

Choline Induced Cardiac Dysfunction by Inhibiting the Production of Endogenous Hydrogen Sulfide in Spontaneously Hypertensive Rats

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Summary

To investigate the exact effects of dietary choline on hypertensive heart disease (HHD) and explore the potential mechanisms, male spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY) were randomly divided into five groups as follows: WKY group, WKY + Choline group, SHR group, SHR + Choline group, and SHR + Choline + NaHS group. In choline treatment groups, rats were fed with 1.3 % (w/v) choline in the drinking water for 3 months. The rats in the SHR + Choline + NaHS group were intraperitoneally injected with NaHS (100 μ mol/kg/day, a hydrogen sulfide (H₂S) donor) for 3 months. After 3 months, left ventricular ejection fraction (LVEF) and fractional shortening (LVFS), the indicators of cardiac function measured by echocardiography, were increased significantly in SHR as compared to WKY, although there was no significant difference in collagen volumes and Bax/Bcl-2 ratio between the two groups, indicating the early stage of cardiac hypertrophy. There was a significant decrease in LVEF and LVFS and an increase in collagen volumes and Bax/Bcl-2 ratio in SHR fed with choline, meanwhile, plasma H₂S levels were significantly decreased significantly in SHR fed with choline accompanying by the decrease of cystathionine- γ -lyase (CSE) activity. Three months of NaHS significantly increased plasma H₂S levels, ameliorated cardiac dysfunction and inhibited cardiac fibrosis and apoptosis in SHR fed with choline. In conclusion, choline aggravated cardiac dysfunction in HHD through inhibiting the production of endogenous H₂S, which was reversed by supplementation of exogenous H₂S donor.

Keywords

Hypertensive heart disease • Choline • Hydrogen sulfide • Cardiac dysfunction

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Introduction

Hypertension is a major risk factor for the development of cardiovascular diseases and remains one of the most important public health problems worldwide. If left untreated, hypertension can lead to an array of long-term end-organ diseases and premature death [1]. As an important target organ, long-lasting pressure overload promotes cardiac hypertrophy and pathologic structural remodeling resulting in hypertensive heart disease (HHD), which ultimately leads to heart failure (HF) [2]. It is reported that several pathophysiologic factors play important roles in HHD, including abnormality of hemodynamic mechanisms, longstanding stimulation of neurohormonal pathways and inappropriate activation of inflammation [3-5]. However, the exact mechanisms are not fully understood.

In recently, gut microbiota has been found to contribute to pathogenesis of hypertension and its end-organ diseases through production of a variety of microbial-derived bioactive metabolites [6-7]. As an essential nutrient for humans, much attention has been given to the potential role of choline and its metabolites in cardiovascular disease. Some studies suggested that

choline exhibited cardioprotective effect against several heart diseases including myocardial infarction, ischemia/reperfusion injury, cardiac hypertrophy and HF [8-10]. On the other hand, it was also shown that choline and its metabolites were associated with HF and had a higher risk of heart diseases [11-12]. In addition, basic study has further confirmed that choline diet and its microbial-derived metabolites, trimethylamine N-oxide (TMAO), aggravated pressure overload-induced HF [13]. Up to now, the connection between dietary choline and HHD was unclear, not to mention the mechanisms involved. As a newly discovered gasotransmitter, hydrogen sulfide (H₂S) has been found to be endogenously generated in cardiovascular system and H₂S deficiency played a critical pathologic role in the development of hypertension and its complications, such as HHD [14]. Our previous studies found that a diet enriched in choline reduced the plasma H₂S levels and induced cardiac dysfunction [15]. In addition, H₂S was also reported to prevent TMAO-induced macrophage inflammation [16]. Therefore, whether H₂S was involved in dietary choline and HHD was worth to be explored.

With this in mind, the aim of present study was to investigate the exact effects of dietary choline on HHD and to explore whether it played a role through H₂S.

Materials and Methods

Animals and treatments

Male spontaneously hypertensive rats (SHR) and Wistar Kyoto rats (WKY), aged 6-weeks were purchased from Vital River Company (Beijing, China). They were kept in an environment with controlled temperature (20-24 °C), humidity (45-55 %) and a regular 12-h light and dark cycle and fed on standard rat chow and tap water *ad libitum* for 2 weeks to adapt the laboratory environment. All animal experiments were performed according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (NIH) and approved by the Ethics Committee for Laboratory Animals Care and Use of Hebei Medical University.

After acclimatization, the rats were randomly divided into five groups, each with six animals as follows: WKY group, WKY + Choline group, SHR group, SHR + Choline group, and SHR + Choline + NaHS group. All rats were fed with 1.3 % (w/v) choline in the drinking water for 3 months, except those in WKY and SHR group which were fed with tap water for the same periods. The rats in the SHR + Choline + NaHS group were intraperitoneally

injected with NaHS (100 μmol/kg/day) for 3 months and the rats in the other groups were injected with the same volume of sterile saline.

After 3 months, cardiac function was assessed by echocardiography. And then, the rats were euthanized by intraperitoneally injecting an overdose of pentobarbital (100 mg/kg). Subsequently, the blood was collected from abdominal aorta. After centrifugation at 1200 g for 10 min, plasma was separated and frozen at -80 °C until further analysis. The heart was rapidly removed to determine the heart mass (heart weight / body weight × 100 %). Left ventricular tissues were frozen at -80 °C and fixed with 4 % paraformaldehyde until further analysis.

Echocardiography

After 3 months treatment, the rats were anaesthetized with 2 % isoflurane and the cardiac function was evaluated by using a VisualSonics Vevo 2100 system (FUJIFILM VisualSonics Inc., Toronto, Canada). M-mode images of the left ventricle were recorded and three consecutive cardiac cycles were selected to measure left ventricular ejection fraction (LVEF) and fractional shortening (LVFS).

Histological analysis

After fixed in 4 % paraformaldehyde for 48 h, the heart tissues were dehydrated, permeabilization, embedded in paraffin, sectioned at 5-μm thickness, and stained with Masson's trichrome to identify collagen deposition, which was shown in blue. The heart sections were examined using an optical microscope (Olympus, Tokyo, Japan) and the collagen volume fraction was calculated as the percentage of collagen (blue-stained area) to the total myocardial area under direct vision.

Measurement of H₂S concentration in plasma

Plasma H₂S concentration was measured using liquid chromatography-mass spectrometry (LC-MS/MS) as previously described [17]. Briefly, 30 μl of plasma were mixed with 80 μl monobromobimane (MBB, Sigma-Aldrich Ltd., USA) and 10 μl 0.1 % ammonia with shaking for 1-h at room temperature for derivatization of sulfide. MBB reacts with sulfide to produce sulfide-dibimane (SDB), which can be separated by gradient elution and analyzed by LC-MS/MS. The reaction was then terminated with 10 μl 20 % formic acid and centrifuged at 15000 g for 10 min. The supernatants were stored at -80 °C until H₂S measurements were performed. H₂S concentrations were determined by using a curve generated with sodium sulfide

(0-40 $\mu\text{mol/l}$) standards.

