# Effects of High-Salt Intake on Glucose Metabolism, Liver Function, and the Microbiome in Rats: Influence of ACE Inhibitors and Angiotensin II Receptor Blockers

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## Abstract

**Background**: High-salt diets (HSD) are known to impact blood pressure and cardiovascular health, but their effects on glucose metabolism, liver function, and gut microbiota remain poorly understood. This study investigates how long-term HSD affects these physiological processes and evaluates the potential therapeutic effects of ACE inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs).

**Methods:** Male Sprague-Dawley rats were fed a normal salt diet (0.3% NaCl), a moderate salt diet (2% NaCl), or a high-salt diet (8% NaCl) for 12 weeks. Two subgroups in the HSD condition received telmisartan or enalapril. We assessed blood pressure, glucose homeostasis, liver inflammation, pancreatic function, and gut microbiota composition.

**Results:** HSD rats exhibited significantly higher blood pressure (130 ± 2 mmHg in ND vs. 144 ± 4 mmHg in HSD; $p < 0.01$), reduced fasting insulin (1.33 ± 0.14 ng/mL in ND vs. 0.60 ± 0.05 ng/mL in HSD; $p < 0.01$), and gut microbiota dysbiosis, with a 71% reduction in Ruminococcus species ($p = 0.018$). Liver inflammation, indicated by an increase in CD68+ macrophages, was also observed in the HSD group. Telmisartan treatment significantly reduced liver inflammation but did not fully restore metabolic homeostasis.

**Conclusion:** HSD disrupts multiple physiological systems, including glucose metabolism and liver function, partly through gut microbiota alterations. ACEIs and ARBs provided partial protection, highlighting the need for multi-targeted interventions to mitigate high-salt diet effects.



**Keywords:** High salt diet, rat physiology, ACE inhibitor, and angiotensin II receptor blocker


## New and Noteworthy

High salt diet induces multi-system disruptions, including liver inflammation, reduced insulin levels, and gut microbiota imbalance. ACEIs and ARBs showed limited efficacy, highlighting the need for comprehensive therapeutic approaches.

## Introduction

High salt intake is well-documented to cause hypertension and cardiovascular diseases (1-3). A wealth of epidemiological studies strongly advocate for reducing salt consumption, aligning with the World Health Organization's recommendation to limit intake to 5 grams per day (4). Despite this, average salt consumption frequently exceeds 10 grams daily in many populations. Excessive salt intake not only disrupts ion homeostasis, but also activates the sympathetic nervous system and suppresses the renin-angiotensin-aldosterone system (RAAS), thereby leading to insulin resistance and a spectrum of other pathophysiological alterations (5). Such changes can adversely affect multiple organs including the heart, brain, kidneys, and blood vessels. Thus, understanding the mechanisms through which excessive salt intake affects health and the pursuit of developing effective treatments represents a pressing concern. In addition, studies on salt sensitivity in hypertension and cardiovascular disease have traditionally relied on salt-sensitive models, such as Dahl-S rats, to explore the impact of excessive salt intake. However, this study primarily focuses on salt-resistant models, such as Sprague-Dawley (SD) rats. While these models offer valuable insights, the consistent plasma sodium levels observed in salt-resistant animals make the mechanisms underlying changes in insulin and glucose homeostasis less clear. This highlights the need for further investigation into how salt intake influences metabolic adaptations in salt-resistant models.

The precise interaction between sodium intake and glucose homeostasis is rarely investigated and remains elusive. In one clinical study, people with type 2 diabetes who consumed over 8 grams of salt daily experienced increases in fasting plasma glucose (by 2.3 mmol/L) and HbA1c (by 0.67%) compared to those consuming 6 grams or less (6). Animal studies highlight that high salt intake may lead to glucose intolerance (7, 8). However, this notion is met with conflicting findings, such as a study where a high-salt diet ameliorated hyperglycemia and insulin resistance in a certain rat model of type 2 diabetes, possibly due to increased adiponectin levels in the plasma (9).

Recent evidence suggests that a high-salt diet (HSD) affects not only cardiovascular health and glucose metabolism but also gut microbiota composition, contributing to metabolic dysfunction (10-12). A high-salt intake reduces beneficial bacteria (Lactobacillus, Bifidobacterium), while increasing pro-inflammatory species like Alistipes and Clostridia, leading to intestinal permeability, inflammation, and altered short-chain fatty acid metabolism(11, 12). Given the gut-liver axis and its role in metabolic regulation, we hypothesize that HSD disrupts glucose

homeostasis and liver function via microbiota dysbiosis. This study systematically examines these effects and evaluates potential therapeutic interventions.

In addition, recent research has highlighted that HSD may have both direct or indirect impacts on the liver, potentially leading to conditions such as fibrosis and fatty liver disease (13-15). Although various mechanisms have been identified through which high-salt diets can cause liver damage, such as disrupting the balance between oxidative stress and the antioxidant system and affecting lipoprotein transport (16), it remains a matter of debate whether these impacts are primarily due to the direct effects of sodium on liver cells or are secondary effects related to changes in systemic health factors like blood pressure and inflammation. Therefore, considering the less clear impact of a high-salt diet on various organs and physiological processes, comprehensive study is essential to gain a more detailed understanding.

ACE inhibitors (ACEIs) and angiotensin receptor blockers (ARBs) are widely used in the treatment of conditions like hypertension and chronic kidney disease (17). Additionally, in clinical settings, ACEI/ARB therapy has been observed to improve liver histology and function in patients with non-alcoholic fatty liver disease (NAFLD) (18, 19). Furthermore, various animal studies have demonstrated the efficacy of ACEI/ARB treatment in decelerating the progression of liver fibrosis (20, 21). Currently, ACEI/ARB has been shown to mitigate some of the harmful effects associated with a high-salt diet (22, 23). One study indicated that in Dahl salt-sensitive rats on a high-salt diet, the combined use of benazepril and valsartan was more effective in enhancing diastolic function and increasing survival rates than using either medication alone (23). While ACEI/ARB combination therapy improves certain cardiovascular outcomes, its effects on liver function, glucose homeostasis, and other consequences of high-salt intake remain underexplored. By modulating the angiotensin II and alternative RAAS pathways, this combination may help restore RAAS balance and mitigate high-salt diet effects, but further investigation is needed.

This study aims to examine key physiological parameters in healthy rats, focusing on glucose homeostasis, liver and pancreatic function, and gut microbiota composition, while investigating the underlying mechanisms in a high-salt diet model. Additionally, it evaluates the potential therapeutic effects of ACE inhibitors/ARBs in mitigating the adverse impact of a high-salt diet on these physiological traits.

