

## Research Article

# Hydrogen Sulfide Mitigates Kidney Injury in High Fat Diet-Induced Obese Mice

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Obesity is prevalent worldwide and is a major risk factor for the development and progression of kidney disease. Hydrogen sulfide (H<sub>2</sub>S) plays an important role in renal physiological and pathophysiological processes. However, whether H<sub>2</sub>S is able to mitigate kidney injury induced by obesity in mice remains unclear. In this study, we demonstrated that H<sub>2</sub>S significantly reduced the accumulation of lipids in the kidneys of high fat diet- (HFD-) induced obese mice. The results of hematoxylin and eosin, periodic acid-Schiff, and Masson's trichrome staining showed that H<sub>2</sub>S ameliorated the kidney structure, decreased the extent of interstitial injury, and reduced the degree of kidney fibrosis in HFD-induced obese mice. We found that H<sub>2</sub>S decreased the expression levels of tumor necrosis factor- $\alpha$ , interleukin- (IL-) 6, and monocyte chemoattractant protein-1 but increased the expression level of IL-10. Furthermore, H<sub>2</sub>S treatment decreased the protein expression of p50, p65, and p-p65 in the kidney of HFD-induced obese mice. In conclusion, H<sub>2</sub>S is able to mitigate renal injury in HFD-induced obese mice through the reduction of kidney inflammation by downregulating the expression of nuclear factor-kappa B. H<sub>2</sub>S or its releasing compounds may serve as a potential therapeutic molecule for obesity-induced kidney injury.

## 1. Introduction

Obesity, well known as a major public health issue worldwide, has been reported to be associated with many diseases, such as hypertension and type 2 diabetes mellitus [1–3]. Accumulating evidence suggests that obesity is also a major risk factor for the development and progression of chronic kidney disease (CKD) [3–5]. Along with the development of obesity, fast body weight gain increases tubular sodium reabsorption in the kidney, which in turn leads to renal vasodilation and glomerular hyperfiltration and eventually results in an increased glomerular filtration rate. Excess body weight gain may raise blood pressure, which can lead to increased renal blood flow. Consequently, these hemodynamic changes cause an increase of glomerular size accompanied by mesangial matrix expansion and podocyte injury [6–8]. Both hemodynamic and morphological changes, together with other factors, such as renal inflammation [9, 10], oxidative stress [9, 11], lipotoxicity [12, 13], insulin resistance [14], and fibrosis

[15, 16], may result in renal dysfunction and ultimately lead to end-stage renal disease (ESRD).

Hydrogen sulfide (H<sub>2</sub>S) is an endogenous gaseous signaling molecule in mammalian tissues, which is enzymatically synthesized by cystathionine  $\beta$ -synthase (CBS), cystathionine  $\gamma$ -lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST) [17–19]. All three enzymes are abundantly expressed in the kidney and are involved in renal H<sub>2</sub>S production [18, 20]. Accumulating evidence supports the roles of H<sub>2</sub>S in renal physiology and pathology [18–21]. Under physiological conditions, basal renal H<sub>2</sub>S regulates tubular functions and renal hemodynamics, including changes of renal blood flow, natriuresis, kaliuresis, glomerular filtration rate, and urinary excretion [18, 19, 22]. H<sub>2</sub>S can also act as an oxygen sensor in the renal medulla, where its accumulation in hypoxic conditions could restore oxygen balance by increasing medullary blood flow, reducing energy requirements for tubular transport, and directly inhibiting mitochondrial respiration [23, 24]. Because medullary hypoxia is a common pathologic

feature of CKD, H<sub>2</sub>S deficiency may contribute to progression of CKD by limiting the important adaptive mechanism [23, 25].

Under pathological conditions, H<sub>2</sub>S plays a renoprotective role in the kidney. For instance, treatment with NaHS (a donor of H<sub>2</sub>S) could effectively attenuate ischemia/reperfusion-induced acute kidney injury by accelerating tubular cell proliferation and reducing superoxide formation and lipid peroxidation [26]. Importantly, the significant reduction of H<sub>2</sub>S-producing enzymes have been found in a rodent model of kidney injury and ESRD patients [23, 27]. H<sub>2</sub>S supplementation has been shown to reduce macrophage infiltration, glomerulosclerosis, and interstitial fibrosis as well as inhibit the blunt upregulation of adhesion molecules and inflammatory mediators [28]. Plasma H<sub>2</sub>S levels are also reduced in overweight subjects and patients with type 2 diabetes [29]. In obese mice, the biosynthesis of H<sub>2</sub>S was significantly reduced in the kidney after 16 weeks of feeding with a high fat diet [30]. Given the potent antioxidative, cytoprotective, and anti-inflammatory properties of H<sub>2</sub>S, we hypothesize that the application of exogenous H<sub>2</sub>S may protect against obesity-related kidney damage.

To test our hypothesis, a diet-induced obesity mouse model was employed in the present study. We found that the administration of NaHS could significantly improve kidney structure and function and suppress inflammation in the kidney of HFD-induced obese mice. Thus, H<sub>2</sub>S or its releasing compounds may possess therapeutic potential in treating obesity-related kidney disease.

## 2. Materials and Methods

**2.1. Animals.** The protocols for animal experiments were reviewed and approved by the Committee of Medical Ethics and Welfare for Experimental Animals of Henan University School of Medicine. Eight-week-old male C57BL/6JNju mice were purchased from the Nanjing Biomedical Research Institute of Nanjing University (Jiangsu, China). Mice were housed in individual ventilated cages in a temperature- and humidity-controlled environment on a 12-hour light/dark cycle with food and water *ad libitum*. The mice were fed either a low fat diet (LFD, 10% kcal as fat, Mediscience Ltd., Jiangsu, China) or a high fat diet (HFD, 45% kcal as fat, Mediscience Ltd., Jiangsu, China) for a total of 16 weeks. After 12 weeks of feeding, the LFD-fed mice were divided into the LFD group (6 mice) and the LFD + H<sub>2</sub>S group (6 mice); the HFD-fed mice were divided into the HFD group (6 mice) and the HFD + H<sub>2</sub>S group (6 mice). The mice from LFD and HFD groups received an intraperitoneal (i.p.) injection of saline; the mice from the LFD + H<sub>2</sub>S group and HFD + H<sub>2</sub>S group received an i.p. injection of NaHS (50 μmol/kg/day, dissolved in saline) for 4 weeks. At the end of experiments, the mice were killed and the plasma was collected. Tissues were rapidly removed from the mice. The tissues were frozen in liquid nitrogen or embedded in FSC 22 frozen section compound (Leica, Buffalo Grove, IL, USA) or immersed in 4% neutral buffered formalin. Frozen tissues and plasma samples were stored at -80°C.

**2.2. Histological Analysis.** Kidney tissues were fixed in formalin, embedded in paraffin, and cut into 5 μm thick sections. The sections were stained with a hematoxylin and eosin (HE) staining kit (Baibo Biotechnology Co., Ltd., Shandong, China), periodic acid-Schiff (PAS) staining kit (Baso Diagnostics Inc., Guangdong, China), and Masson's trichrome (MT) staining kit (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) according to the manufacturer's protocols. The PAS score is defined as follows: 0 = no deposits of PAS-positive material, 1 = up to one-third, 2 = one-third to two-thirds, and 3 = more than two-thirds of the glomerular cross section stain positive for PAS. Tubulointerstitial injury scores are defined as follows: 0 = no injury, 1 = less than 25%, 2 = 25–50%, 3 = 50–75%, and 4 = more than 75% [31]. Renal interstitial fibrosis (RIF) was scored from 0 to 3 as follows: 0 = absent ( $n_0$ ), 1 = less than 25% of the area ( $n_1$ ), 2 = 25–50% of the area ( $n_2$ ), and 3 = more than 50% of the area ( $n_3$ ). The RIF index was calculated according to the following equation: RIF index =  $(0 \times n_0 + 1 \times n_1 + 2 \times n_2 + 3 \times n_3) / (n_0 + n_1 + n_2 + n_3) \times 100\%$  [32]. All sections were scanned using an Olympus BX51 microscope (Olympus, Tokyo, Japan) and analyzed using Image J software (National Institutes of Health, Bethesda, MD, USA).

**2.3. Biochemical Analysis.** Kidney triglyceride (TG) was measured by enzymatic colorimetric method using a commercial kit according to the manufacturer's protocols (Applygen Technologies Inc., Beijing, China). Serum TG, cystatin C (Cys-C), creatinine (Cre), blood urea nitrogen (BUN), and kidney injury molecule-1 (KIM-1) were measured using a Roche Cobas 8000 automatic biochemical analyzer (Roche Diagnostics, Basel, Switzerland) according to the manufacturer's protocols. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), monocyte chemoattractant protein-1 (MCP-1), and IL-10 were determined using ELISA kits (Wuhan Elabscience Biotechnology Co., Ltd., Hubei, China) according to the manufacturer's protocols.