Measurement of the cystathionine- γ -lyase (CSE) activity

The activity of CSE in heart tissues was measured according to the previously described methods with some modified [18]. Briefly, heart tissues were homogenized in ice-cold PBS and centrifuged at 12,000 g for 20 min at 4 °C. The supernatant was immediately used to measure the activity of CSE, and proteins in the supernatant were quantified using the BCA reagent. To measure the CSE activity, the enzyme substrate L-cysteine (10 mmol/l) and the cofactor pyridoxal-5'-phosphate (2 mmol/l) were added to the supernatant for an incubation of 0.5 h. Then H₂S concentrations in the reaction system were measured using LC-MS/MS and the amount of H₂S produced per microgram protein per hour was calculated as the activity of CSE.

Western blot analysis

Frozen heart tissues were cut into small fragments and homogenized with ice-cold RIPA lysis buffer. Proteins were extracted and quantified by the BCA method. Equal amount of protein samples were separated on 10 % SDS-PAGE gels and transferred to a polyvinylidene fluoride

membrane, which was blocked with 5 % non-fat milk for 1 h. After that, the primary antibody specific for CSE (1:1000, Proteintech Biotechnology, USA), Bax (1:1000, Proteintech Biotechnology, USA), Bcl-2 (1:1000, Proteintech Biotechnology, USA), and GAPDH (1:5000, Proteintech Biotechnology, USA) were added to the membranes and incubated for at 4 °C overnight. Next, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies at room temperature for 1 h after washing with TBST. The intensity of the protein bands was assessed on the ECL detection system (Thermo, USA) and quantified using the Image J software (Image J 1.52, NIH, USA).

Statistical analysis

Results were presented as mean \pm SEM. Statistical analysis was performed using an SPSS software package, version 13.0 (SPSS, Inc., USA). One-way ANOVA followed by least significant difference t-test (LSD) was used to compare values between multiple groups. The Kruskal-Wallis rank sum test was used for the results with small sample size and followed by LSD post hoc comparisons. $P < 0.05$ was considered statistically significant.

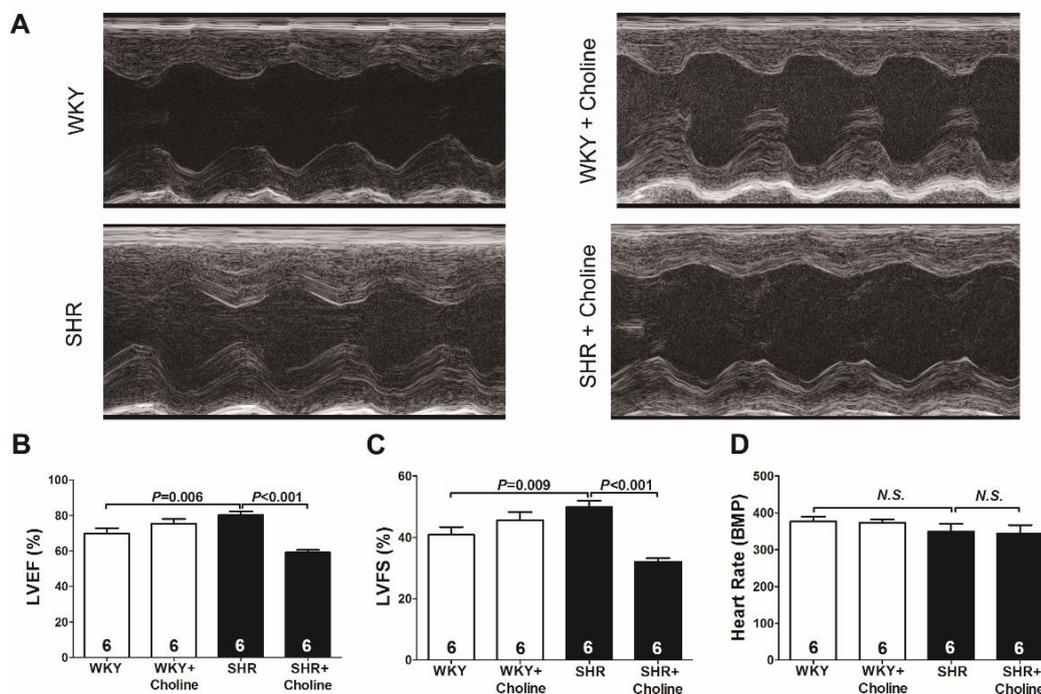


Fig. 1. Dietary choline aggravated cardiac dysfunction in spontaneously hypertensive rats.

(A) Representative M-mode images.

(B) The changes of left ventricular ejection fraction (LVEF).

(C) The changes of left ventricular fractional shortening (LVFS).

(D) The changes of heart rate (HR).

Results are expressed as mean \pm SEM. A P of < 0.05 was considered significant.

Results

Dietary choline aggravated cardiac dysfunction in SHR

As was shown in Fig. 1A-C, LVEF and LVFS, the

indicators of cardiac function measured by echocardiography, were significantly increased in SHR group as compared to those in WKY group, but they were significantly decreased in SHR fed with choline. There

was no significant difference in heart rate among the four groups (Fig. 1D).

Dietary choline exacerbated cardiac fibrosis and apoptosis in SHR

Masson's trichrome staining showed although there was no significant difference between WKY and

SHR group, interstitial collagen volumes were markedly increased in SHR after fed with choline (Fig. 2A-B). The heart mass was higher in SHR group than those in WKY group, but there was no significant difference between SHR and SHR + Choline group (Fig. 2C). In addition, Bax/Bcl-2 ratio was also significantly increased in the myocardium in SHR after fed with choline (Fig. 2D-G).

