

This study demonstrated that *Gochujang* did not increase SBP regardless of its high salt content as it did change the mRNA level pattern involved in RAAS, electrocyte excretion, and Na^{+} transporter level. However, the current study has limitations regarding the isocaloric diet because the contents of indigestible carbohydrates in *Gochujang* were not evaluated, which might affect the available carbohydrate content and the energy value in the experimental diet. Thus, in future studies, it will be critical to analyze dietary fiber contents in *Gochujang* to make an accurate experimental diet, but also understand its possible effects on *Gochujang*'s diverse health-beneficial outcomes, including *Gochujang*'s blood pressure control efficacy.

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Ethics statement

All animal protocols were approved by the Institutional Animal Care and Use Committees (IACUC) of Jeonbuk National University (JBNU 2016-0030).

Data availability statement

All data presented in the study be made publicly available at or before the time of acceptance.

CRediT authorship contribution statement

Jung Eun Park: Writing – original draft, Formal analysis, Data curation. **Anna Han:** Writing – review & editing, Writing – original draft, Data curation. **Eun-Gyung Mun:** Writing – review & editing, Formal analysis, Data curation. **Youn-Soo Cha:** Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30451.

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