**2.4. RNA Extraction and RT-PCR.** Total RNA was isolated from kidney tissues using TRIzol reagent (Life Technologies, Rockville, MD, USA), treated with DNase I (Roche Applied Science, Mannheim, Germany), and purified using RNA clean-up kit (Cwbiotech, Beijing, China). One microgram of total RNA was applied for cDNA synthesis using a cDNA reverse transcription kit (Cwbiotech, Beijing, China). Primers were designed according to the primer design principles with Primer Premier 5.0 (Premier Biosoft, Palo Alto, CA, USA): TNF- $\alpha$ , forward 5'-GACGTGGAAGACTGGCA-GAAGAG-3' and reverse 5'-TTGGTGGTTTGTGAGTGT-GAG-3'; IL-6, forward 5'-TAGTCCTTCCCTACCCCAA-TTCC-3' and reverse 5'-TAAGACAATGGATCGGTC-TAC-3'; MCP-1, forward 5'-CAGCCAGATGCAGTTAAC-GC-3' and reverse 5'-GCCTACTCATTGGGATCATCTTG-3'; IL-10, forward 5'-GCTCTTACTGACTGGCATGAG-3' and reverse 5'-CGCAGCTCTAGGAGCATGTG-3'; and 18S rRNA, forward 5'-AGAGTCGGCATCGTTTATGGTC-3' and reverse 5'-CGAAAGCATTGCCAAGAAT-3'. The PCR reactions were performed in a total volume of 20 μL using the following thermal cycling parameters: 95°C for

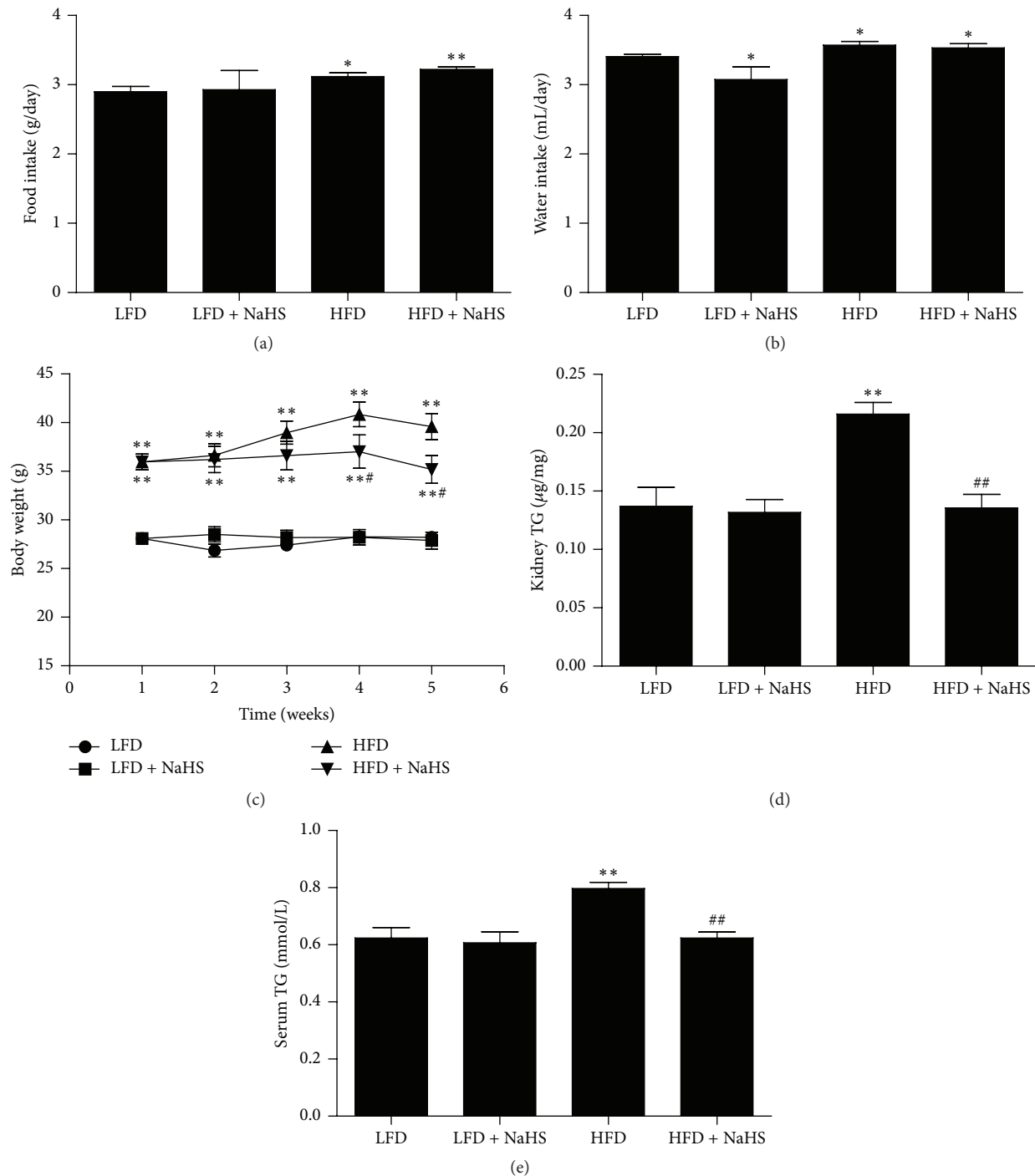


FIGURE 1: Effect of H<sub>2</sub>S on the body weight and TG levels of mice. ((a), (b)) Food intake and water intake were calculated. (c) The body weight of mice. (d) Kidney TG levels of mice. (e) Serum TG levels of mice. Values were presented as mean  $\pm$  SEM ( $n = 6$ ); \* $P < 0.05$ ; \*\* $P < 0.01$  compared with the LFD group; # $P < 0.05$ ; ## $P < 0.01$  compared with the HFD group.

1 min, 40 cycles of 94°C for 45 s, 58°C for 45 s, and 72°C for 1 min. The mRNA expression levels of the test genes were normalized to 18S rRNA levels.

**2.5. Western Blot Analysis.** Kidney tissues were homogenized in RIPA buffer (Sigma Chemical, St. Louis, MO, USA). Protein concentrations of kidney homogenates were measured

with the BCA protein assay kit (Beyotime Institute of Biotechnology, Shanghai, China). The extracted proteins (40  $\mu$ g) were separated on SDS-PAGE gel and transferred to a nitrocellulose membrane. After blocking, the membranes were incubated with anti-TNF- $\alpha$  antibody (Beyotime Institute of Biotechnology, Shanghai, China), antinuclear factor-kappa B (NF- $\kappa$ B), p65 antibody (Wuhan Boster Biotech Co., Ltd.,