## Materials and Methods

## Animals

Six-week-old male Sprague-Dawley (SD) rats were obtained from Hunan SJA Laboratory Animal (Changsha, China). Male rats were chosen to avoid variability from hormonal fluctuations in females that could affect glucose metabolism, liver function, and other physiological parameters. These rats were allotted a week to acclimatize to their new surroundings before being housed in environmentally controlled cages, maintaining optimal temperature and equipped with 12-hour cycles of light and darkness. The rats had unrestricted access to food and water. The experimental procedures were authorized by the Experimental Animal Center at Hunan Normal University, under permit number D2020008, ensuring adherence to ethical standards and animal welfare guidelines.

## Study design

A total of 65 male SD rats were divided into five groups and underwent different treatments over 12 weeks: (1) rats were fed a 0.3% NaCl control diet (ND group, n=10); (2) rats were fed a 2% NaCl medium-salt diet (MSD group, n=10); (3) rats were fed an 8% NaCl very high-salt diet (HSD group, n=15); (4) rats were fed 8% NaCl diet along with telmisartan (5 mg/kg) (HSD+TELM group, n=15); (5) rats were fed 8% NaCl diet along with enalapril (10 mg/kg) (HSD+ENAL group, n=15). The composition of the control diet (D10001), 2% NaCl diet (RD20072101), and 8% NaCl diet (D05011703, Shenzhen Ruidi Biotechnology Co., Ltd.) is shown in Supplementary Table 1. All diets used in the study were irradiated. The drugs telmisartan (J20090089) from Boehringer Ingelheim Pharma GmbH & Co. KG and enalapril (H32026567) from Yangtze River Pharmaceutical Group Co., Ltd. were administered to the animals via gavage at dosages of 5 mg/kg and 10 mg/kg, respectively. A flow chart detailing the steps of the animal experiment is included in Supplementary Figure 1. The body weight was measured every two weeks and food intake was recorded every week. At the beginning and end of the study, the CODA tail-cuff (Kent Scientific Corp., Torrington, CT, USA) blood pressure system was used to measure the systolic blood pressure (SBP). In the $9^{th}$ week, an oral glucose tolerance test (OGTT) was conducted. In brief, the animals underwent an 8-hour fasting period, followed by a gavage of 2mg glucose per gram of body weight. Blood samples were then collected from the tail vein at 0, 30, 60, and 120-minute intervals to measure blood glucose, following previously established protocols. The 0–120-minute area under the curve (AUC) for glucose concentrations was calculated using the trapezoidal rule. This method involves approximating the region beneath the curve as a series of trapezoids and

summing their areas to determine the total(24). Before sacrifice, the 24-hour urine was collected by a metabolic cage. The cage, equipped with a specialized funnel to separate urine from feces and minimize contamination, housed one animal with unrestricted access to food and water. Urine was collected over 24 hours, and its volume recorded. Feces were collected and placed in sterilized cryopreservation tubes and then stored at −80 °C. In the $12^{th}$ week, the animals were sacrificed by intraperitoneal injection of a solution of sodium pentobarbital 3% (wt/vol.). Blood, liver, and pancreas tissues were collected and stored for biochemical, morphological, and molecular analysis.

**Serum indicators measurement**

Blood was centrifuged at 3000rpm for 10 min to obtain serum. Cholesterol (CHOL), triacylglycerols (TG), high-density lipoproteins (HDL), and low-density lipoproteins (LDL) were measured using a Hitachi 7020 automatic biochemistry analyzer (Hitachi High-Technologies, Tokyo, Japan). Serum levels of insulin and C-peptide were determined via enzyme-linked immunosorbent assay (ELISA) kits (Shanghai Jianglai Biological Co., Ltd., Shanghai, China) according to the manufacturer's instructions. The concentration of sodium (Na) in serum and urine is determined by ion-specific electrode (ISE) technology.

**Liver morphology**

The liver samples were initially rinsed with phosphate-buffered saline (PBS), subsequently fixed in 4% paraformaldehyde, and then embedded in paraffin. Slices of 3 μm thickness were cut using a Microm HM230 Microtome and stained with several techniques: Staining with Hematoxylin and Eosin (H&E) was applied for the detection of lipid droplets and inflammatory cells, while Sirius Red was utilized to identify fibrosis. The Periodic Acid-Schiff (PAS) technique was used for glycogen detection, and CD68 immunofluorescence for macrophages. For each staining type, 30 images per slide were captured with an Olympus BH-2 microscope (200×) using a CFW-1310C digital camera. Quantitative analysis of the samples was performed using ImageJ software (version 1.410), facilitating the meticulous evaluation of the histological features.

**Pancreas morphology**

a) H&E Staining: pictures of whole tissue slides, and every islet of Langerhans were taken using the Zeiss Axiovert 100 microscope (25×/200×) and the Leica EC3 digital camera. The number of cells per islet was counted, and the islet area was measured using ImageJ software.

b) Pancreas immunofluorescence: The insulin and glucagon content of islets of Langerhans was measured using immunofluorescence staining. We used an antibody against insulin (1:200,

ab181547, Abcam, Cambridge, UK), an antibody against glucagon (1:200, ab92517, Abcam, Cambridge, UK), and a secondary antibody (1:500, ab150075 and ab150077, Abcam, Cambridge, UK) diluted in an antibody diluent (Dako, Glostrup, DK). All islets per slide were photographed using an Olympus BH-2 microscope (200×) and a CFW-1310C digital camera. 30 images for each sample were taken. The insulin and glucagon-positive areas were determined using ImageJ.

**Cell Cultures**

Mouse-derived pancreatic β-cells (MIN6 cells) were kindly supplied by Dr. Dominik Wigger (Institute of Pharmacy, Freie Universität Berlin, Berlin, Germany). The cells were maintained in DMEM Medium (containing 4.5 g/L D-Glucose and 6.4 g/L NaCl), supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 50 mmol/L β-mercaptoethanol under an atmosphere of 5% CO2 and 95% humidified air at 37°C. The medium was changed every 2-3 days. Experiments on MIN6 cells were performed between passages 21-30.

**Glucose-stimulated insulin secretion (GSIS)**

The GSIS experiment was performed following the methodology described in previous studies(25-27). Cells were seeded in 12-well plates with $1 \times 10^5$/mL and then incubated for 24h. Subsequently, cells were treated with 0, 20, 40, and 80 mM NaCl added to the medium, and the osmolarity was adjusted across the different salt concentrations by adding an inert osmotic agent, mannitol. The cells were then incubated continuously for 48 hours. The medium in the wells was removed and the cells were washed with glucose-free Krebs-Ringer bicarbonate HEPES (KRBH) buffer (115mMNaCl, 4.7mM KCl, 1.2mM MgSO4, 1.2mM KH2PO4, 1.3mM CaCl2, 24mM NaHCO3, 0.1%BSA, and 10mM Hepes, pH 7.4) twice. Following incubation for 1 h in glucose-free KRBH, the MIN6 cells were treated for 1 h in KRBH buffer with low (3.3 mM) or stimulatory (16.7 mM) glucose concentrations. Three replications were performed for each treatment group. Conditioned media were collected and centrifuged at 2500 rpm for 5 min. The supernatants were removed for analysis with insulin ELISA kits (ALPCO, USA) following the manufacturer's instructions.