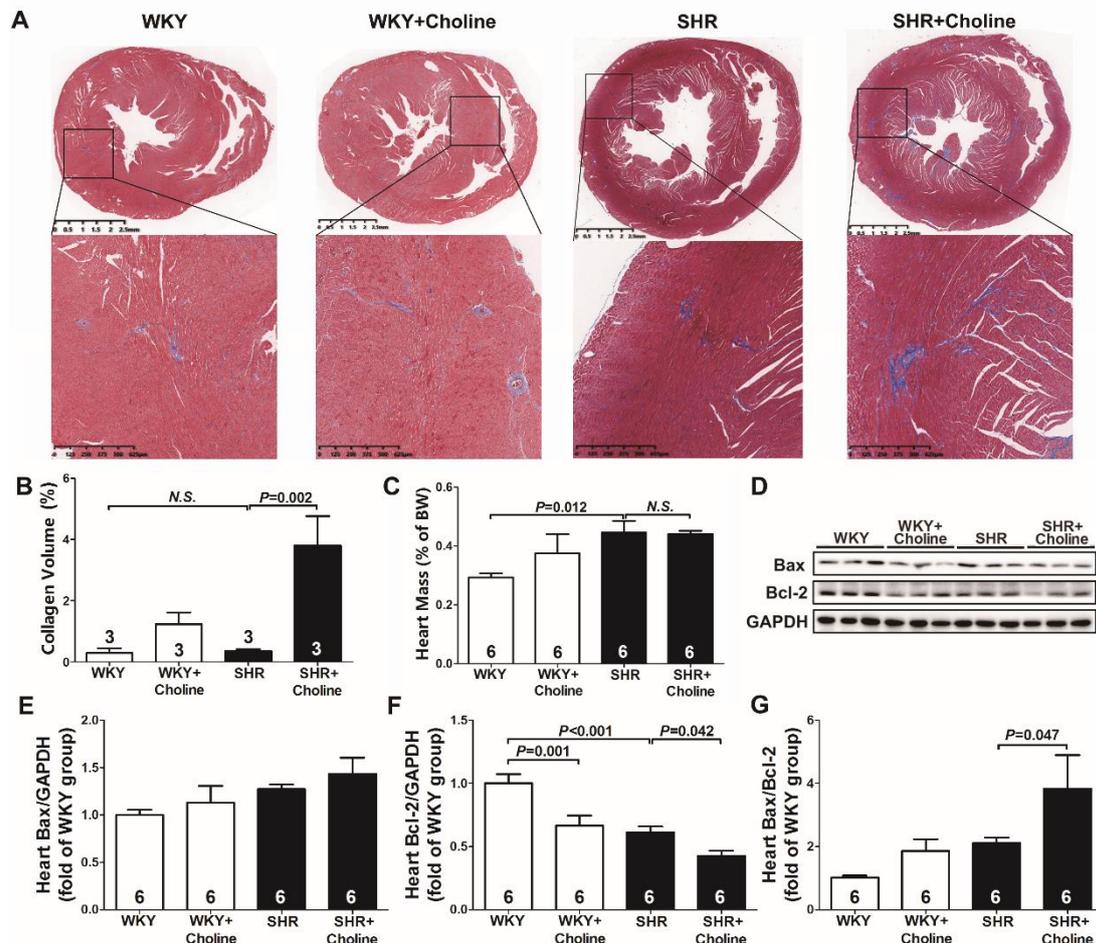


Fig. 2. Dietary choline exacerbated cardiac fibrosis and apoptosis in spontaneously hypertensive rats. (A) Representative Masson-stained myocardial sections. (B) Quantitative analysis of collagen volume fraction. (C) The changes of heart mass (heart weight / body weight \times 100 %). (D-G) Representative western blots and quantitative analysis for Bax and Bcl-2 protein expression in heart tissues. GAPDH was used as the internal control. Results are expressed as mean \pm SEM. A P of <0.05 was considered significant.

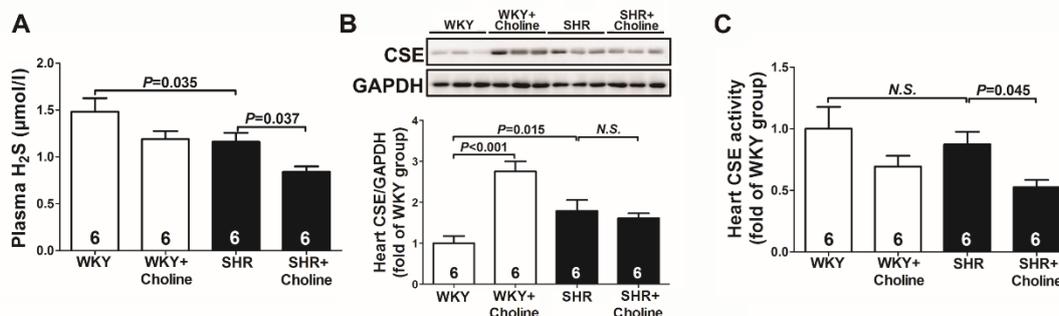


Fig. 3. Dietary choline inhibited the endogenous production of H₂S in spontaneously hypertensive rats. (A) H₂S levels in plasma. (B) Representative western blots and quantitative analysis for CSE protein expression in heart tissues. GAPDH was used as the internal control. (C) CSE activity in heart tissues. Results are expressed as mean \pm SEM. A P of <0.05 was considered significant.

Dietary choline inhibited the endogenous production of H₂S in SHR

As was shown in Fig. 3A, plasma H₂S levels were significantly lower in SHR group than those in WKY group, which were decreased significantly further in SHR + Choline group. As compared with WKY group, the protein expressions of CSE, the main enzyme for H₂S production in the cardiovascular system, were significantly increased both in WKY + Choline group and SHR group, while there was no significant difference between SHR and SHR + Choline group (Fig. 3B). Although there was no significant difference between WKY and SHR group, the CSE activity was significantly decreased in SHR fed with choline (Fig. 3C).

NaHS improved choline-induced cardiac dysfunction in SHR

To investigate whether H₂S plays a role in

choline-induced cardiac dysfunction in SHR, NaHS, a H₂S donor, was used. As was shown in Fig. 4A, NaHS treatment significantly increased plasma H₂S levels in SHR fed with choline; meanwhile, it also increased LVEF and LVFS in SHR fed with choline (Fig. 4B-C). There was no significant difference in heart rate among the three groups (Fig. 4E)

NaHS alleviated choline-induced cardiac fibrosis and apoptosis in SHR

Masson's trichrome staining showed that interstitial collagen volumes were markedly decreased in SHR + Choline + NaHS group as compared to those in SHR + Choline group (Fig. 5A-B). The heart mass was lower in SHR + Choline + NaHS group than those in SHR + Choline group (Fig. 5C). In addition, Bax/Bcl-2 ratio was also significantly decreased in the myocardium in SHR fed with choline after NaHS treatment (Fig. 5D-G).