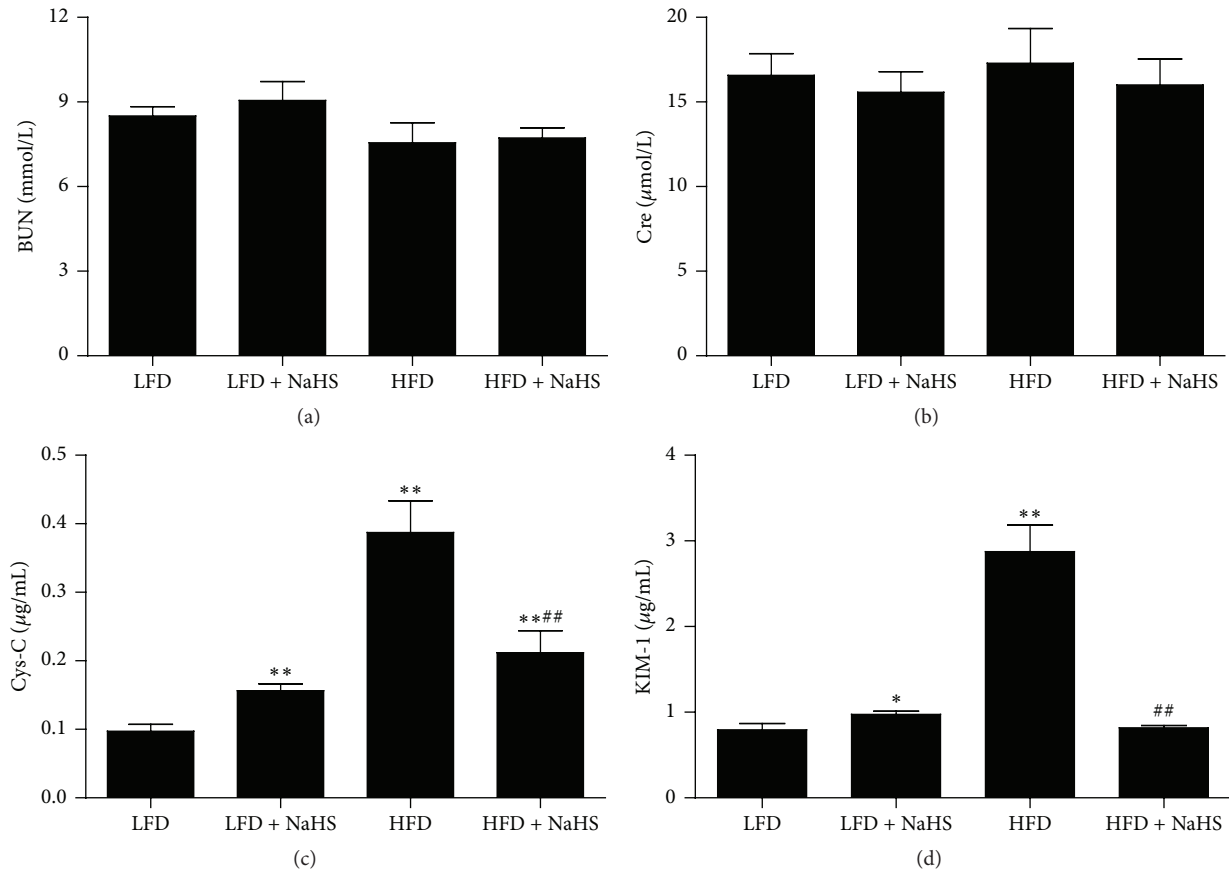


FIGURE 2: Effect of H<sub>2</sub>S on the kidney function of mice. The serum levels of BUN (a), Cre (b), Cys-C (c), and KIM-1 (d) were analyzed in each group of mice. Values were presented as mean  $\pm$  SEM ( $n = 6$ ); \* $P < 0.05$ ; \*\* $P < 0.01$  compared with the LFD group; ## $P < 0.01$  compared with the HFD group.

Hubei, China), anti-NF- $\kappa$ B p50 antibody (Wuhan Boster Biotech Co., Ltd., Hubei, China), anti-phospho-NF- $\kappa$ B p65 (Ser536) antibody (Beyotime Institute of Biotechnology, Shanghai, China), and anti- $\beta$ -actin antibody (Proteintech, Hubei, China). After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibody (Beyotime Institute of Biotechnology, Shanghai, China). The reaction was visualized using an enhanced chemiluminescence system (Thermo Fisher Scientific, Rockford, IL, USA). Immunoblots were quantified by densitometry using Quantity One software (Bio-Rad, CA, USA).

**2.6. Statistical Analysis.** Data were presented as the means  $\pm$  standard error of the mean (SEM). Differences between groups were analyzed by one-way analysis of variance (ANOVA) using SPSS 17.0 software, followed by Tukey's test. A  $P$  value of less than 0.05 was considered to be statistically significant.

### 3. Results

**3.1. H<sub>2</sub>S Reduces Body Weight and TG Levels in HFD-Induced Obese Mice.** As shown in Figures 1(a)–1(c), in comparison with mice fed with LFD, HFD-fed mice exhibited increased food intake, water intake, and body weight. H<sub>2</sub>S treatment

significantly decreased the body weight and TG levels of mice fed HFD, whereas H<sub>2</sub>S treatment did not change the body weight and TG levels of mice fed LFD (Figures 1(c)–1(e)).

**3.2. H<sub>2</sub>S Treatment Improves the Kidney Function in HFD-Induced Obese Mice.** In clinical practice, serum BUN and Cre are the most frequently used markers of kidney function [33, 34]. Cys-C and KIM-1 are considered sensitive and specific biomarkers in early kidney injury [33]. To investigate the effects of HFD and NaHS treatment on kidney function, the plasma levels of BUN, Cre, Cys-C, and KIM-1 in these mice were analyzed using biochemical methods. The results showed that there were no significant differences in plasma BUN and Cre levels between the LFD and LFD + NaHS group (Figures 2(a) and 2(b)). After 12 weeks of HFD feeding followed by 4 weeks of H<sub>2</sub>S treatment, the levels of plasma BUN and Cre were not changed, suggesting that HFD did not cause end-stage kidney injury. However, HFD induced 3- to 4-fold increases in plasma Cys-C and KIM-1 levels (Figures 2(c) and 2(d)), indicating that the kidney injury induced by HFD was in the early stage. After 4 weeks of H<sub>2</sub>S treatment, the plasma Cys-C and KIM-1 levels were significantly reduced, indicating that H<sub>2</sub>S has a therapeutic effect on early kidney injury induced by HFD.



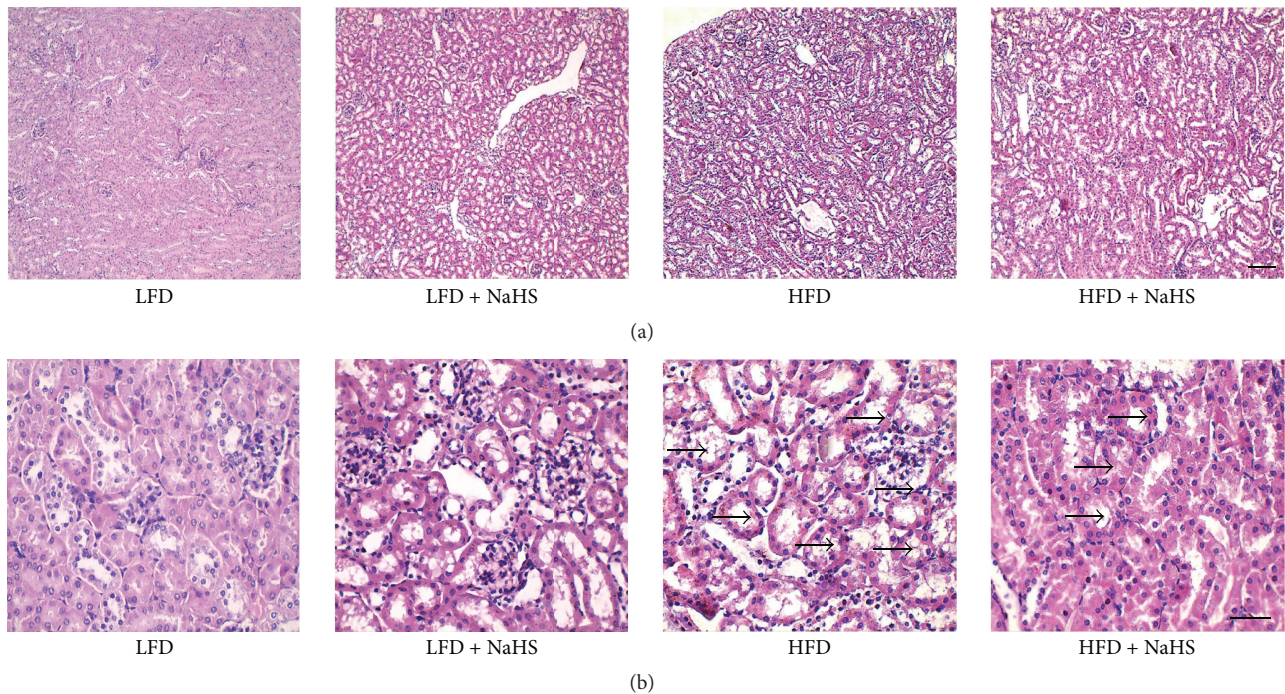


FIGURE 3: Evaluation of the kidney structure of mice using HE staining. Representative photographs of HE-stained kidney sections in each group of mice ( $n = 6/\text{group}$ ). (a) Original magnification,  $\times 100$ ; scale bar =  $200 \mu\text{m}$ . (b) Original magnification,  $\times 400$ ; scale bar =  $20 \mu\text{m}$ .

**3.3.  $\text{H}_2\text{S}$  Ameliorates Kidney Structure in HFD-Induced Obese Mice.** To investigate the effect of  $\text{H}_2\text{S}$  on kidney structure in HFD-induced obese mice, HE, PAS, and MT staining were performed. HE staining demonstrated that the kidney of the LFD control group showed clear tubular and glomerular structures and the tubular endothelial cells presented a good arrangement (Figures 3(a) and 3(b)). Upon HFD, the glomerular volume was increased, glomerular structure was unclear, Bowman's capsule was reduced, and sclerosis was observed in the glomerulus. The tubular endothelial cells swelled and became vacuoles, part of the tubular cavity expanded (Figures 3(a) and 3(b)). After NaHS treatment, the structure of the kidney was significantly improved, the glomerular volume was reduced, Bowman's cavity and the structure of the glomerulus became clear, and the tubular endothelial cells were arranged favorably (Figures 3(a) and 3(b)).

**3.4.  $\text{H}_2\text{S}$  Decreases the Carbohydrate Content and Interstitial Injury in HFD-Induced Obese Mice.** PAS staining can be used to stain structures containing a high proportion of carbohydrate macromolecules, such as glycoprotein, glycogen, and proteoglycans [35]. Compared with the LFD, HFD increased the amount of PAS-positive staining in both glomerular and tubular structures (Figures 4(a)–4(c)), suggesting that the carbohydrate content was increased in the kidney of mice fed HFD. After NaHS treatment, the PAS-positive staining in both glomerular and tubular structures was reduced, demonstrating that NaHS treatment could effectively decrease the carbohydrate content in HFD-induced obese mice. Furthermore, as shown in Figure 4(d), HFD increased the interstitial

injury, while NaHS treatment significantly decreased the interstitial injury.