**RNA-seq and data analysis**

The UID RNA-seq experiment, including sequencing and data analysis, was conducted by Seqhealth Technology Co., Ltd., Wuhan, China. Total RNA was extracted from liver and pancreas tissues from ND and HSD group (5 samples from each group) using TRIzol Reagent (Invitrogen, catalog number 15596026), followed by DNA digestion with DNaseI. RNA quality was assessed using the Nanodrop™ OneC spectrophotometer (Thermo Fisher Scientific, Inc.) and 1.5% agarose

gel electrophoresis. RNA was quantified with Qubit 3.0 using the Qubit™ RNA Broad Range Assay kit (Life Technologies, Q10210). For library preparation, 2 μg of total RNA was used with the KC-Digital™ Stranded mRNA Library Prep Kit for Illumina® (Wuhan Seqhealth Co., Ltd., China), which utilizes an 8-base UMI to label pre-amplified cDNA, reducing PCR bias. Libraries of 200–500 bp were enriched, quantified, and sequenced on the Novaseq 6000 sequencer (Illumina) using the PE150 model. Analysis of the sequencing data included the generation of volcano plots and heat maps for differentially expressed genes and performing GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) enrichment analysis.

**Real-time quantitative PCR**

Candidate genes were selected based on the RNA sequencing data (top 10 differentially expressed genes) and their functions. Expression of these candidate genes in liver and pancreas tissues was assessed by real-time quantitative PCR using a standard protocol. Briefly, total RNA was isolated from the liver and pancreas using Trizol reagent (TaKaRa, Dalian, Liaoning, China). The cDNA was synthesized using oligo-dT and random primers (TaKaRa, Dalian, Liaoning, China). Primer sequences are shown in Supplementary Table 2. The PCR was carried out on Roche Lightcycle 480 with Power SYBR Green PCR Master Mix in accordance with the instructions. All samples were analyzed in triplicate. The $2^{-\Delta\Delta Ct}$ method was used to quantify the expression of genes.

**Fatty acid detection in liver tissues**

12 liver tissues (6 from the ND group and 6 from the HSD group) were used to detect the content of 7 Short-chain fatty acids and 51 free fatty acids.

(a) Short-chain fatty acid measurement: Acetic, propionic, butyric, isobutyric, valeric, and isovaleric acids were prepared as 100 mg/mL stock solutions in water, and caproic acid in either, then diluted to create working solutions. Liver samples were placed in 1.5 mL centrifuge tubes with 500 μL water, and 100 mg glass beads, and homogenized for 1 minute. After centrifuging at 4°C, 12000 rpm for 10 minutes, 200 μL supernatant was mixed with 100 μL 15% phosphoric acid, 20 μL of 375 μg/mL 4-methylvaleric acid, and 280 μL ether, then homogenized and centrifuged again. The final supernatant was analyzed using a Thermo ISQ LT mass spectrometer and Thermo Trace 1310 gas chromatography system with an EI source and SIM scanning at 70 eV electron energy.

(b) Free fatty acid measurement: A 51-component fatty acid methyl ester mix (4000 μg/mL) was prepared in n-hexane to create ten concentration gradients. Samples were transferred to 2 mL

centrifuge tubes, mixed with 1 mL chloroform-methanol (2:1), 100 mg glass beads, and frozen in liquid nitrogen for 2-3 minutes. After thawing and homogenizing, the samples were centrifuged at 12000 rpm, 4°C for 5 minutes. The supernatant was transferred to a 15 mL tube, mixed with 2 mL 1% methanolic sulfuric acid, and esterified at 80°C for 30 minutes. Post-cooling, 1 mL n-hexane was added for extraction, followed by 5 mL $H_2O$ wash, and centrifuged at 3500 rpm, 4°C for 10 minutes. 700 μL of the supernatant, mixed with anhydrous sodium sulfate, was centrifuged again. This diluted sample was mixed with 15 μL 500 ppm methyl salicylate as an internal standard, and 200 μL of the supernatant was used for analysis with a Thermo TSQ 7000 mass spectrometer/Thermo Trace 1300 gas chromatography system using a Thermo TG-FAME capillary column and EI source at 70 eV.

**Absolute quantification of 16S rRNA amplicon sequencing**

Accurate 16S absolute quantification sequencing was conducted by Genesky Biotechnologies Inc., Shanghai. Total genomic DNA was extracted from 12 feces samples (6 from the ND group and 6 from the HSD group) using the FastDNA® SPIN Kit for Feces (MP Biomedicals, Santa Ana, CA) and assessed for integrity via agarose gel electrophoresis. DNA concentration and purity were measured using Nanodrop 2000 and Qubit3.0 Spectrophotometers. Artificial spike-ins, mimicking natural 16S rRNA genes but with random sequences (~40% GC content), were added to the sample DNA in known gradients. The V3-V4 regions of the 16S rRNA gene and spike-ins were amplified using primers 341F and 805R and sequenced on an Illumina NovaSeq 6000 sequencer.

**Bioinformatic Analysis**

In QIIME2, the raw sequencing reads were first processed. Adaptors and primers were trimmed with cutadapt, and the DADA2 plugin handled quality control, identifying amplicon sequence variants (ASVs). ASV sequences were taxonomically classified with a Naive Bayes classifier, trained on SILVA (version 138.1), using a confidence threshold of 0.8. After identifying and counting spike-in sequences, a standard curve was created for each sample by correlating read counts to spike-in numbers. This curve was then used to calculate the absolute copy number of each ASV in the samples, based on the ASV-specific read counts.

**Statistics**

Unless otherwise noted, all data are shown as mean ± SEM. By using the Shaprio-Wilk test, the distribution of data was checked for normality. Data meeting the assumption of homogeneity of variance were analyzed using one-way ANOVA followed by Tukey's post-hoc test. Dietary (ND,

MSD, HSD) and pharmacological (HSD, HSD+TELM, HSD+ENAL) comparisons were conducted as separate independent analyses. And a Kruskal-Wallis test plus a post hoc analysis (Dunn multiple comparison test) was used for variables not passing a normality or equal variance test. The unpaired Student's t-test and Pearson correlation analysis were applied for normally distributed data, while the Mann-Whitney U test and Spearman correlation analysis were used for non-normally distributed data. We regarded <0.05 as a significant *P*-value. GraphPad Prism version 8 (GraphPad Software, San Diego, CA, USA) and SPSS version 22.0 (SPSS, Chicago, IL, USA) were used for data analysis.