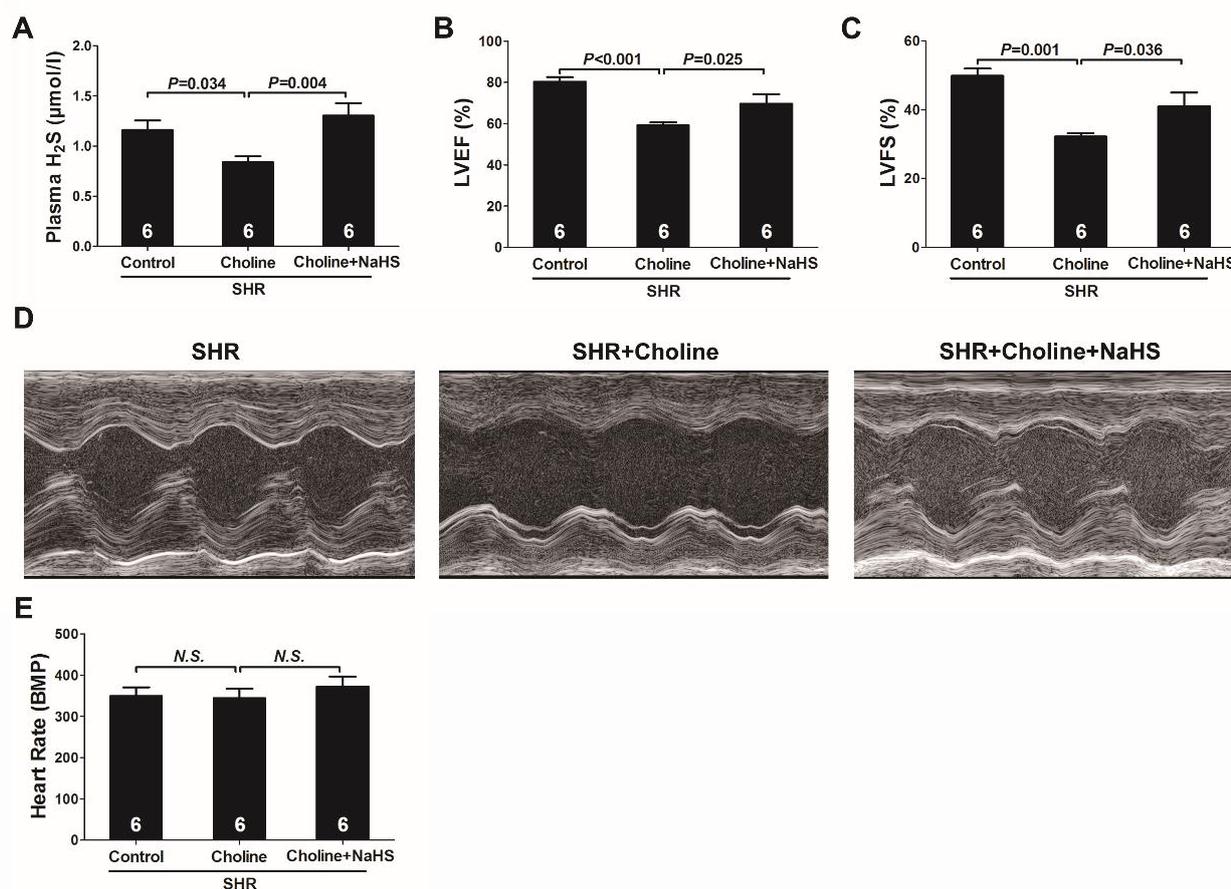


Fig. 4. NaHS improved choline-induced cardiac dysfunction in spontaneously hypertensive rats. **(A)** H₂S levels in plasma. **(B)** The changes of left ventricular ejection fraction (LVEF). **(C)** The changes of left ventricular fractional shortening (LVFS). **(D)** Representative M-mode images. **(E)** The changes of heart rate (HR). Results are expressed as mean ± SEM. A *P* of <0.05 was considered significant.

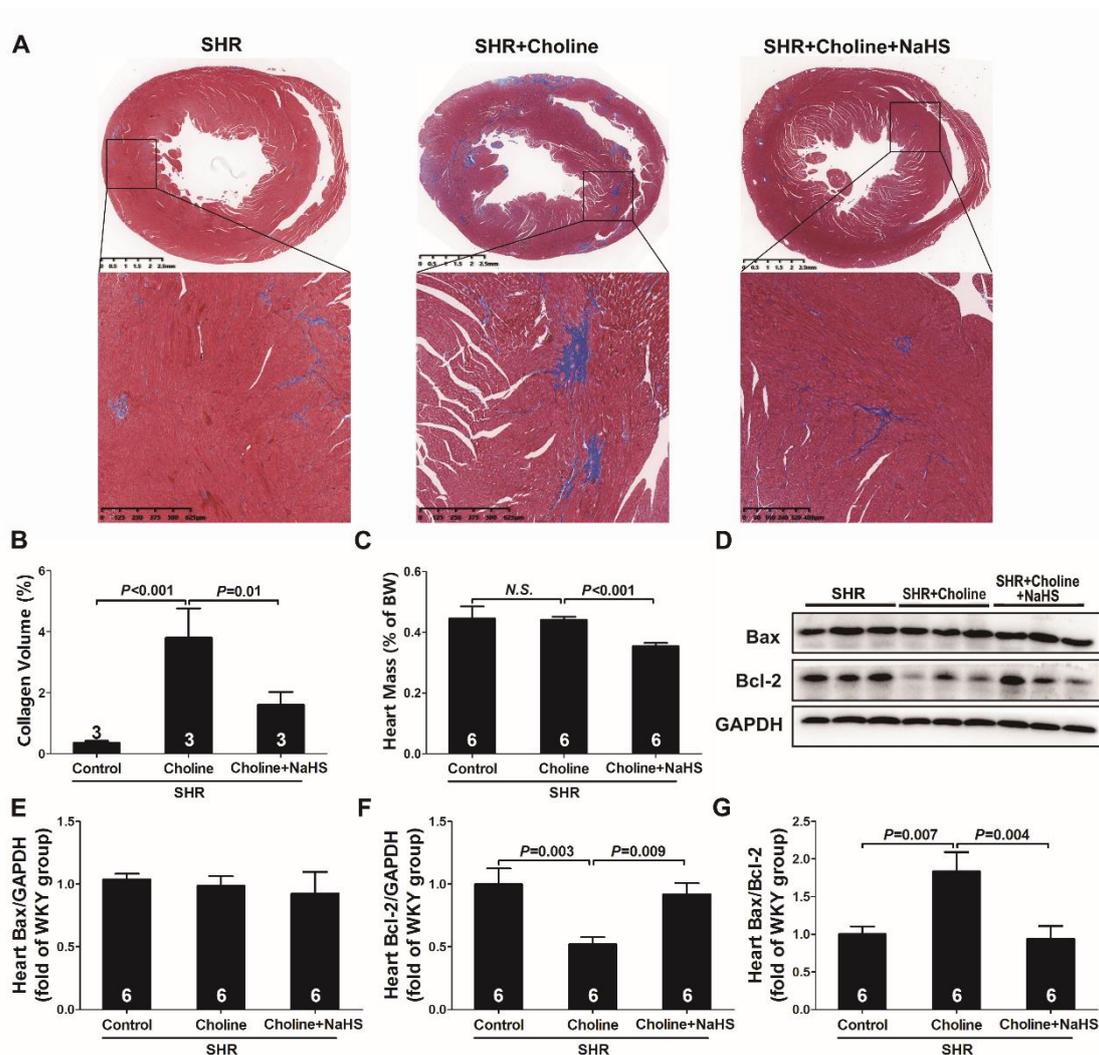


Fig. 5. NaHS alleviated choline-induced cardiac fibrosis and apoptosis in spontaneously hypertensive rats. (A) Representative Masson-stained myocardial sections. (B) Quantitative analysis of collagen volume fraction. (C) The changes of heart mass (heart weight / body weight \times 100 %). (D-G) Representative western blots and quantitative analysis for Bax and Bcl-2 protein expression in heart tissues. GAPDH was used as the internal control. Results are expressed as mean \pm SEM. A P of <0.05 was considered significant.

Discussion

In the present study, we found that choline aggravated cardiac dysfunction in HHD through inhibiting the production of endogenous H_2S , which was reversed by supplementation of exogenous H_2S .

HHD is one of the most common complications of hypertension and its progression is largely dependent on the stage of hypertension. In the early stage of hypertension, the increased left ventricular wall stress caused by hypertension-induced pressure overload leads to myocardial hypertrophy as a compensatory mechanism to maintain and even improve cardiac function. In the late stage of hypertension, sustained pressure overload induces cardiomyocyte apoptosis and ventricular remodeling which further leads to eventual decompensation of cardiac

function [19]. As a most widely used animal model of essential hypertension and its cardiovascular complications, SHR was used to explore the progression of HHD in the current study. It was reported that SHR progressively developed hypertension starting around 5-6 weeks after birth and an active phase of cardiac hypertrophy was observed between 16 and 20 weeks with enhanced cardiac function [20]. At the end of the experiment, the rats were about 20 weeks old in the present study. And then LVEF and LVFS, used as measures of systolic function, were increased significantly in SHR as compared to WKY; meanwhile the heart mass was higher in SHR than WKY, indicating the existence of cardiac hypertrophy in SHR. In agreement with our study, Lee et al. reported that the maximal rate of LV pressure rise ($+dP/dt$), an indicator of systolic function, was increased

significantly and the left ventricular mass index for body weight and cardiomyocyte sizes were also increased in 16-week-old SHR, indicating the early development of ventricular hypertrophy [21]. Long-lasting cardiac hypertrophy could induce collagen accumulation, increased fibrosis, and cardiomyocyte apoptosis in the ventricle wall, but our result showed that SHR had no significant myocardial fibrosis and apoptosis as compared to WKY, also indicating the early stage of cardiac hypertrophy. On the contrary, in Li's study [22], LVEF was measured in SHR and WKY using *in vivo* cardiac magnetic resonance imaging and LVEF was significantly decreased in SHR as compared with that in WKY at 8 weeks of age. However, in our study, LVEF and LVFS, which was measured by echocardiography, were significantly increased in SHR group as compared to those in WKY group. Our results were also in line with Conrad's study which found that active tension was greater in the 12-month SHR group than in the 12-month WKY group and the 20-month SHR group [23]. In addition, Li's study found that LVEF significantly decreased in SHR but did not change in WKY rats from 1 to 2 months of age. However, previous study had reported that the EF index-afterload relations *i.e.*, a measure of the contractile state, of the 6- and 12-month-old SHR were similar to those of the normotensive rats of all ages and a depression in the contractile state of the SHR occurred at 18 months [24]. The difference in cardiac function detection methods might be the reason for the different results between the studies.