**3.5.  $\text{H}_2\text{S}$  Ameliorates the Fibrosis of the Kidney in HFD-Induced Obese Mice.** Masson's trichrome is a three-color staining protocol used in histology, which produces red fibers, blue collagen, pink cytoplasm, and dark brown nuclei [32]. In the LFD control group, kidney tubular endothelial cells presented fullness of pink cytoplasm. By HFD, the amounts of blue collagen staining and red fiber staining were increased in kidney glomerular basement membrane and tubular interstitial area (Figure 5(a)), suggesting the accumulation of collagen and connective tissue fibers in the kidney. After NaHS treatment, the amounts of both blue collagen staining and red fiber staining were reduced (Figure 5(a)), and the RIF index was decreased (Figure 5(b)), indicating that  $\text{H}_2\text{S}$  could play a role in ameliorating the fibrosis of kidney.

**3.6. Treatment of NaHS Reduces Kidney Inflammation in HFD-Induced Obese Mice.** Our results demonstrated that HFD induced injuries in kidney structure and function, and the injuries could be ameliorated by the administration of NaHS. To investigate the underlying mechanism, we examined kidney inflammation by analyzing the cytokine levels in the kidney using ELISA techniques. Compared with the LFD, HFD increased the expression levels of  $\text{TNF-}\alpha$ , IL-6, and MCP-1 in the kidney (Figures 6(a)–6(c)). Treatment with NaHS significantly decreased the levels of  $\text{TNF-}\alpha$ , IL-6, and MCP-1 (Figures 6(a)–6(c)), whereas it increased the IL-10 level (Figure 6(d)). The expressions of cytokine genes were further detected by RT-PCR. As shown in Figures 7(a)–7(d),

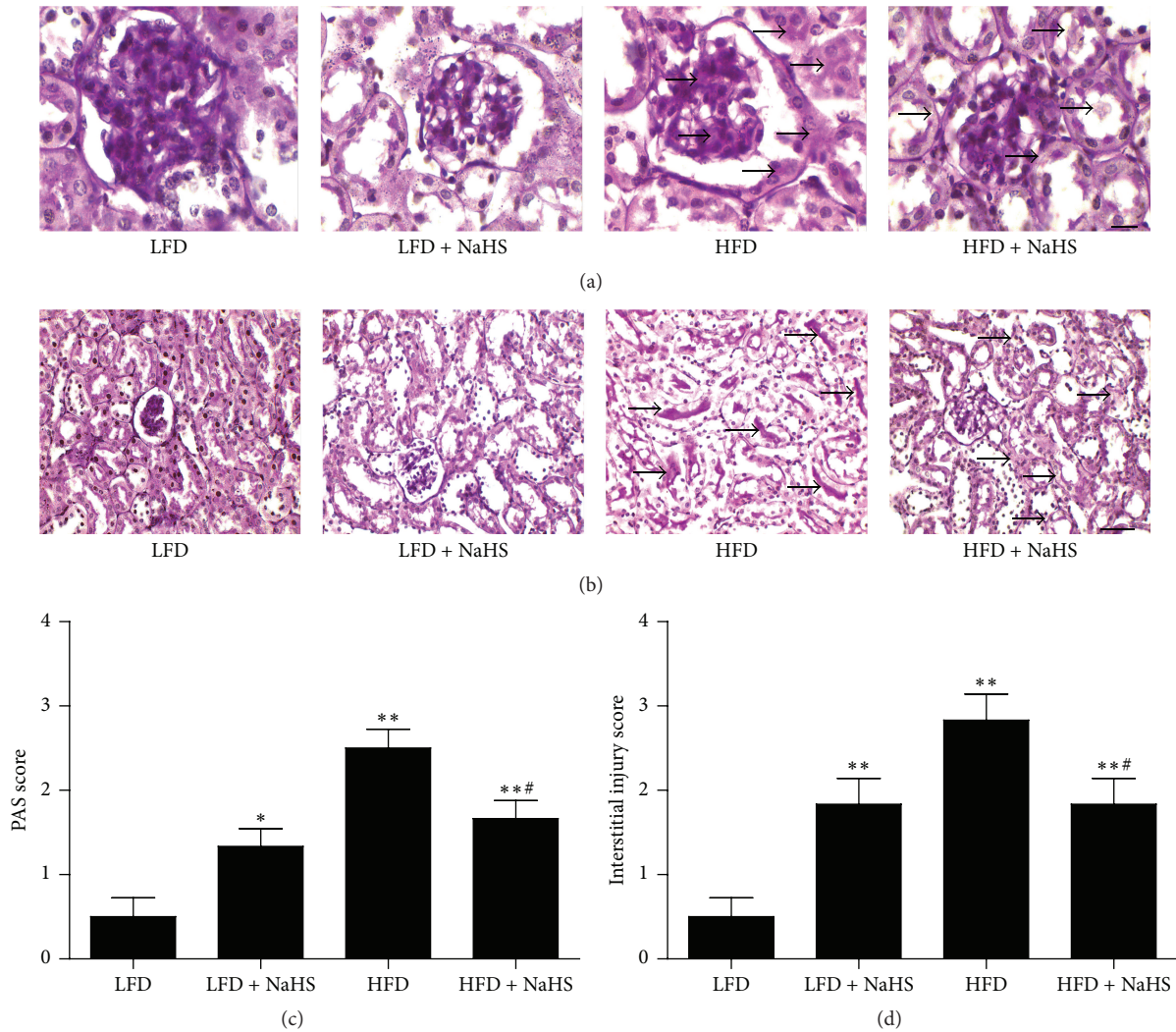


FIGURE 4: Evaluation of carbohydrate content in the kidney of mice using PAS staining. Representative photographs of PAS-stained glomerulus ((a) original magnification,  $\times 1000$ ; scale bar =  $5 \mu\text{m}$ ) and renal tubular ((b) original magnification,  $\times 400$ ; scale bar =  $20 \mu\text{m}$ ). Quantitative analysis of PAS-positive deposits (c) and interstitial injury (d) in each group of mice. Values were presented as mean  $\pm$  SEM ( $n = 6$ ); \* $P < 0.05$ ; \*\* $P < 0.01$  compared with the LFD group; # $P < 0.05$  compared with the HFD group.

compared with the LFD, HFD increased the gene expressions of TNF- $\alpha$ , IL-6, and MCP-1 in the kidney. Treatment of NaHS significantly decreased kidney TNF- $\alpha$ , IL-6, and MCP-1 gene expressions (Figures 7(a)–7(d)), whereas it increased kidney IL-10 expression (Figure 7(e)). These results indicated that H<sub>2</sub>S could reduce the kidney inflammation in HFD-induced obese mice.

**3.7. H<sub>2</sub>S Reduces the Expression of NF- $\kappa$ B in the Kidney of Obese Mice.** To investigate the mechanisms of NaHS on cytokine regulation, the expression level of NF- $\kappa$ B was detected. NF- $\kappa$ B is a transcription factor that plays a key role in the regulation of the expression of multiple cytokine genes involved in a variety of physiological processes [36]. The p50/p65 heterodimer is the most abundant and well understood of the NF- $\kappa$ B dimers in most cells [37, 38]. We examined the protein expressions of p50, p65, and p-p65 in the kidneys of mice. As shown in Figures 8(a)–8(e), compared

with the LFD, HFD increased kidney p50, p65, and p-p65 protein expressions and the p-p65/p65 ratio. Treatment with NaHS significantly decreased the protein expressions of p50, p65, and p-p65, as well as the ratio of p-p65/p65 in the kidney (Figures 8(a)–8(e)). The results indicated that HFD increased kidney inflammation through the upregulation of NF- $\kappa$ B expression, and treatment with NaHS effectively reduced kidney inflammation by downregulating NF- $\kappa$ B expression.