## Results

### Effects of HSD on physiological characteristics in rats and intervention effect of ACEI/ARB

The average daily food intake per animal was significantly higher in the HSD group (Figure 1A). The body weight of rats in the HSD group decreased significantly compared to the control group from the 2$^{nd}$ week, and this trend was also observed in the MSD group starting from the 6$^{th}$ week (Supplementary Table 3). The body weight was significantly lower in the HSD group (Figure 1B). The liver weight of rats did not significantly differ among groups (Supplementary Table 3). Rats on HSD depicted a decreased heart rate and an increased systolic blood pressure (SBP) in the 12$^{th}$ week compared to rats with the control diet (Figure 1C and Supplementary Table 3). In addition, we observed that animals on HSD consumed more water and produced a higher urine volume compared to control animals (Supplementary Table 3). The concentration of sodium in the serum and urine of rats with HSD was significantly higher than that in the ND group (Supplementary Table 3). As expected, in animals fed with HSD, the administration of telmisartan and enalapril for 12 weeks significantly reduced SBP compared to those animals not receiving treatment (Figure 1C and Supplementary Table 3). However, treatment with both telmisartan and enalapril had no impact on food intake, body weight, liver weight, or serum sodium levels (Supplementary Table 3).

### Effects of HSD on glucose homeostasis in rats and intervention effect of ACEI/ARB

Rats on an HSD exhibited a significant reduction in fasting blood glucose levels compared to those on a normal diet (Figure 1D). The OGTT results further revealed that, at intervals of 0-, 60-, and 120 minutes post-glucose intake, the HSD group had notably lower blood glucose levels than the ND group (Figure 1E and Supplementary Table 3). Additionally, the area under the curve (AUC) for blood glucose in the HSD group showed a significant decline (Figure 1F). However, no

significant difference in blood glucose was observed between the MSD group and the ND group. No significant difference in glucose content in 24-hour urine was observed among groups (Supplementary Table 3). Regarding the intervention, treatments with telmisartan and enalapril in animals on HSD did not affect blood glucose levels (Figure 1D-F).

**Effects of HSD on insulin and C-peptide concentration in the serum and pancreas morphology in rats and intervention effect of ACEI/ARB**

Using ELISA, we observed that both insulin and C-peptide levels in the serum were significantly reduced in rats fed with HSD compared to the control group (Supplementary Table 3). Additionally, the fasting glucose/ insulin ratio was significantly higher in the HSD group (Supplementary Table 3). However, upon morphological examination of the pancreas, we found no significant differences in the average area of the islets or the relative expression of insulin and glucagon in pancreatic tissue among groups (Figure 2A and B, Supplementary Table 4). A notable observation was that the number of cells per islet was significantly higher in the HSD group compared to the control group (Supplementary Table 4). Furthermore, in rats fed with HSD, treatment with either telmisartan or enalapril did not influence serum insulin and C-peptide levels, or pancreatic morphology when compared to untreated animals (Figure 2A-D, Supplementary Table 3 and 4). This suggests that while HSD affects serum insulin and C-peptide levels, its impact on pancreatic morphology is limited and that ACEI/ARB treatments do not modify these effects.

**High sodium concentration inhibited the insulin secretion of pancreatic cells *in vitro***

To investigate the impact of HSD on the insulin secretion capabilities of pancreatic cells, we conducted an *in vitro* experiment focusing on glucose-stimulated insulin secretion (GSIS). For this purpose, MIN6 cells were treated with varying concentrations of NaCl, adding 0, 20, 40, and 80 mM to the medium, respectively, and then were stimulated with glucose to induce insulin secretion. The experimental results revealed a significant decrease in insulin concentration in the supernatant of cells treated with 80 mM NaCl, under both normal and high-dose glucose stimulation (as shown in Figure 2E). This finding suggests that high sodium concentrations may inhibit the insulin secretion function of pancreatic cells.

**HSD affected the liver function and led to liver inflammation in rats and ARB alleviated inflammation induced by HSD**

The concentration of triglyceride and low-density lipoprotein (LDL) in serum did not differ among groups. However, there was a notable reduction in total cholesterol (CHOL) levels in rats fed with a MSD, and a significant decrease in high-density lipoprotein (HDL) in rats with HSD, both compared to the control group (Supplementary Table 3). Morphological analysis of liver sections revealed no significant differences in the number of lipid droplets per section, the area of these droplets, the extent of fibrosis, or glycogen content among the groups (Supplementary Table 4). Nevertheless, we observed a significantly higher number of inflammatory cells per liver section in the animals fed with MSD and HSD, compared to those on a normal diet, highlighting an inflammatory response (Figure 3A and B, Supplementary Table 4). Additionally, immunofluorescence results for CD68, a marker of macrophage activity, showed a significant increase in the CD68-positive area in animals on HSD (Figure 3A and C, Supplementary Table 4). Regarding the intervention, in animals on HSD, treatment with telmisartan or enalapril did not significantly impact the serum markers related to liver function (Supplementary Table 3). However, telmisartan treatment notably reduced the number of inflammatory cells per liver section (Figure 3B), and there was a trend toward a decrease in the CD68-positive area in the HSD+telmisartan group, although this did not reach statistical significance compared to the HSD group (Figure 3C). No significant difference in liver inflammation parameters was observed between the HSD+enalapril group and the HSD group (Supplementary Table 4). The above results indicated that high salt intake had a bad impact on the liver function of rats. Furthermore, high salt intake resulted in inflammation in the liver tissues of rats, which could be alleviated by the administration of telmisartan.

**HSD had an impact on the gene expression profile in the pancreas and liver of rats**

We performed RNA sequencing on pancreatic and liver tissues from rats in the HSD group and the ND group to compare their gene expression profiles. Based on the screening criteria of $|\log_2 (\text{fold change})| > 1$ and p-value $< 0.05$, we identified that 9 genes were upregulated, and 78 genes were downregulated in the pancreatic tissues between the two groups (Figure 4A). To validate these findings, we selected candidate genes based on their differential expression ranking and functional relevance. We then verified their expression in the pancreatic tissues of rats through quantitative real-time PCR (qRT-PCR). This analysis revealed that the expression of the *Rorc* gene was significantly increased, while that of the *Scn7a* gene was significantly decreased in the pancreatic tissues of rats fed with HSD, in comparison to the control group (Figure 4B and C). Correlation

analysis showed that the expression of *Rorc* was positively associated to the fasting glucose/insulin ratio while the expression of Scn7a negatively associated to the fasting glucose/insulin ratio (Figure 4D and E).