Although choline was an essential dietary nutrient and plays a wide range of physiological roles in human health, contradictory findings were published on choline and its metabolites and heart diseases. Prolonged choline deficiency was reported to induce cardiac dysfunction [25] and choline, as a methyl donor, alleviated cardiac hypertrophy in SHR by regulating DNA methylation [26]. On the other hand, it was found that plasma levels of choline and its microbial-derived metabolites, TMAO, were both elevated in patients with chronic HF [27]. In addition, high intake of choline exacerbated cardiac dysfunction, fibrosis, and inflammation in HF with preserved ejection fraction [28]. In line with this finding, our study also found that there was a significant decrease in cardiac function and an increase in fibrosis and apoptosis in SHR fed with choline. In addition, although there was no significant difference in hypertrophy, apoptosis, and cardiac function between WKY and WKY + Choline group, the mean values of these measurements

were higher in WKY fed with choline, and similar to those in SHR. The above results indicated that choline administration might induce myocardial hypertrophy in WKY and it accelerated the transition from a compensatory to decompensatory stage in HHD of SHR, however the mechanisms behind this remained to be elucidated.

H₂S, as one of the gasotransmitters, is endogenously biosynthesized from L-cysteine by CSE in cardiovascular system and plays a protective role in HHD [29-30]. Consistent with previous reports [31-32], we found that plasma H₂S levels were significantly lower in SHR group than those in WKY group; meanwhile it was decreased significantly further in SHR fed with choline. Notably, CSE were markedly decreased in humans with hypertension [33], and the similar results also were found in SHR [34]. In addition, the use of DL-propargylglycine (a CSE inhibitor) [35] or knockout of CSE protein [36] dramatically elevated the level of basal blood pressure in animal research. Therefore, the CSE expression or activity was involved in the pathogenesis of hypertension and its complications. In present study, the protein expression of CSE did not change between SHR and SHR + Choline group, but it was upregulated both in WKY + Choline group and SHR group. The increased expression of CSE protein could be explained as a compensatory mechanism; however, this compensation did not increase plasma H₂S levels due to oxidative stress that accelerated the metabolism of H₂S in HHD. This compensatory mechanism had also been reported by previous studies in which a higher CSE expression was observed in myocardial tissue, but H₂S level was decreased in plasma [37]. Then, we detected the activity of CSE and found that the CSE activity was significantly decreased in SHR fed with choline. The above results indicated that choline inhibited the CSE activity to decrease H₂S plasma levels in SHR. The catalytic activity of CSE was found to be inhibited by inflammatory stimuli and activated by Akt signaling pathway or intracellular calcium [38]. It was reported that choline could induce inflammation by NLRP3 inflammasome activation and IL-1 β production [39]. Choline also could reduce the overload of intracellular Ca²⁺ in isolated myocytes [40]. Meanwhile the decrease of intracellular Ca²⁺ could inhibit the activation of AKT pathway [41]. All of this might be responsible for the choline-induced decrease in CSE activity. Subsequently, to confirm our hypothesis that choline exacerbated HHD in SHR by inhibiting endogenous H₂S production, NaHS, an exogenous H₂S

donor, was used in our study. The results showed that NaHS treatment significantly increased plasma H₂S levels, ameliorated cardiac dysfunction and inhibited cardiac fibrosis and apoptosis in SHR fed with choline. In HHD, sustained pressure overload led to cardiomyocyte hypertrophy and cardiac fibroblasts proliferation, migration, and activation ensuing in cardiac hypertrophy and fibrosis. There was some overlap between the inducing mechanisms of cardiac hypertrophy and fibrosis including over-activated sympathetic nervous (SNS) and renin-angiotensin-aldosterone (RAAS) systems, increased inflammation and oxidative stress, and altered energy metabolism [42-43]. Accumulating evidence suggested that H₂S played a protective role in cardiovascular disease by inhibiting SNS and RAAS over-activation, reducing inflammation and oxidative stress and ameliorating energy metabolism [44-45]. The pathological mechanisms of cardiac hypertrophy and fibrosis involved complex cellular and molecular signaling cascades which shared dissimilarities as well as commonalities. There were some common molecular targets between cardiac hypertrophy and fibrosis, such as mitogen-activated protein kinase (MAPK), Wnt, AMP-activated protein kinase (AMPK), and sirtuins [46-49]. While, H₂S had been reported to not only inhibit the MAPK and Wnt signaling pathway, but also activate the AMPK and sirtuins signaling pathway [50-53]. The difference between cardiac hypertrophy and fibrosis was that increases in intracellular Ca²⁺ ([Ca²⁺]_i) concentration and Ca²⁺-dependent signaling pathways, including calcineurin/nuclear factor of activated T cells signaling and calmodulin-dependent kinase II signaling, was the main cause of cardiac hypertrophy [54]; while the transforming growth factor-β (TGF-β) and matrix metalloproteinases (MMPs) signaling pathway was the major canonical pathway in cardiac fibrosis [43, 55]. Recent advancements demonstrated that H₂S significantly

reduced [Ca²⁺]_i concentration [56], inhibiting TGF-β pathway [57], and suppressed MMP hyperactivity [58] by S-sulhydrating the cysteine switch motif, which might be beneficial for improving cardiac hypertrophy and fibrosis from different perspectives. Uncontrolled pathological cardiac hypertrophy and fibrosis was usually associated with increased cellular senescence and cell death that would promote systolic and diastolic dysfunction, eventually leading to HF. Regarding the molecular pathogenesis, modes of regulated necrosis, such as apoptosis, ferroptosis, necroptosis and pyroptosis played important roles in HF [59]; while H₂S was been found to alleviate the above death modes [60-63]. Therefore, the above pathway might be the key target of H₂S in the treatment of HHD.

There were several weakness and limitations in the present study. Firstly, direct evidence of how choline regulated CSE activity needs to be found. Secondly, the exact mechanism of H₂S improving HHD in our study needed to be further explored in future studies.