## 4. Discussion

H<sub>2</sub>S has been widely considered to be an endogenous gaseous signaling molecule, along with carbon monoxide and nitric oxide [17, 20, 21, 39]. Recently, an increasing number of studies suggest that H<sub>2</sub>S plays important and complex roles in renal physiology and pathophysiology, including the regulation of baseline hemodynamics and tubular properties [18, 19, 22], action as an oxygen sensor/transducer [28, 40],



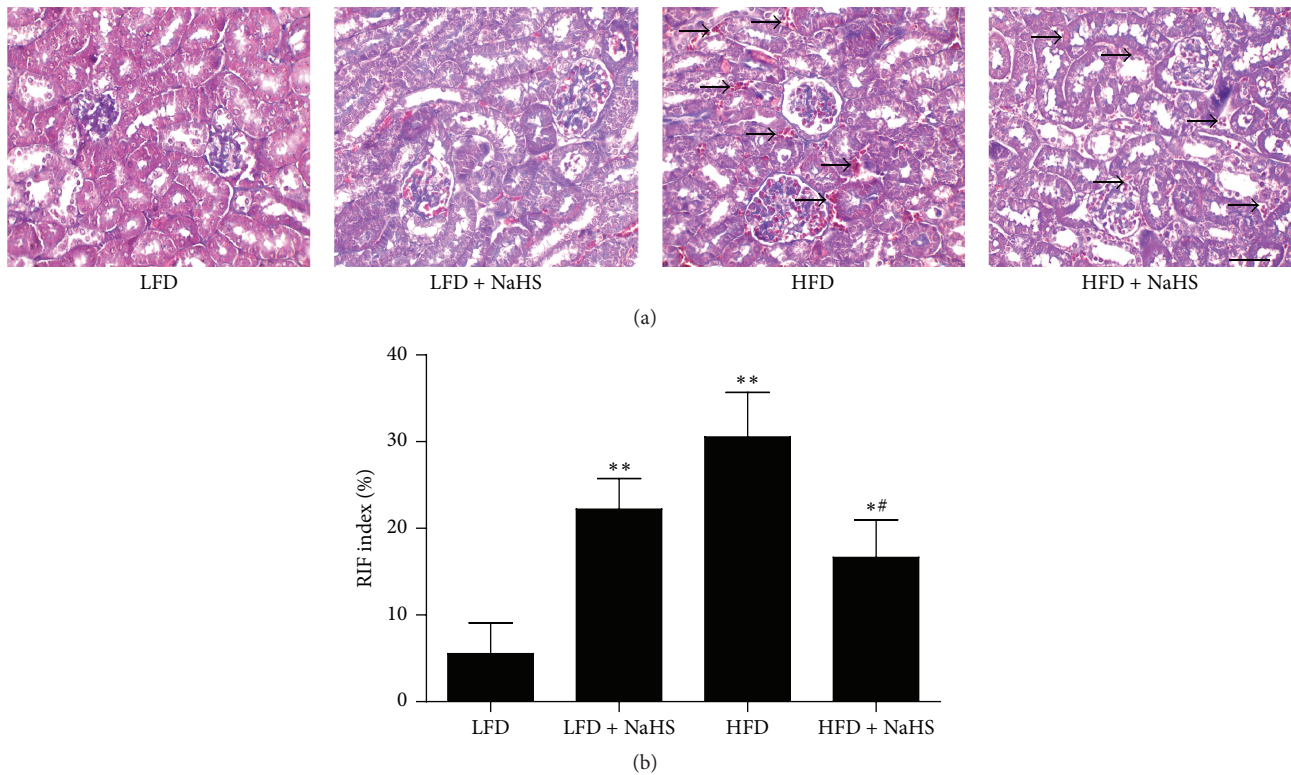


FIGURE 5: Evaluation of renal collagen deposition using MT staining (blue: collagen, pink: cytoplasm, and brown-black: nuclei). (a) Representative samples of MT staining (original magnification,  $\times 400$ ; scale bar =  $20 \mu\text{m}$ ) in each group of mice. (b) The extent of the renal lesions is represented by the RIF index. Values were presented as mean  $\pm$  SEM ( $n = 6$ ); \* $P < 0.05$ ; \*\* $P < 0.01$  compared with the LFD group; # $P < 0.05$  compared with the HFD group.

protection against renal I/R injury [21, 26, 41], attenuation of renal fibrosis [18, 42], and protection against glomerulosclerosis [28, 43]. Recent studies indicate that the  $\text{H}_2\text{S}$  level was significantly reduced in overweight patients with type 2 diabetes and HFD-induced obese mice [29, 30]. In addition, there is increasing evidence that the obesity epidemic has coincided with an increased incidence of CKD [3, 8]. Therefore, we speculate that  $\text{H}_2\text{S}$  deficiency may potentially contribute to the progression of CKD and the administration of exogenous  $\text{H}_2\text{S}$  could mitigate CKD induced by obesity.

In the current study, we demonstrated that  $\text{H}_2\text{S}$  significantly reduced the body weight and kidney TG levels of mice fed with HFD, suggesting that  $\text{H}_2\text{S}$  plays an important role in lipid metabolism. However, the precise molecular mechanisms behind the role of  $\text{H}_2\text{S}$  in the regulation of lipid metabolism need to be further investigated. A recent study showed that intrarenal arterial infusion of NaHS increased glomerular filtration rate, renal blood flow, urinary sodium, and potassium excretion, indicating that  $\text{H}_2\text{S}$  is involved in the control of renal function [22]. Our data indicated that the kidney injury induced by HFD was in the early stage and  $\text{H}_2\text{S}$  could maintain normal renal function by reducing the plasma levels of specific biomarkers in early kidney injury, such as Cys-C and KIM-1.

HE staining is the most common method used in the anatomic pathology diagnosis [44]. Our results showed

that HFD increased the glomerular volume, reduced the Bowman's capsule, and resulted in glomerulosclerosis, suggesting that HFD could successfully induce renal injury. After treatment with  $\text{H}_2\text{S}$ , the structure of the kidney was significantly improved in HFD-induced obese mice. It has been reported that the glomerular tuft area and the mesangial matrix area in mice fed with HFD tended to be larger than that in mice fed with LFD [45]. Similarly, we found that HFD increased the amount of PAS-positive staining in both glomerular and tubular structures, while  $\text{H}_2\text{S}$  significantly reduced the PAS-positive staining in the kidney. Furthermore,  $\text{H}_2\text{S}$  could decrease the interstitial injury in HFD-induced obese mice. A recent study revealed that  $\text{H}_2\text{S}$  exhibited potent antifibrotic effects on obstructed nephropathy and inhibited the proliferation and differentiation of renal fibroblasts both *in vitro* and *in vivo* [18]. Our results indicated that HFD increased the red fiber staining in the glomerular basement membrane and tubular interstitial area and  $\text{H}_2\text{S}$  effectively reduced the degree of renal fibrosis.

Obesity-related nephropathy is associated with regenerative cell proliferation, monocyte infiltration, and increased renal expressions of systemic proinflammatory  $\text{TNF-}\alpha$ , IL-6, and MCP-1 [1, 9]. Similarly, our data indicated that HFD increased the expression levels of  $\text{TNF-}\alpha$ , IL-6, and MCP-1 in the kidney. The administration of NaHS significantly decreased the expression levels of these