In the liver, we identified that 58 genes were upregulated, and 67 genes were downregulated between the two groups (Figure 5A). GO and KEGG pathway analysis revealed that these differentially expressed genes predominantly participated in fatty acid biosynthesis and metabolism processes and were significantly involved in the fatty acid metabolism pathway (Figure 5B). We focused on genes within the fatty acid metabolism pathway that exhibited a high fold change to verify their expression in the liver tissue of each group by qRT-PCR. The results indicated a significant decrease in the expression levels of *Fasn, Acly*, and *Srebf1* in the animals fed with HSD, compared to the control group (Figure 5C-E). Correlation analysis showed that the expression of *Fasn* and *Acly* had a negative correlation with the CD68 expression in the liver (Figure 5F-H). However, it was noted that treatment with telmisartan and enalapril did not affect the expression of these candidate genes in either the pancreas or liver tissues of animals on a high-salt diet. Further details about gene expression changes in the pancreas and liver tissues are shown in Supplementary Table 5.

**HSD led to a decreased fatty acid content in the liver of rats**

Building on our findings about the function of differentially expressed genes in the liver, we next analyzed the fatty acid content in liver tissues from rats in both the HSD group and the ND group. For this purpose, we utilized mass spectrometry to quantify the levels of 7 short-chain fatty acids and 51 free fatty acids. Our analysis revealed a significant reduction in the levels of two specific short-chain fatty acids— acetic acid and caproic acid—as well as 24 free fatty acids in the liver tissues of rats fed with the HSD, compared to those in the control group (Figure 6A and B). We found that the expression of *Fasn* had a positive correlation with the content of acetic acid and caproic acid (Figure 6C and D). Additionally, Figures 6E and F present a cluster heatmap and a correlation heatmap, respectively, for the 51 free fatty acids in the liver tissues of both groups. These heat maps provide a visual representation of the differences in free fatty acid profiles between the two dietary groups. Further details about fatty acid content in liver tissues are shown in Supplementary Table 6.

**HSD caused the imbalance of intestinal microbiota of rats**

To further investigate the effects of HSD, we conducted microbiota sequencing of fecal samples from rats in both the HSD group and the ND group, aiming to compare the composition of their intestinal microbiota. The effective sequences from all samples were clustered into operational taxonomic units (OTUs) with a similarity threshold of 97%. Species annotation was performed based on these representative OTU sequences. The Venn diagram, as presented in Figure 7A, revealed a greater number of OTUs in the fecal samples of animals on HSD than in the control group, with 999 OTUs common to both groups. To evaluate the richness and alpha diversity of the rats' intestinal microbiota, we used various indices including Chao1, ACE, Shannon, Simpson, and coverage. The analysis showed a significant increase in the Chao1, ACE, and Shannon indices for the animals on HSD, indicating that both the richness and alpha-diversity of the intestinal microbiota were higher in these HSD-fed animals compared to the controls (Figure 7B). Furthermore, the taxonomic compositions of the intestinal microbiota in each group were analyzed at both the phylum and genus levels, based on relative and absolute abundance (Figures 7C and D). At the phylum level, the dominant communities identified were Firmicutes, Bacteroidetes, Verrucomicrobia, Desulfobacterota, Proteobacteria, and Campylobacteria, as shown in Figure 7C. These phyla represent a broad spectrum of bacteria commonly found in the gut, each playing a unique role in the gut ecosystem. Further analysis at the genus level revealed that the most abundant communities were Ruminococcus, Bacteroides, UCG-005, Akkermansia, Lachnospiraceae_NK4A136_group, Alloprevotella, Romboutsia, Colidextribacter, Helicobacter, and Psychrobacter, as depicted in Figure 7D. These genera include both commensal and potentially pathogenic bacteria, indicating a diverse bacterial population within the rat intestinal microbiota. This detailed breakdown at the genus level provides a more nuanced understanding of the microbial composition in the gut of rats subjected to different dietary conditions.

Further analysis using the Linear Discriminant Analysis Effect Size (LEfSe) method provided a more in-depth understanding of the differences in intestinal microbiota between the two groups. The cladograms generated by LEfSe and the bar chart with a Linear Discriminant Analysis (LDA) score greater than 2 clearly demonstrated significant alterations in the intestinal microbiota at all levels between the HSD group and the control group, as illustrated in Figures 7E and F. Specifically, compared to the control group, high salt intake in the HSD group resulted in a notable increase in the absolute abundance of the phylum Actinobacteria (Figure 7E). At the genus level, the impact of high salt intake was marked by a significant decrease in the relative abundance of Ruminococcus

and Bacteroides and a significant increase in the abundance of several genera, including Faecalibaculum, Staphylococcus, Facklamia, A2, Allobaculum, Psychrobacter, Adlercreutzia, and Aerococcus. The top 10 altered microbiomes at the genus level were shown in Figure 8A. The correlation analysis between ten altered microbiomes and changes in phenotypic parameters revealed significant associations. Specifically, Ruminococcus and Bacteroides exhibited positive correlations with both the AUC of glucose and fasting insulin levels. Conversely, these microbes showed a negative correlation with the fasting glucose to insulin ratio. Additionally, Allobaculum demonstrated a negative correlation with the AUC of glucose, as illustrated in Figure 8B. Further analysis indicated that Ruminococcus and Bacteroides were also positively correlated with the hepatic content of acetic acid. In contrast, Psychrobacter displayed a negative association with the hepatic content of caproic acid, as depicted in Figure 8C.

## Discussion

### Summary of key findings

The current study provides compelling evidence of the harmful impacts of a high salt diet on glucose homeostasis, insulin secretion, liver function, liver fatty acid content and gut microbiota in rats, thereby enhancing our understanding of the effects of high salt consumption. Mechanically, the changes in gene expression patterns in the pancreas and liver as well as dysbiosis of intestinal microbiota contributed to the phenotypic alterations observed in animals on a high salt diet. In addition, we revealed that the treatments with telmisartan and enalapril were effective in mitigating the high blood pressure induced by the high salt diet. Telmisartan also demonstrated potential in reversing liver inflammation caused by the diet. The key findings of the current study were shown in Figure 9.

### Effect of high salt diet on body weight

Studies on HSD report conflicting body weight outcomes. Short-term HSD in mice/humans increases food intake (hyperphagia) but stabilizes weight via compensatory energy expenditure (28). However, our study found weight loss in HSD-fed animals, possibly due to heightened energy use or increased water intake reducing caloric absorption (29). For example, rams on a 12% salt diet showed reduced weight gain with elevated water consumption (30). Proposed mechanisms like leptin resistance (31) or hypercatabolism remain speculative, as causal evidence is limited. Experimental variables (salt concentration, species differences) further complicate interpretations.