In conclusion, choline induced cardiac dysfunction in HHD through inhibiting the production of endogenous H₂S, which was reversed by supplementation of exogenous H₂S donor. Therefore, H₂S was a potential therapeutic agent for HHD.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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References

1. Harrison DG, Coffman TM, Wilcox CS. Pathophysiology of hypertension: the mosaic theory and beyond. *Circ Res.* 2021;128(7):847-863. <https://doi.org/10.1161/CIRCRESAHA.121.318082>
2. Drazner MH. The progression of hypertensive heart disease. *Circulation.* 2011;123(3):327-334. <https://doi.org/10.1161/CIRCULATIONAHA.108.845792>
3. Kario K, Williams B. Angiotensin receptor-neprilysin inhibitors for hypertension-hemodynamic effects and relevance to hypertensive heart disease. *Hypertens Res.* 2022;45(7):1097-1110. <https://doi.org/10.1038/s41440-022-00923-2>
4. Han C, Qian X, Ren X, Zhang S, Hu L, Li J, Huang Y, Huang R, Ooi K, Lin H, Xia C. Inhibition of cGAS in paraventricular nucleus attenuates hypertensive heart injury via regulating microglial autophagy. *Mol Neurobiol.* 2022;59(11):7006-7024. <https://doi.org/10.1007/s12035-022-02994-1>

5. Higashikuni Y, Liu W, Numata G, Tanaka K, Fukuda D, Tanaka Y, Hirata Y, Imamura T, Takimoto E, Komuro I, Sata M. NLRP3 Inflammasome activation through heart-brain interaction initiates cardiac inflammation and hypertrophy during pressure overload. *Circulation*. 2023;147(4):338-355. <https://doi.org/10.1161/CIRCULATIONAHA.122.060860>
6. Richards EM, Li J, Stevens BR, Pepine CJ, Raizada MK. Gut microbiome and neuroinflammation in hypertension. *Circ Res*. 2022;130(3):401-417. <https://doi.org/10.1161/CIRCRESAHA.121.319816>
7. Muralitharan RR, Jama HA, Xie L, Peh A, Snelson M, Marques FZ. Microbial peer pressure: the role of the gut microbiota in hypertension and its complications. *Hypertension*. 2020;76(6):1674-1687. <https://doi.org/10.1161/HYPERTENSIONAHA.120.14473>
8. Hang P, Zhao J, Su Z, Sun H, Chen T, Zhao L, Du Z. Choline inhibits ischemia-reperfusion-induced cardiomyocyte autophagy in rat myocardium by activating Akt/mTOR signaling. *Cell Physiol Biochem*. 2018;45(5):2136-2144. <https://doi.org/10.1159/000488049>
9. Xu M, Xue RQ, Lu Y, Yong SY, Wu Q, Cui YL, Zuo XT, Yu XJ, Zhao M, Zang WJ. Choline ameliorates cardiac hypertrophy by regulating metabolic remodelling and UPRmt through SIRT3-AMPK pathway. *Cardiovasc Res*. 2019;115(3):530-545. <https://doi.org/10.1093/cvr/cvy217>
10. Yang B, Lin H, Xu C, Liu Y, Wang H, Han H, Wang Z. Choline produces cytoprotective effects against ischemic myocardial injuries: evidence for the role of cardiac m3 subtype muscarinic acetylcholine receptors. *Cell Physiol Biochem*. 2005;16(4-6):163-174. <https://doi.org/10.1159/000089842>
11. Papandreou C, Bulló M, Hernández-Alonso P, Ruiz-Canela M, Li J, Guasch-Ferré M, Toledo E, Clish C, Corella D, Estruch R, Ros E, Fitó M, Alonso-Gómez A, Fiol M, Santos-Lozano JM, Serra-Majem L, Liang L, Martínez-González MA, Hu FB, Salas-Salvadó J. Choline metabolism and risk of atrial fibrillation and heart failure in the PREDIMED study. *Clin Chem*. 2021;67(1):288-297. <https://doi.org/10.1093/clinchem/hvaa224>
12. Wei H, Zhao M, Huang M, Li C, Gao J, Yu T, Zhang Q, Shen X, Ji L, Ni L, Zhao C, Wang Z, Dong E, Zheng L, Wang DW. FMO3-TMAO axis modulates the clinical outcome in chronic heart-failure patients with reduced ejection fraction: evidence from an Asian population. *Front Med*. 2022;16(2):295-305. <https://doi.org/10.1007/s11684-021-0857-2>
13. Organ CL, Otsuka H, Bhushan S, Wang Z, Bradley J, Trivedi R, Polhemus DJ, Tang WH, Wu Y, Hazen SL, Lefer DJ. Choline Diet and Its Gut Microbe-Derived Metabolite, Trimethylamine N-Oxide, Exacerbate Pressure Overload-Induced Heart Failure. *Circ Heart Fail*. 2016;9(1):e002314. <https://doi.org/10.1161/CIRCHEARTFAILURE.115.002314>
14. Wang R. Roles of hydrogen sulfide in hypertension development and its complications: what, so what, now what. *Hypertension*. 2023;80(5): 936-944. <https://doi.org/10.1161/HYPERTENSIONAHA.122.19456>
15. Bai L, Dai J, Xia Y, He K, Xue H, Guo Q, Tian D, Xiao L, Zhang X, Teng X, Wu Y, Jin S. Hydrogen sulfide ameliorated high choline-induced cardiac dysfunction by inhibiting cGAS-STING-NLRP3 inflammasome pathway. *Oxid Med Cell Longev*. 2022;2022:1392896. <https://doi.org/10.1155/2022/1392896>
16. Liu MH, Lin XL, Xiao LL. Hydrogen sulfide attenuates TMAO-induced macrophage inflammation through increased SIRT1 sulfhydration. *Mol Med Rep*. 2023;28(1):129. <https://doi.org/10.3892/mmr.2023.13016>
17. Tan B, Jin S, Sun J, Gu Z, Sun X, Zhu Y, Huo K, Cao Z, Yang P, Xin X, Liu X, Pan L, Qiu F, Jiang J, Jia Y, Ye F, Xie Y, Zhu YZ. New method for quantification of gasotransmitter hydrogen sulfide in biological matrices by LC-MS/MS. *Sci Rep*. 2017;7:46278. <https://doi.org/10.1038/srep46278>
18. Tao BB, Liu SY, Zhang CC, Fu W, Cai WJ, Wang Y, Shen Q, Wang MJ, Chen Y, Zhang LJ, Zhu YZ, Zhu YC. VEGFR2 functions as an H₂S-targeting receptor protein kinase with its novel Cys1045-Cys1024 disulfide bond serving as a specific molecular switch for hydrogen sulfide actions in vascular endothelial cells. *Antioxid Redox Signal*. 2013;19(5):448-464. <https://doi.org/10.1089/ars.2012.4565>
19. Gradman AH, Alfayoumi F. From left ventricular hypertrophy to congestive heart failure: management of hypertensive heart disease. *Prog Cardiovasc Dis*. 2006;48(5):326-341. <https://doi.org/10.1016/j.pcad.2006.02.001>
20. Meng C, Jin X, Xia L, Shen SM, Wang XL, Cai J, Chen GQ, Wang LS, Fang NY. Alterations of mitochondrial enzymes contribute to cardiac hypertrophy before hypertension development in spontaneously hypertensive rats. *J Proteome Res*. 2009;8(5):2463-2475. <https://doi.org/10.1021/pr801059u>