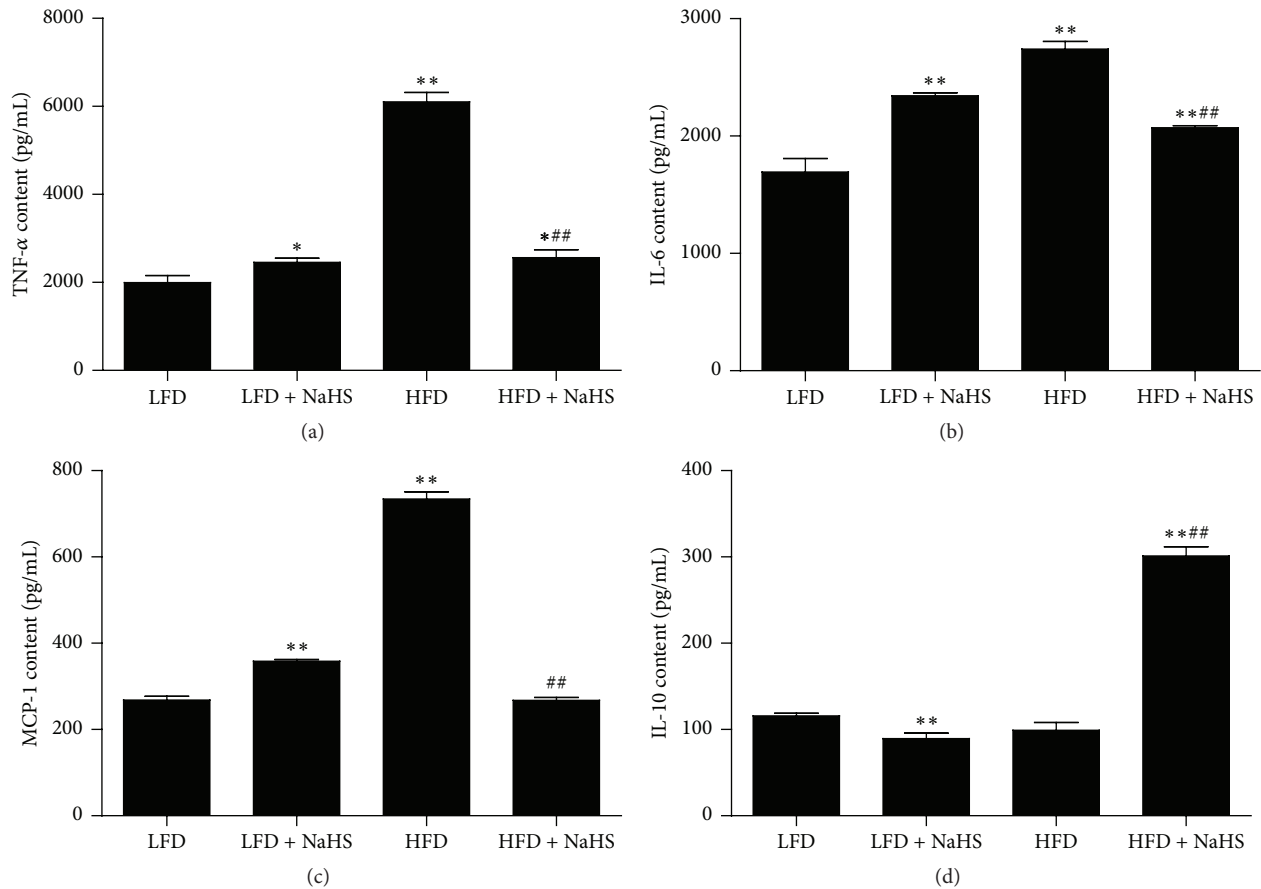


FIGURE 6: Effect of  $H_2S$  on the cytokine levels in the kidney of mice was assayed using ELISA techniques. The expression levels of TNF- $\alpha$  (a), IL-6 (b), MCP-1 (c), and IL-10 (d) were analyzed. Values were presented as mean  $\pm$  SEM ( $n = 6$ ); \* $P < 0.05$ ; \*\* $P < 0.01$  compared with the LFD group; ## $P < 0.01$  compared with the HFD group.

proinflammatory cytokines. IL-10 is a Th<sub>2</sub>-type cytokine that is produced by a number of immunological cell types, including macrophages/monocytes, and it is a potent inhibitor of proinflammatory cytokines and chemokines [46, 47]. Notably, we found increased expression levels of IL-10. Furthermore, the gene expression of TNF- $\alpha$ , IL-6, MCP-1, and IL-10 exhibited similar changes. Thus, when the TNF- $\alpha$ /IL-6/MCP-1 results were contrasted with the IL-10 results, it appeared that the relative balance between proinflammatory and anti-inflammatory was “tipped” toward a proinflammatory state. The proinflammatory state played a mechanistic role in the progression of kidney injury. In contrast, treatment with  $H_2S$  resulted in an anti-inflammatory state, which played an inhibitory role in the progression of kidney injury. However, it should also be noted that the administration of  $H_2S$  increased the expression levels of TNF- $\alpha$ , IL-6, and MCP-1 and decreased the expression levels of IL-10 in LFD-fed mice. Furthermore,  $H_2S$  increased the plasma levels of Cys-C and KIM-1 and enhanced the interstitial injury and fibrosis of the kidney in LFD-fed mice, which may be attributed to the opposite effect of  $H_2S$  on inflammation [20, 48, 49]. Based on these studies, we hypothesize that  $H_2S$  is able to exhibit an anti-inflammatory effect in HFD-fed mice and exert a proinflammatory effect in LFD-fed mice.

The NF- $\kappa$ B network plays a crucial role in human health, and aberrant NF- $\kappa$ B activation contributes to the development of a wide range of autoimmune, inflammatory, and malignant disorders, including atherosclerosis, multiple sclerosis, rheumatoid arthritis, inflammatory bowel diseases, and malignant tumors [36, 38, 50]. The NF- $\kappa$ B family comprises five major members: c-Rel, p50, p52, p65 (Rel A), and Rel B [36, 38]. The p50/p65 heterodimer is the most important transcription factor of the canonical NF- $\kappa$ B pathway and is commonly referred to as NF- $\kappa$ B [38, 51]. A recent study demonstrated that high body adiposity could induce an inflammatory and proliferative microenvironment in the rat kidney [1]. Similarly, our data showed that HFD increased kidney p50, p65, and p-p65 protein expressions and the p-p65/p65 ratio, suggesting that HFD induced an inflammatory microenvironment in the kidneys of mice. However, the administration of  $H_2S$  significantly decreased the protein expressions of p50, p65, and p-p65, as well as the ratio of p-p65/p65 in the kidney. These results indicate that  $H_2S$  could reduce kidney inflammation by downregulating NF- $\kappa$ B expression.

In conclusion, our results suggest that  $H_2S$  is able to reduce kidney lipids, improve kidney function, and reduce the interstitial injury and fibrosis of the kidney through the

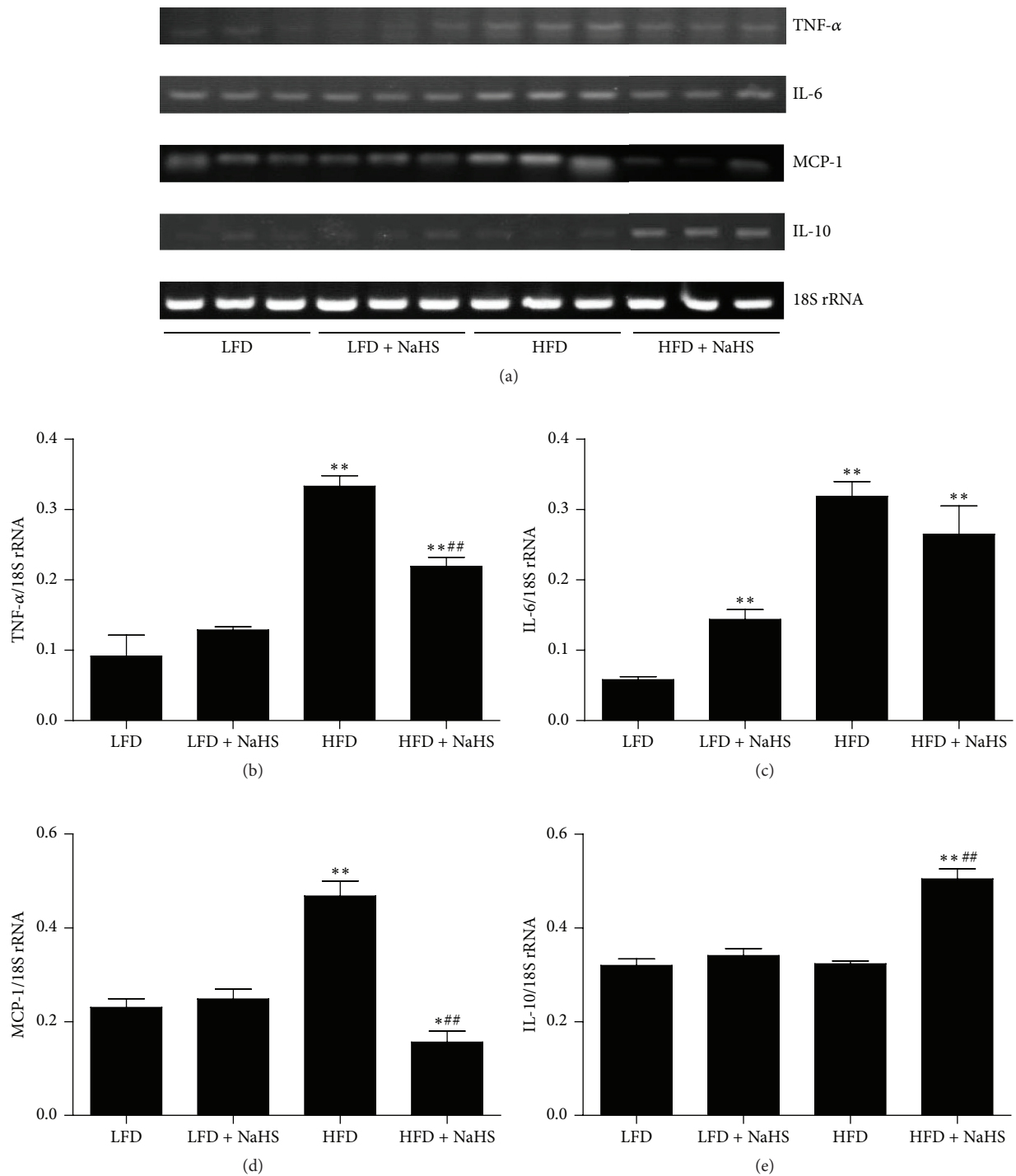


FIGURE 7: Effect of  $H_2S$  on the cytokine levels in the kidney of mice was assayed using RT-PCR techniques. (a) The expression levels of TNF- $\alpha$ , IL-6, MCP-1, and IL-10 were measured by RT-PCR. 18S rRNA was used as an internal control. Bar graphs showed the quantification of TNF- $\alpha$  (b), IL-6 (c), MCP-1 (d), and IL-10 (e). Values were presented as mean  $\pm$  SEM ( $n = 3$ ); \* $P < 0.05$ ; \*\* $P < 0.01$  compared with the LFD group; \*\*\* $P < 0.01$  compared with the HFD group.