While HSD may disrupt energy balance, rigorous studies are needed to validate mechanisms and clarify inconsistencies.

**Potential underlying mechanism: how a high salt diet impacts glucose homeostasis**

The relationship between salt intake and glucose metabolism remains incompletely understood, with studies reporting conflicting effects of HSD on insulin sensitivity and glycemic control. HSD has been linked to insulin resistance in rodent models. Rats fed HSD exhibited impaired insulin-stimulated glucose uptake in muscle, reduced hepatic glycogen synthase activity, and amplified insulin signaling dysfunction in salt-sensitive strains (32, 33). Similarly, combining HSD with a high-fat diet worsened glucose intolerance and suppressed pancreatic β-cell expansion, impairing insulin secretion (8). Conversely, chronic salt overload improved glucose metabolism in some models without altering insulin sensitivity (34), while HSD reduced plasma insulin and ameliorated hyperglycemia in diabetic rats, possibly via elevated adiponectin (9). Rams fed a 12% salt diet also showed lower insulin levels (30), suggesting species-specific or context-dependent effects. Human studies mirror this controversy. HSD (≥8 g/day) increased fasting glucose (+2.3 mmol/L) and HbA1c (+0.67%) in type 2 diabetes patients compared to low-salt intake (6). Acute HSD also reduced insulin sensitivity in healthy subjects (35). However, normotensive individuals displayed enhanced insulin sensitivity on HSD, with lower insulin levels despite unchanged glucose (36). These disparities may stem from differences in baseline health, salt sensitivity, or compensatory mechanisms.

In the HSD group, lower serum glucose and insulin levels may reflect several physiological adaptations to chronic high-salt intake. While high-salt diets are typically associated with insulin resistance, chronic sodium loading might enhance insulin sensitivity in tissues like the liver and skeletal muscle, possibly through alterations in insulin receptor function (37). Additionally, reduced insulin concentrations could indicate an adaptive response of pancreatic β-cells to lower glucose demand, rather than β-cell dysfunction (8, 38). Furthermore, high salt intake may promote osmotic diuresis, increasing renal glucose clearance and contributing to reduced blood glucose levels(39, 40). Thus, while high-salt intake alters glucose homeostasis, the observed changes in glucose and insulin do not necessarily reflect improved metabolic health but rather adaptive responses.

Targeting RAS (e.g., telmisartan) improves inflammation and glucose metabolism (41), but direct effects on insulin secretion remain limited. This highlights the need for multi-targeted approaches.

**High salt diet caused liver inflammation and the reduction of fatty acids**

In our study, we observed that HSD also induced liver inflammation in rats, aligning with the findings of other research in this area (15, 16, 42-44). A study has indicated that high salt intake can increase the risk of liver damage and fibrosis in both adults and developing embryos. The underlying pathological mechanism is thought to be a result of an imbalance between oxidative stress and the body's antioxidant system (16). Another study revealed that a high-salt diet worsened nonalcoholic steatohepatitis (NASH) in LOX-1 transgenic /apoE knockout mice fed a high-fat diet. This exacerbation was linked to the stimulation of oxidative and inflammatory processes (43). A recent study indicated a significant increase in both the proportion and activation of macrophages in the livers of mice fed with HSD (15). Promotes inflammation in liver tissues, marked by increased infiltration of CD68-positive macrophages and pro-inflammatory mediators. This inflammatory state disrupts insulin signaling pathways, leading to hepatic insulin resistance.

Moreover, our findings indicate a downregulation in the expression of *Fasn* (Fatty Acid Synthase), *Acly* (ATP Citrate Lyase), and *Srebf1* (Sterol Regulatory Element-Binding Transcription Factor 1) in the liver of rats on a high salt diet. These genes are primarily involved in regulating fatty acid metabolism. Similarly, a study that conducted transcriptome analysis found that the expression of genes related to lipid and steroid biosynthesis, including *Fasn, Scd1* (Stearoyl-CoA Desaturase-1), and *Cyp7a1* (Cytochrome P450 Family 7 Subfamily A Member 1), was significantly reduced in the livers of HSD mice (45). Another transcriptome analysis revealed that a high-salt diet impacts at least 15 enzymatic activities and various signaling and metabolic pathways. These include retinol metabolism, linoleic acid metabolism, and steroid hormone biosynthesis, among others (46). We subsequently verified that HSD resulted in a reduction of two short-chain fatty acids and 24 free fatty acids in the liver. Short-chain fatty acids (SCFAs), mainly produced by gut bacteria, play a vital role in gut health, immune modulation, and metabolic regulation. Free fatty acids, originating from dietary or body fats, are a crucial energy source and are involved in the regulation of glucose and lipid metabolism. The decreased levels of these fatty acids observed in this study may be linked to abnormal energy metabolism in animals fed with HSD. Similarly, one study showed that high concentrations of NaCl significantly reduced intracellular triglyceride and free fatty acid levels while significantly increasing lipolysis activity in 3T3-L1 cells (47). In summary, the decrease in SCFAs in the liver exacerbates metabolic disturbances, as SCFAs are key regulators of glucose and lipid metabolism. Downregulation of genes involved in fatty acid synthesis (e.g.,

Fasn, Acly, Srebf1) suggests impaired lipid-glucose cross-talk, a hallmark of metabolic dysfunction in insulin-resistant states.

**Gut microbiome imbalance caused by a high salt diet may contribute to liver inflammation and disrupt glucose homeostasis**

Several studies have indicated that HSD may disrupt gut microbial balance (10-12). In this study, we particularly noted a marked decrease in Ruminococcus and Bacteroides in HSD-fed animals. As Bacteroides are key producers of SCFAs like butyrate and propionate, their reduced abundance may be tied to the lower SCFAs levels in these animals. Ruminococcus is also involved in the production of SCFAs, such as acetate, propionate, and butyrate(48). Dysbiosis, characterized by a reduction in beneficial bacteria such as Ruminococcus and Bacteroides, leads to lower production of SCFAs like butyrate, which plays a crucial role in maintaining glucose homeostasis. The reduction in SCFAs impairs insulin sensitivity and increases systemic inflammation, which are both linked to the development of metabolic syndrome. Furthermore, gut-derived metabolites, such as secondary bile acids, that modulate glucose metabolism through farnesoid X receptor (FXR) signaling pathways, are disrupted under high salt conditions(49). This further impairs glucose tolerance.