21. Lee TM, Lin MS, Chou TF, Tsai CH, Chang NC. Effect of pravastatin on development of left ventricular hypertrophy in spontaneously hypertensive rats. *Am J Physiol Heart Circ Physiol*. 2005;289(1):H220-H227. <https://doi.org/10.1152/ajpheart.00417.2004>
22. Li J, Kemp BA, Howell NL, Massey J, Mińczuk K, Huang Q, Chordia MD, Roy RJ, Patrie JT, Davogusto GE, Kramer CM, Epstein FH, Carey RM, Taegtmeier H, Keller SR, Kundu BK. Metabolic changes in spontaneously hypertensive rat hearts precede cardiac dysfunction and left ventricular hypertrophy. *J Am Heart Assoc*. 2019;8(4):e010926. <https://doi.org/10.1161/JAHA.118.010926>
23. Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OH. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. *Circulation*. 1995;91(1):161-170. <https://doi.org/10.1161/01.CIR.91.1.161>
24. Mirsky I, Pfeffer JM, Pfeffer MA, Braunwald E. The contractile state as the major determinant in the evolution of left ventricular dysfunction in the spontaneously hypertensive rat. *Circ Res*. 1983;53(6):767-78. <https://doi.org/10.1161/01.RES.53.6.767>
25. Strilakou AA, Lazaris AC, Perelas AI, Mourouzis IS, Douzis ICh, Karkalousos PL, Stylianaki AT, Pantos CI, Liapi CA. Heart dysfunction induced by choline-deficiency in adult rats: the protective role of L-carnitine. *Eur J Pharmacol*. 2013;709(1-3):20-27. <https://doi.org/10.1016/j.ejphar.2013.03.025>
26. Liu L, He X, Zhao M, Yang S, Wang S, Yu X, Liu J, Zang W. Regulation of DNA methylation and 2-OG/TET signaling by choline alleviated cardiac hypertrophy in spontaneously hypertensive rats. *J Mol Cell Cardiol*. 2019;128:26-37. <https://doi.org/10.1016/j.yjmcc.2019.01.011>
27. Trøseid M, Ueland T, Hov JR, Svardal A, Gregersen I, Dahl CP, Aakhus S, Gude E, Bjørndal B, Halvorsen B, Karlsen TH, Aukrust P, Gullestad L, Berge RK, Yndestad A. Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure. *J Intern Med*. 2015;277(6):717-726. <https://doi.org/10.1111/joim.12328>
28. Shuai W, Wen J, Li X, Wang D, Li Y, Xiang J. High-choline diet exacerbates cardiac dysfunction, fibrosis, and inflammation in a mouse model of heart failure with preserved ejection fraction. *J Card Fail*. 2020;26(8):694-702. <https://doi.org/10.1016/j.cardfail.2020.04.017>
29. Nguyen ITN, Wiggerhauser LM, Bulthuis M, Hillebrands JL, Feelisch M, Verhaar MC, van Goor H, Joles JA. Cardiac Protection by Oral Sodium Thiosulfate in a Rat Model of L-NNA-Induced Heart Disease. *Front Pharmacol*. 2021;12:650968. <https://doi.org/10.3389/fphar.2021.650968>
30. Jin S, Teng X, Xiao L, Xue H, Guo Q, Duan X, Chen Y, Wu Y. Hydrogen sulfide ameliorated L-NAME-induced hypertensive heart disease by the Akt/eNOS/NO pathway. *Exp Biol Med (Maywood)*. 2017;242(18):1831-1841. <https://doi.org/10.1177/1535370217732325>
31. Cui C, Fan J, Zeng Q, Cai J, Chen Y, Chen Z, Wang W, Li SY, Cui Q, Yang J, Tang C, Xu G, Cai J, Geng B. CD4+ T-Cell Endogenous Cystathionine γ Lyase-Hydrogen Sulfide Attenuates Hypertension by Sulfhydrating Liver Kinase B1 to Promote T Regulatory Cell Differentiation and Proliferation. *Circulation*. 2020;142(18):1752-1769. <https://doi.org/10.1161/CIRCULATIONAHA.119.045344>
32. Li J, Teng X, Jin S, Dong J, Guo Q, Tian D, Wu Y. Hydrogen sulfide improves endothelial dysfunction by inhibiting the vicious cycle of NLRP3 inflammasome and oxidative stress in spontaneously hypertensive rats. *J Hypertens*. 2019;37(8):1633-1643. <https://doi.org/10.1097/HJH.0000000000002101>
33. Xiao L, Dong JH, Teng X, Jin S, Xue HM, Liu SY, Guo Q, Shen W, Ni XC, Wu YM. Hydrogen sulfide improves endothelial dysfunction in hypertension by activating peroxisome proliferator-activated receptor delta/endothelial nitric oxide synthase signaling. *J Hypertens*. 2018;36(3):651-665. <https://doi.org/10.1097/HJH.0000000000001605>
34. Cui C, Fan J, Zeng Q, Cai J, Chen Y, Chen Z, Wang W, Li SY, Cui Q, Yang J, Tang C, Xu G, Cai J, Geng B. CD4+ T-Cell Endogenous Cystathionine γ Lyase-Hydrogen Sulfide Attenuates Hypertension by Sulfhydrating Liver Kinase B1 to Promote T Regulatory Cell Differentiation and Proliferation. *Circulation*. 2020;142(18):1752-1769. <https://doi.org/10.1161/CIRCULATIONAHA.119.045344>
35. Al-Magableh MR, Kemp-Harper BK, Hart JL. Hydrogen sulfide treatment reduces blood pressure and oxidative stress in angiotensin II-induced hypertensive mice. *Hypertens Res*. 2015;38(1):13-20. <https://doi.org/10.1038/hr.2014.125>

36. Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S, Snyder SH, Wang R. H₂S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science*. 2008;322(5901):587-590. <https://doi.org/10.1126/science.1162667>
37. Chang L, Geng B, Yu F, Zhao J, Jiang H, Du J, Tang C. Hydrogen sulfide inhibits myocardial injury induced by homocysteine in rats. *Amino Acids*. 2008;34(4):573-585. <https://doi.org/10.1007/s00726-008-0027-8>
<https://doi.org/10.1007/s00726-007-0011-8>
38. Cirino G, Szabo C, Papapetropoulos A. Physiological roles of hydrogen sulfide in mammalian cells, tissues, and organs. *Physiol Rev*. 2023;103(1):31-276. <https://doi.org/10.1152/physrev.00028.2021>
39. Sanchez-Lopez E, Zhong Z, Stubelius A, Sweeney SR, Booshehri LM, Antonucci L, Liu-Bryan R, Lodi A, Terkeltaub R, Lacial JC, Murphy AN, Hoffman HM, Tiziani S, Guma M, Karin M. Choline uptake and metabolism modulate macrophage IL-1 β and IL-18 production. *Cell Metab*. 2019;29(6):1350-1362.e7. <https://doi.org/10.1016/j.cmet.2019.03.011>
40. Liu Y, Sun HL, Li DL, Wang LY, Gao Y, Wang YP, Du ZM, Lu YJ, Yang BF. Choline produces antiarrhythmic actions in animal models by cardiac M₃ receptors: improvement of intracellular Ca²⁺ handling as a common mechanism. *Can J Physiol Pharmacol*. 2008;86(12):860-865. <https://doi.org/10.1139/Y08-094>
41. Koo BH, Won MH, Kim YM, Ryoo S. Arginase II protein regulates Parkin-dependent p32 degradation that contributes to Ca²⁺-dependent eNOS activation in endothelial cells. *Cardiovasc Res*. 2022;118(5):1344-1358. <https://doi.org/10.1093/cvr/cvab163>
42. Nakamura M, Sadoshima J. Mechanisms of physiological and pathological cardiac hypertrophy. *Nat Rev Cardiol*. 2018;15(7):387-407. <https://doi.org/10.1038/s41569-018-0007-y>
43. Frangogiannis NG. Cardiac fibrosis. *Cardiovasc Res*. 2021;117(6):1450-1488. <https://doi.org/10.1093/cvr/cvaa324>
44. Lv B, Chen S, Tang C, Jin H, Du J, Huang Y. Hydrogen sulfide and vascular regulation - An update. *J Adv Res*. 2020;27:85-97. <https://doi.org/10.1016/j.jare.2020.05.007>
45. Murphy B, Bhattacharya R, Mukherjee P. Hydrogen sulfide signaling in mitochondria and disease. *FASEB J*. 2019;33(12):13098-13125. <https://doi.org/10.1096/fj.201901304R>
46. Zhao T, Kee HJ, Bai L, Kim MK, Kee SJ, Jeong MH. Selective HDAC8 Inhibition Attenuates Isoproterenol-Induced Cardiac Hypertrophy and Fibrosis via p38 MAPK Pathway. *Front Pharmacol*. 2021;12:677757. <https://doi.org/10.3389/fphar.2021.677757>
47. Nayakanti SR, Friedrich A, Sarode P, Jafari L, Maroli G, Boehm M, Bourgeois A, Grobs Y, Khassafi F, Kuenne C, Guenther S, Dabral S, Wilhelm J, Weiss A, Wietelmann A, Kojonazarov B, Janssen W, Looso M, de Man F, Provencher S, Tello K, Seeger W, Bonnet S, Savai R, Schermuly RT, Pullamsetti SS. Targeting Wnt- β -catenin-FOSL signaling ameliorates right ventricular remodeling. *Circ Res*. 2023;132(11):1468-1485. <https://doi.org/10.1161/CIRCRESAHA.122.321725>
48. Chen J, Chen S, Zhang B, Liu J. SIRT3 as a potential therapeutic target for heart failure. *Pharmacol Res*. 2021;165:105432. <https://doi.org/10.1016/j.phrs.2021.105432>
49. Feng Y, Zhang Y, Xiao H. AMPK and cardiac remodelling. *Sci China Life Sci*. 2018;61(1):14-23. <https://doi.org/10.1007/s11427-017-9197-5>
50. Tian D, Dong J, Jin S, Teng X, Wu Y. Endogenous hydrogen sulfide-mediated MAPK inhibition preserves endothelial function through TXNIP signaling. *Free Radic Biol Med*. 2017;110:291-299. <https://doi.org/10.1016/j.freeradbiomed.2017.06.016>
51. Bhattacharjee D, Raina K, Mandal TK, Thummer RP, Bhabak KP. Targeting Wnt/ β -catenin signaling pathway in triple-negative breast cancer by benzylic organotrithiols: Contribution of the released hydrogen sulfide towards potent anti-cancer activity. *Free Radic Biol Med*. 2022;191:82-96. <https://doi.org/10.1016/j.freeradbiomed.2022.08.029>
52. Wang M, Tang W, Zhu YZ. An update on AMPK in hydrogen sulfide pharmacology. *Front Pharmacol*. 2017;8:810. <https://doi.org/10.3389/fphar.2017.00810>
53. Guan R, Wang J, Cai Z, Li Z, Wang L, Li Y, Xu J, Li D, Yao H, Liu W, Deng B, Lu W. Hydrogen sulfide attenuates cigarette smoke-induced airway remodeling by upregulating SIRT1 signaling pathway. *Redox Biol*. 2020 ;28:101356. <https://doi.org/10.1016/j.redox.2019.101356>

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54. Oldfield CJ, Duhamel TA, Dhalla NS. Mechanisms for the transition from physiological to pathological cardiac hypertrophy. *Can J Physiol Pharmacol.* 2020;98(2):74-84. <https://doi.org/10.1139/cjpp-2019-0566>
 55. Sygitowicz G, Maciejak-Jastrzębska A, Sitkiewicz D. A review of the molecular mechanisms underlying cardiac fibrosis and atrial fibrillation. *J Clin Med.* 2021;10(19):4430. <https://doi.org/10.3390/jcm10194430>
 56. Dai L, Qian Y, Zhou J, Zhu C, Jin L, Li S. Hydrogen sulfide inhibited L-type calcium channels (CaV1.2) via up-regulation of the channel sulfhydration in vascular smooth muscle cells. *Eur J Pharmacol.* 2019;858:172455. <https://doi.org/10.1016/j.ejphar.2019.172455>
 57. Yang R, Qu C, Zhou Y, Konkell JE, Shi S, Liu Y, Chen C, Liu S, Liu D, Chen Y, Zandi E, Chen W, Zhou Y, Shi S. Hydrogen Sulfide Promotes Tet1- and Tet2-Mediated Foxp3 Demethylation to drive regulatory T cell differentiation and maintain immune homeostasis. *Immunity.* 2015; 43(2):251-263. <https://doi.org/10.1016/j.immuni.2015.07.017>
 58. Zhu J, Wang Y, Rivett A, Li H, Wu L, Wang R, Yang G. Deficiency of cystathionine gamma-lyase promotes aortic elastolysis and medial degeneration in aged mice. *J Mol Cell Cardiol.* 2022;171:30-44. <https://doi.org/10.1016/j.yjmcc.2022.06.011>
 59. Del Re DP, Amgalan D, Linkermann A, Liu Q, Kitsis RN. Fundamental mechanisms of regulated cell death and implications for heart disease. *Physiol Rev.* 2019;99(4):1765-1817. <https://doi.org/10.1152/physrev.00022.2018>
 60. Zhang J, Cen L, Zhang X, Tang C, Chen Y, Zhang Y, Yu M, Lu C, Li M, Li S, Lin B, Zhang T, Song X, Yu C, Wu H, Shen Z. MPST deficiency promotes intestinal epithelial cell apoptosis and aggravates inflammatory bowel disease via AKT. *Redox Biol.* 2022;56:102469. <https://doi.org/10.1016/j.redox.2022.102469>
 61. Liang Z, Miao Y, Teng X, Xiao L, Guo Q, Xue H, Tian D, Jin S, Wu Y. Hydrogen Sulfide Inhibits Ferroptosis in Cardiomyocytes to Protect Cardiac Function in Aging Rats. *Front Mol Biosci.* 2022;9:947778. <https://doi.org/10.3389/fmolb.2022.947778>
 62. Kar S, Shahshahan HR, Hackfort BT, Yadav SK, Yadav R, Kambis TN, Lefer DJ, Mishra PK. Exercise training promotes cardiac hydrogen sulfide biosynthesis and mitigates pyroptosis to prevent high-fat diet-induced diabetic cardiomyopathy. *Antioxidants (Basel).* 2019;8(12):638. <https://doi.org/10.3390/antiox8120638>
 63. Gong W, Zhang S, Chen Y, Shen J, Zheng Y, Liu X, Zhu M, Meng G. Protective role of hydrogen sulfide against diabetic cardiomyopathy via alleviating necroptosis. *Free Radic Biol Med.* 2022;181:29-42. <https://doi.org/10.1016/j.freeradbiomed.2022.01.028>
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