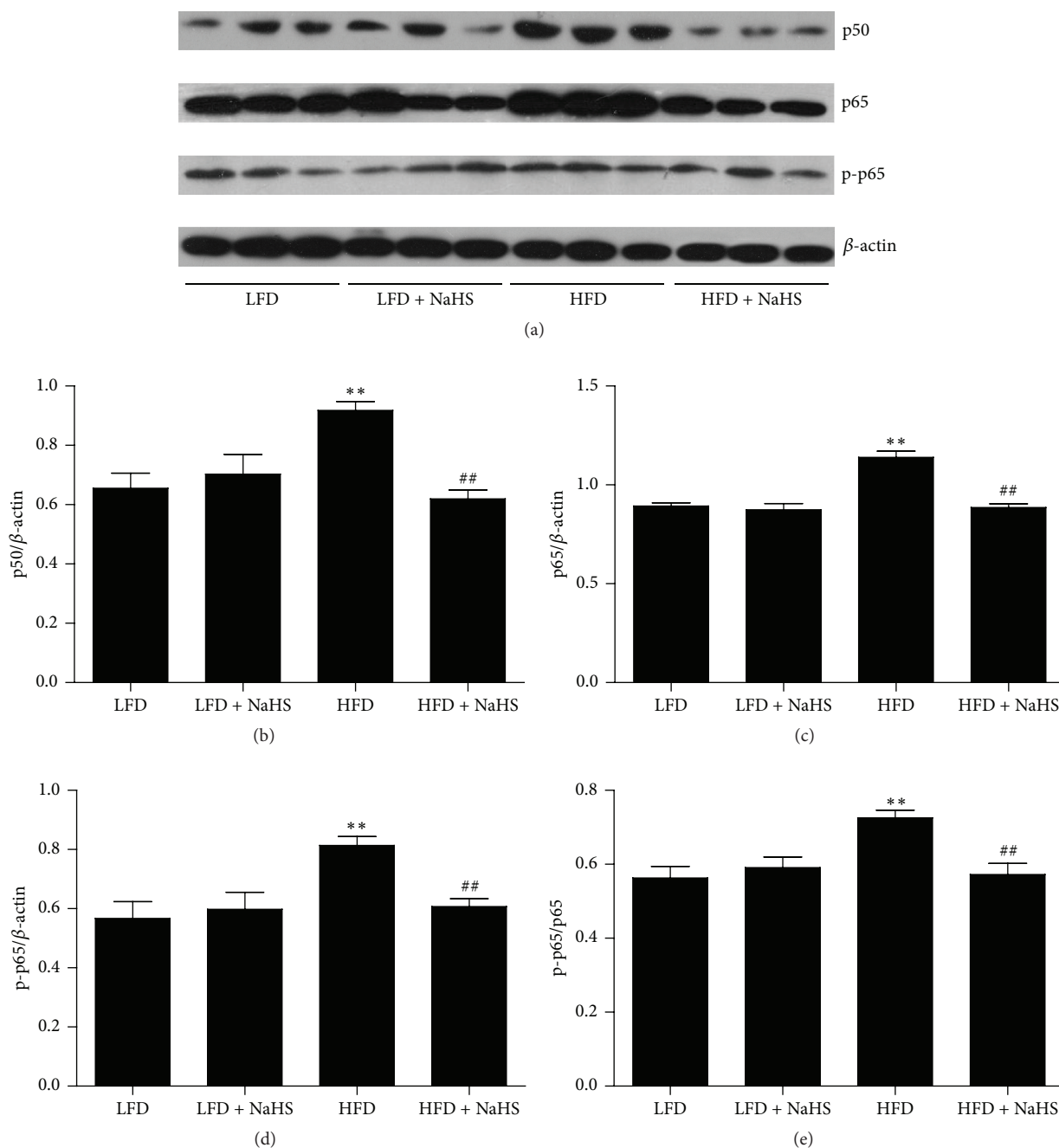


FIGURE 8: Effect of H<sub>2</sub>S on the protein level of NF- $\kappa$ B in the kidney of mice. (a) The expression levels of p50, p65, and p-p65 were measured by Western blot.  $\beta$ -actin was used as an internal control. Bar graphs showed the quantification of p50 (b), p65 (c), p-p65 (d), and p-p65/p65 (e). Values were presented as mean  $\pm$  SEM ( $n = 3$ ); \*\*  $P < 0.01$  compared with the LFD group; ##  $P < 0.01$  compared with the HFD group.

reduction of kidney inflammation by downregulating NF- $\kappa$ B expression. Therefore, H<sub>2</sub>S or its releasing compounds may serve as a potential therapeutic molecule for obesity-induced kidney injury.

### Competing Interests

The authors declare that they have no competing interests related to this work.

### Authors' Contributions

Dongdong Wu and Biao Gao contributed equally to this work.

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## References

- [1] K. Stemmer, D. Perez-Tilve, G. Ananthakrishnan et al., “High-fat-diet-induced obesity causes an inflammatory and tumor-promoting microenvironment in the rat kidney,” *Disease Models and Mechanisms*, vol. 5, no. 5, pp. 627–635, 2012.
- [2] K. Sharma, “Obesity, oxidative stress, and fibrosis in chronic kidney disease,” *Kidney International Supplements*, vol. 4, no. 1, pp. 113–117, 2014.
- [3] E. Börgeson and K. Sharma, “Obesity, immunomodulation and chronic kidney disease,” *Current Opinion in Pharmacology*, vol. 13, no. 4, pp. 618–624, 2013.
- [4] M. A. Reddy and R. Natarajan, “Recent developments in epigenetics of acute and chronic kidney diseases,” *Kidney International*, vol. 88, no. 2, pp. 250–261, 2015.
- [5] J. F. O’Toole and J. R. Sedor, “Kidney disease: new technologies translate mechanisms to cure,” *The Journal of Clinical Investigation*, vol. 124, no. 6, pp. 2294–2298, 2014.
- [6] E. Ritz and N. Koleganova, “Obesity and chronic kidney disease,” *Seminars in Nephrology*, vol. 29, no. 5, pp. 504–511, 2009.
- [7] A.-E. Declèves and K. Sharma, “Obesity and kidney disease: differential effects of obesity on adipose tissue and kidney inflammation and fibrosis,” *Current Opinion in Nephrology and Hypertension*, vol. 24, no. 1, pp. 28–36, 2015.
- [8] R. Nistala, J. Habibi, G. Lastra et al., “Prevention of obesity-induced renal injury in male mice by DPP4 inhibition,” *Endocrinology*, vol. 155, no. 6, pp. 2266–2276, 2014.
- [9] S. Ruiz, P. E. Pergola, R. A. Zager, and N. D. Vaziri, “Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease,” *Kidney International*, vol. 83, no. 6, pp. 1029–1041, 2013.
- [10] S. K. Sinha, M. Shaheen, T. B. Rajavashisth, D. Pan, K. C. Norris, and S. B. Nicholas, “Association of race/ethnicity, inflammation, and albuminuria in patients with diabetes and early chronic kidney disease,” *Diabetes Care*, vol. 37, no. 4, pp. 1060–1068, 2014.
- [11] J. P. Kooman, P. Kotanko, A. M. W. J. Schols, P. G. Shiels, and P. Stenvinkel, “Chronic kidney disease and premature ageing,” *Nature Reviews Nephrology*, vol. 10, no. 12, pp. 732–742, 2014.
- [12] R. A. Zager, A. C. M. Johnson, and K. Becker, “Acute unilateral ischemic renal injury induces progressive renal inflammation, lipid accumulation, histone modification, and ‘end-stage’ kidney disease,” *American Journal of Physiology—Renal Physiology*, vol. 301, no. 6, pp. F1334–F1345, 2011.
- [13] J. M. Weinberg, “Lipotoxicity,” *Kidney International*, vol. 70, no. 9, pp. 1560–1566, 2006.
- [14] S. S. Thomas, L. Zhang, and W. E. Mitch, “Molecular mechanisms of insulin resistance in chronic kidney disease,” *Kidney International*, 2015.
- [15] M. G. Wong and C. A. Pollock, “Biomarkers in kidney fibrosis: are they useful?” *Kidney International Supplements*, vol. 4, no. 1, pp. 79–83, 2014.
- [16] B. D. Humphreys, F. Xu, V. Sabbisetti et al., “Chronic epithelial kidney injury molecule-1 expression causes murine kidney fibrosis,” *The Journal of Clinical Investigation*, vol. 123, no. 9, pp. 4023–4035, 2013.
- [17] C. Szabo, C. Coletta, C. Chao et al., “Tumor-derived hydrogen sulfide, produced by cystathionine- $\beta$ -synthase, stimulates bioenergetics, cell proliferation, and angiogenesis in colon cancer,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 110, no. 30, pp. 12474–12479, 2013.
- [18] K. Song, F. Wang, Q. Li et al., “Hydrogen sulfide inhibits the renal fibrosis of obstructive nephropathy,” *Kidney International*, vol. 85, no. 6, pp. 1318–1329, 2014.
- [19] B. S. Kasinath, “Hydrogen sulfide to the rescue in obstructive kidney injury,” *Kidney International*, vol. 85, no. 6, pp. 1255–1258, 2014.
- [20] R. Wang, “Physiological implications of hydrogen sulfide: a whiff exploration that blossomed,” *Physiological Reviews*, vol. 92, no. 2, pp. 791–896, 2012.
- [21] E. M. Bos, R. Wang, P. M. Snijder et al., “Cystathionine  $\gamma$ -lyase protects against renal ischemia/reperfusion by modulating oxidative stress,” *Journal of the American Society of Nephrology*, vol. 24, no. 5, pp. 759–770, 2013.
- [22] M. Xia, L. Chen, R. W. Muh, P.-L. Li, and N. Li, “Production and actions of hydrogen sulfide, a novel gaseous bioactive substance, in the kidneys,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 329, no. 3, pp. 1056–1062, 2009.
- [23] M. A. Aminzadeh and N. D. Vaziri, “Downregulation of the renal and hepatic hydrogen sulfide ( $H_2S$ )-producing enzymes and capacity in chronic kidney disease,” *Nephrology Dialysis Transplantation*, vol. 27, no. 2, pp. 498–504, 2012.
- [24] J. Beltowski, “Hypoxia in the renal medulla: Implications for hydrogen sulfide signaling,” *Journal of Pharmacology and Experimental Therapeutics*, vol. 334, no. 2, pp. 358–363, 2010.
- [25] T. Tanaka and M. Nangaku, “The role of hypoxia, increased oxygen consumption, and hypoxia-inducible factor-1 alpha in progression of chronic kidney disease,” *Current Opinion in Nephrology and Hypertension*, vol. 19, no. 1, pp. 43–50, 2010.
- [26] S. J. Han, J. I. Kim, J.-W. Park, and K. M. Park, “Hydrogen sulfide accelerates the recovery of kidney tubules after renal ischemia/reperfusion injury,” *Nephrology Dialysis Transplantation*, vol. 30, no. 9, pp. 1497–1506, 2015.
- [27] Z. Xu, G. Prathapasinghe, N. Wu, S.-Y. Hwang, Y. L. Siow, and O. Karmin, “Ischemia-reperfusion reduces cystathionine- $\beta$ -synthase-mediated hydrogen sulfide generation in the kidney,” *American Journal of Physiology—Renal Physiology*, vol. 297, no. 1, pp. F27–F35, 2009.
- [28] I. Lobb, E. Sonke, G. Aboalsamh, and A. Sener, “Hydrogen sulphide and the kidney: important roles in renal physiology and pathogenesis and treatment of kidney injury and disease,” *Nitric Oxide*, vol. 46, pp. 55–65, 2015.
- [29] M. Whiteman, K. M. Gooding, J. L. Whatmore et al., “Adiposity is a major determinant of plasma levels of the novel vasodilator hydrogen sulphide,” *Diabetologia*, vol. 53, no. 8, pp. 1722–1726, 2010.
- [30] M. T. Peh, A. B. Anwar, D. S. W. Ng, M. Shirhan Bin Mohamed Atan, S. D. Kumar, and P. K. Moore, “Effect of feeding a high fat diet on hydrogen sulfide ( $H_2S$ ) metabolism in the mouse,” *Nitric Oxide—Biology and Chemistry*, vol. 41, pp. 138–145, 2014.
- [31] Q. Zhang, H. Luan, L. Wang et al., “Galectin-9 ameliorates anti-GBM glomerulonephritis by inhibiting th1 and th17 immune responses in mice,” *American Journal of Physiology—Renal Physiology*, vol. 306, no. 8, pp. F822–F832, 2014.
- [32] T.-B. Zhou, C. Ou, Y.-H. Qin, F.-Y. Lei, W.-F. Huang, and G. P. C. Drummen, “LIM homeobox transcription factor 1B expression affects renal interstitial fibrosis and apoptosis in unilateral