Our study also found increased levels of Staphylococcus, Psychrobacter, and Aerococcus in HSD-fed animals, bacteria often associated with inflammation. Additionally, Bacteroides help regulate immune response and maintain gut balance, possibly offering protection against allergies and autoimmune diseases(50). This disruption, termed gut dysbiosis, leads to an increase in pro-inflammatory bacteria that activate Th17 cells, promoting a systemic inflammatory response. The resulting inflammation impairs insulin signaling pathways, contributing to insulin resistance and glucose intolerance. However, further research is needed to confirm these findings.

**Effect of ACE Inhibitor and ARBs treatment on adverse physiological processes induced by high salt diet**

In our study, we discovered that treatments with telmisartan and enalapril could reverse high blood pressure caused by a high-salt diet, yet they did not significantly affect other physiological processes. Uniquely, telmisartan was effective in reducing liver inflammation induced by a high-salt diet. While ACE inhibitors and ARBs are known to counter some negative effects of a high-salt diet, such as hypertension and cardiovascular issues (19, 20), research on their impact on liver function and glucose homeostasis is limited. Our findings are pioneering in confirming

Telmisartan's capability to alleviate high-salt diet-induced liver inflammation. Previous studies have shown that telmisartan reduces reactive oxygen species in mouse pancreatic β cells (51). And both ARBs (like telmisartan or valsartan) and ACE inhibitors (such as perindopril) have been reported to enhance pancreatic islet function in various diabetes models (52-54). However, in our study, we did not observe a significant effect of telmisartan on insulin secretion and glucose homeostasis in the context of a high-salt diet. This suggests that while telmisartan can be beneficial for certain aspects of metabolic health, its impact on glucose homeostasis under high-salt conditions may be limited.

**Interconnected effects of salt on different organ systems**

The effects of high salt intake on various organ systems are interconnected through complex physiological and biochemical pathways. High salt intake has been widely reported to impact cardiovascular health, kidney function, and neural regulation. In our study, we also revealed that high salt intake has detrimental effects on pancreatic and liver function, as well as gut health, in rats. Hypertension, driven by increased blood volume from sodium retention, elevates oxidative stress and systemic inflammation, which can damage pancreatic beta cells and reduce insulin secretion (55). In the liver, excessive salt exacerbates conditions such as non-alcoholic fatty liver disease (NAFLD) through mechanisms like hepatic steatosis and inflammation. These changes impair liver function, affecting overall metabolism and nutrient processing, which in turn impacts cardiovascular health and kidney function. Additionally, high salt intake disrupts gut health by altering the gut microbiota composition, reducing populations of beneficial bacteria, and promoting gut inflammation. This microbiome disruption compromises the gut barrier function, leading to systemic inflammation and affecting immune responses(56). These inflammatory processes can influence liver function, pancreatic health, and cardiovascular health. The interplay between these organ systems demonstrates how high salt intake can initiate a cascade of adverse health effects, highlighting the importance of managing dietary salt to prevent systemic health issues.

**Study limitations**

Our study has certain limitations. Firstly, while our data indicates that high salt intake influences glucose homeostasis and insulin secretion, it is important to recognize that glucose regulation is a multifaceted process involving various pathways beyond insulin signaling. These include renal, hepatic, and inflammatory mechanisms, which can independently or interactively affect glucose

metabolism. Given the complexity of glucose homeostasis, the observed reduction in blood glucose cannot be solely attributed to changes in insulin levels. We acknowledge that further dynamic and longitudinal studies are needed to more comprehensively understand how high-salt diets impact glucose regulation through these diverse and interconnected pathways. In addition, our observations were based on male rats, and sex-based differences in glucose homeostasis, liver function, and responses to high salt intake or RAAS inhibition warrant further investigation in female models for a more comprehensive understanding. Our research did not thoroughly examine changes in fatty acid content and intestinal microbiota in the telmisartan and enalapril treatment groups, given that we observed only limited efficacy of these treatments in mitigating the negative impacts of a high-salt diet. Consequently, further research in these areas is necessary.

### Conclusions

Our study highlights the detrimental effects of a high-salt diet on glucose homeostasis, insulin secretion, liver function, fatty acid content, and gut microbiota in rats. Notably, alterations in the gene expression within pancreatic and hepatic tissues and gut microbiota dysbiosis were mechanistically linked to the phenotypic modifications observed in subjects consuming a diet high in salt. This study provides valuable insights into the multisystem effects of a high-salt diet and the limited efficacy of ACE inhibitors and ARBs in addressing these broader impacts beyond blood pressure regulation.

## SUPPLEMENTARY MATERIALS

The supplementary materials for this study are available on Figshare.

Supplementary Fig. S1: https://doi.org/10.6084/m9.figshare.28600256.

Supplementary Tables S1-S6: https://doi.org/10.6084/m9.figshare.28400801.

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## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the methods and supplementary material of this article.

## DISCLOSURES

All authors declared no competing interests. This study was funded by Boehringer Ingelheim AG, which also provided the investigational drugs used in the research. The authors declare no other competing financial or personal interests.

## AUTHOR CONTRIBUTIONS

Berthold Hocher conceptualized and designed the study, supervised data collection, and critically reviewed the manuscript.

Xiaoli Zhang, Liping Liu did animal experiments.

Xiaoli Zhang analyzed data and drafted the initial manuscript.

Mohamed M. S. Gaballa contributed significantly to the histomorphological analysis.

Ahmed A. Hasan, Yvonne Liu, Johann-Georg Hocher, Xin Chen, Dominik Wigger, Jian Li, Bernhard K. Krämer and Burkhard Kleuser critically reviewed the manuscript for important intellectual content.

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## Figures

**Figure 1. Effect of high salt diet on food intake, body weight, blood pressure and blood glucose of rats and intervention effect of ACEI/ARB.** (A): Average daily food intake per animal in each group; (B-D): Comparison of body weight (B), SBP(C) and fasting blood glucose (D) among groups; (E-F): Blood glucose concentrations at different time points (E) and AUC of blood glucose (F) in OGTT. N=6-8 in ND group, n=9-10 in MSD group and n=9-15 in HSD, HSD+TELM and HSD+ENAL groups. The data were presented as mean ± SEM. Comparisons among dietary groups (ND, MSD, HSD) and drug treatment groups (HSD, HSD+TELM, HSD+ENAL) were conducted separately using one-way ANOVA followed by Tukey's post-hoc test. For the AUC of blood glucose data, Levene's test was used to check the equality of variances. **: $p<0.01$ vs. ND group; ** in B and E: $p<0.01$, HSD vs. ND; ##: $p<0.01$ vs. HSD group.
ND: normal diet; MSD: medium-salt diet; HSD: high-salt diet; TELM: telmisartan; ENAL: enalapril; AUC: area under curve; SBP: systolic blood pressure; OGTT: oral glucose tolerance test.

**Figure 2. Effects of high salt diet on pancreas morphology of rats and insulin concentration *in vivo* and *in vitro*.** (A): Representative images of H&E and insulin staining of pancreas tissue in each group (magnification: 200× and scale bar: 50μm); (B): Relative insulin expression in pancreas tissue in each group; (C): Insulin concentration in serum in each group; (D): Fasting glucose/insulin ratio in each group; (E): Insulin concentration in supernatant of MIN6 cells treated by different concentrations of NaCl under the stimulation of low-dose and high-dose glucose. Three replications for each treatment. N=6-10 in ND group, n=7-10 in MSD group and n=11-15 in HSD, HSD+TELM and HSD+ENAL groups. The data were presented as mean ± SEM. Comparisons among dietary groups (ND, MSD, HSD) and drug treatment groups (HSD, HSD+TELM, HSD+ENAL) were conducted separately using one-way ANOVA followed by Tukey's post-hoc test. *: $p<0.05$ vs. ND group; **: $p<0.01$ vs. ND group.
ND: normal diet; MSD: medium-salt diet; HSD: high-salt diet; TELM: telmisartan; ENAL: enalapril.

**Figure 3. High salt diet led to liver inflammation in rats, which could be alleviated by telmisartan treatment.** (A): Representative images of H&E and CD68 staining of liver tissue in each group (magnification: 200× and scale bar: 50μm); (B): Number of inflammatory cells per liver section in H&E images for each group; (C): CD68 positive area per liver section in immunofluorescence images for each group. N=8-9 in ND group, n=8-10 in MSD group and n=8-12 in HSD, HSD+TELM and HSD+ENAL groups. The data were presented as mean ± SEM. Comparisons among dietary groups (ND, MSD, HSD) and drug treatment groups (HSD, HSD+TELM, HSD+ENAL) were conducted separately using one-way ANOVA followed by Tukey's post-hoc test. *: $p<0.05$ vs. ND group; **: $p<0.01$ vs. ND group; #: $p<0.05$ vs. HSD group.

ND: normal diet; MSD: medium-salt diet; HSD: high-salt diet; TELM: telmisartan; ENAL: enalapril.

**Figure 4. High salt diet had an impact on the gene expression profile in the pancreas of rats** (A): Volcano plot of differentially expressed genes in pancreas between HSD group and ND group; (B-C): Expression of differentially expressed genes in pancreas tissues of each group measured by qRT-PCR. N=7 in each group. The data were presented as mean ± SEM. Comparisons among dietary groups (ND, MSD, HSD) and drug treatment groups (HSD, HSD+TELM, HSD+ENAL) were conducted separately using one-way ANOVA followed by Tukey's post-hoc test. (D-E): Correlation analysis between altered genes and fasting glucose/insulin ratio. *: $p<0.05$ vs. ND group.
ND: normal diet; MSD: medium-salt diet; HSD: high-salt diet; TELM: telmisartan; ENAL: enalapril.

**Figure 5. High salt diet had an impact on the gene expression profile in the liver of rats** (A): Volcano plot of differentially expressed genes in liver between HSD group and ND group; (B): Functional analysis of differentially expressed genes by using GO and KEGG; (C-E): Expression of differentially expressed genes in liver tissues of each group measured by qRT-PCR. N=6-7 in each group. The data were presented as mean ± SEM. Comparisons among dietary groups (ND, MSD, HSD) and drug treatment groups (HSD, HSD+TELM, HSD+ENAL) were conducted separately using one-way ANOVA followed by Tukey's post-hoc test. (F-H) Correlation analysis between altered genes and CD68 expression in liver. *: $p<0.05$ vs. ND group; **: $p<0.01$ vs. ND group.

ND: normal diet; MSD: medium-salt diet; HSD: high-salt diet; TELM: telmisartan; ENAL: enalapril.

**Figure 6. High salt diet led to a decreased fatty acid content in the liver of rats.** (A-B): Content of acetic acid (A) and caproic acid (B) in HSD group and ND group. N=5-6 in each group. (C-D): Correlation analysis between Fasn expression and the content of acetic acid (C) and caproic acid (D) in liver. (E): Cluster heatmap of free fatty acids in liver tissues. The relative abundance is indicated by varying colors, with redder colors representing higher levels and bluer colors indicating lower levels. The columns represent samples, the rows represent metabolites. (F): Correlation heatmap of differential metabolites. The vertical and oblique coordinates represent the names of the differential metabolites. The color of the dots indicates the degree of correlation, with red representing positive correlation and blue indicating negative correlation. The deeper the color, the greater the absolute value of the correlation coefficient. Dots of different sizes represent the magnitude of the p value.

ND: normal diet; HSD: high-salt diet.

**Figure 7. High salt diet caused the imbalance of intestinal microbiota of rats.** (A): Venn diagram of OTUs in each group; (B): Comparison of index of alpha-diversity of intestinal microbiota between groups; (C-D): Intestinal microbial composition of the rat in each group, at the phylum (C) and genus (D) levels. (E): Cladograms generated by LEfSe indicating differences in the bacterial taxa of rats between groups; (F): LDA scores for the differentially abundant bacterial taxa between two groups. LDA scores>2.0 are shown. N=6 in each group.

ND: normal diet; HSD: high-salt diet; OTU: operational taxonomic units.

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869 **Figure 8. Correlation analysis between altered microbiome and changed phenotypic parameters.** (A):
870 Top 10 altered microbiomes at genus levels by using Wilcoxon analysis; (B): Correlation analysis between
871 altered microbiome and blood glucose and insulin; (C): Correlation analysis between altered microbiome
872 and short-chain fatty acid.
873 ND: normal diet; HSD: high-salt diet. *: $p<0.05$.

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**Figure 9. Graphical overview: effects of high salt diet on rat physiological traits and molecular mechanisms as revealed in this study.** High salt intake led to increased blood pressure, reduced serum insulin levels, as well as a decline in fasting blood glucose and the AUC for blood glucose. In addition, high salt consumption caused liver inflammation and a decrease in two short-chain fatty acids (acetic acid and caproic acid) and 24 free fatty acids. Furthermore, the study identified the dysregulation of three genes (Fasn, Acly, and Srebf1) in the liver and two genes (Rorc and Scn7a) in the pancreas. Importantly, gut microbiome imbalance caused by high salt diet may contribute to liver inflammation and disrupt glucose homeostasis. Regarding the intervention with ACEI and ARB, treatments with telmisartan and enalapril were effective in mitigating the high blood pressure induced by the high salt diet. Telmisartan also demonstrated potential in reversing liver inflammation caused by the diet. However, neither treatment significantly altered the other detrimental effects induced by the high salt intake.