- ureteral obstructed rats,” *American Journal of Physiology—Renal Physiology*, vol. 306, no. 12, pp. F1477–F1488, 2014.
- [33] O. Arun, G. Celik, B. Oc et al., “Renal effects of coronary artery bypass graft surgery in diabetic and non-diabetic patients: a study with urinary neutrophil gelatinase-associated lipocalin and serum Cystatin C,” *Kidney and Blood Pressure Research*, vol. 40, no. 2, pp. 141–152, 2015.
- [34] Y. Niu, L. Na, R. Feng et al., “The phytochemical, EGCG, extends lifespan by reducing liver and kidney function damage and improving age-associated inflammation and oxidative stress in healthy rats,” *Aging Cell*, vol. 12, no. 6, pp. 1041–1049, 2013.
- [35] S. H. Lam, C. Y. Ung, M. M. Hlaing et al., “Molecular insights into 4-nitrophenol-induced hepatotoxicity in zebrafish: transcriptomic, histological and targeted gene expression analyses,” *Biochimica et Biophysica Acta—General Subjects*, vol. 1830, no. 10, pp. 4778–4789, 2013.
- [36] S. Nakajima and M. Kitamura, “Bidirectional regulation of NF- $\kappa$ B by reactive oxygen species: a role of unfolded protein response,” *Free Radical Biology and Medicine*, vol. 65, pp. 162–174, 2013.
- [37] C. K. Shrum, D. Defrancisco, and M. K. Meffert, “Stimulated nuclear translocation of NF- $\kappa$ B and shuttling differentially depend on dynein and the dynactin complex,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 8, pp. 2647–2652, 2009.
- [38] W. Yan, R. Li, J. He, J. Du, and J. Hou, “Importin  $\beta$ 1 mediates nuclear factor- $\kappa$ B signal transduction into the nuclei of myeloma cells and affects their proliferation and apoptosis,” *Cellular Signalling*, vol. 27, no. 4, pp. 851–859, 2015.
- [39] D. Wu, W. Si, M. Wang, S. Lv, A. Ji, and Y. Li, “Hydrogen sulfide in cancer: friend or foe?” *Nitric Oxide*, vol. 50, pp. 38–45, 2015.
- [40] K. R. Olson, “Hydrogen sulfide as an oxygen sensor,” *Antioxidants & Redox Signaling*, vol. 22, no. 5, pp. 377–397, 2015.
- [41] D. Wu, J. Wang, H. Li, M. Xue, A. Ji, and Y. Li, “Role of hydrogen sulfide in ischemia-reperfusion injury,” *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 186908, 16 pages, 2015.
- [42] K. Song, Q. Li, X.-Y. Yin, Y. Lu, C.-F. Liu, and L.-F. Hu, “Hydrogen sulfide: a therapeutic candidate for fibrotic disease?” *Oxidative Medicine and Cellular Longevity*, vol. 2015, Article ID 458720, 10 pages, 2015.
- [43] U. Sen, C. Munjal, N. Qipshidze, O. Abe, R. Gargoum, and S. C. Tyagi, “Hydrogen sulfide regulates homocysteine-mediated glomerulosclerosis,” *American Journal of Nephrology*, vol. 31, no. 5, pp. 442–455, 2010.
- [44] B. D. Llewellyn, “Nuclear staining with alum hematoxylin,” *Biotechnic and Histochemistry*, vol. 84, no. 4, pp. 159–177, 2009.
- [45] N. Deji, S. Kume, S.-I. Araki et al., “Structural and functional changes in the kidneys of high-fat diet-induced obese mice,” *American Journal of Physiology—Renal Physiology*, vol. 296, no. 1, pp. F118–F126, 2009.
- [46] E.-G. Hong, H. J. Ko, Y.-R. Cho et al., “Interleukin-10 prevents diet-induced insulin resistance by attenuating macrophage and cytokine response in skeletal muscle,” *Diabetes*, vol. 58, no. 11, pp. 2525–2535, 2009.
- [47] K. Gotoh, M. Inoue, T. Masaki et al., “A novel anti-inflammatory role for spleen-derived interleukin-10 in obesity-induced inflammation in white adipose tissue and liver,” *Diabetes*, vol. 61, no. 8, pp. 1994–2003, 2012.
- [48] K. L. Flannigan, T. A. Agbor, R. W. Blackler et al., “Impaired hydrogen sulfide synthesis and IL-10 signaling underlie hyperhomocysteinemia-associated exacerbation of colitis,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 111, no. 37, pp. 13559–13564, 2014.
- [49] D. R. Linden, “Hydrogen sulfide signaling in the gastrointestinal tract,” *Antioxidants and Redox Signaling*, vol. 20, no. 5, pp. 818–830, 2014.
- [50] A. Ma and B. A. Malynn, “A20: linking a complex regulator of ubiquitylation to immunity and human disease,” *Nature Reviews Immunology*, vol. 12, no. 11, pp. 774–785, 2012.
- [51] M. Zhuang, M. Zhao, H. Qiu et al., “Effusarin E suppresses nasopharyngeal carcinoma cell growth by inhibiting NF- $\kappa$ B and COX-2 signaling,” *PLoS ONE*, vol. 9, no. 10, Article ID e109951, 2014.