

Research Article

Hydrogen Sulfide Ameliorated High Choline-Induced Cardiac Dysfunction by Inhibiting cGAS-STING-NLRP3 Inflammasome Pathway

Lu Bai ¹, Jing Dai ², Yuxuan Xia ¹, Kaichuan He ¹, Hongmei Xue ¹, Qi Guo ¹,
Danyang Tian ¹, Lin Xiao ¹, Xiangjian Zhang ³, Xu Teng ¹, Yuming Wu ^{1,3},
and Sheng Jin ¹

¹Department of Physiology, Hebei Medical University, Hebei 050017, China

²Department of Clinical Diagnostics, Hebei Medical University, Hebei 050017, China

³Hebei Collaborative Innovation Center for Cardio-Cerebrovascular Disease, 050017 Hebei, China

Correspondence should be addressed to Sheng Jin; jinshengsheng@126.com

Received 2 June 2022; Accepted 12 July 2022; Published 22 July 2022

Academic Editor: Tao Zheng

Copyright © 2022 Lu Bai et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Although it is an essential nutrient, high choline intake directly or indirectly via its metabolite is associated with increased risk of cardiovascular disease, the mechanism of which remains to be elucidated. The present study was performed to investigate whether hydrogen sulfide (H₂S) was involved in high choline-induced cardiac dysfunction and explore the potential mechanisms. We found that ejection fraction (EF) and fractional shortening (FS), the indicators of cardiac function measured by echocardiography, were significantly decreased in mice fed a diet containing 1.3% choline for 4 months as compared to the control, while applying 3,3-dimethyl-1-butanol (DMB) to suppress trimethylamine N-oxide (TMAO, a metabolite of choline) generation ameliorated the cardiac function. Subsequently, we found that feeding choline or TMAO significantly increased the protein levels of cyclic GMP-AMP (cGAMP) synthase (cGAS), stimulator of interferon genes (STING), NOD-like receptor protein 3 (NLRP3), caspase-1, and interleukin-1 β (IL-1 β) as compared to the control, which indicated the activation of cGAS-STING-NLRP3 inflammasome axis. Moreover, the protein expression of cystathionine γ -lyase (CSE), the main enzyme for H₂S production in the cardiovascular system, was significantly increased after dietary supplementation with choline, but the plasma H₂S levels were significantly decreased. To observe the effect of endogenous H₂S, CSE knockout (KO) mice were used, and we found that the EF, FS, and plasma H₂S levels in WT mice were significantly decreased after dietary supplementation with choline, while there was no difference between CSE KO+control and CSE KO+choline group. To observe the effect of exogenous H₂S, mice were intraperitoneally injected with sodium hydrosulfide (NaHS, a H₂S donor) for 4 months, and we found that NaHS improved the cardiac function and reduced the protein levels of cGAS, STING, NLRP3, caspase-1, and IL-1 β in mice receiving dietary choline. In conclusion, our studies revealed that high choline diet decreased plasma H₂S levels and induced cardiac dysfunction via cGAS-STING-NLRP3 inflammasome axis while H₂S treatment could restore the cardiac function by inhibiting cGAS-STING-NLRP3 inflammasome axis.

1. Introduction

Choline is an essential bioactive micronutrient abundant in egg yolk, red meat, fish, dairy products, and soybean. Although it can be formed *de novo* by methylation of phosphatidylethanolamine, additional dietary intake of choline is

also required or else will develop a deficiency state [1]. Because of its wide-ranging roles in biological processes including cholinergic neurotransmission, lipid transport, membrane phospholipids synthesis, and methyl group metabolism, inadequate intake or abnormal metabolism of choline can lead to neurological disorders, cancers, and

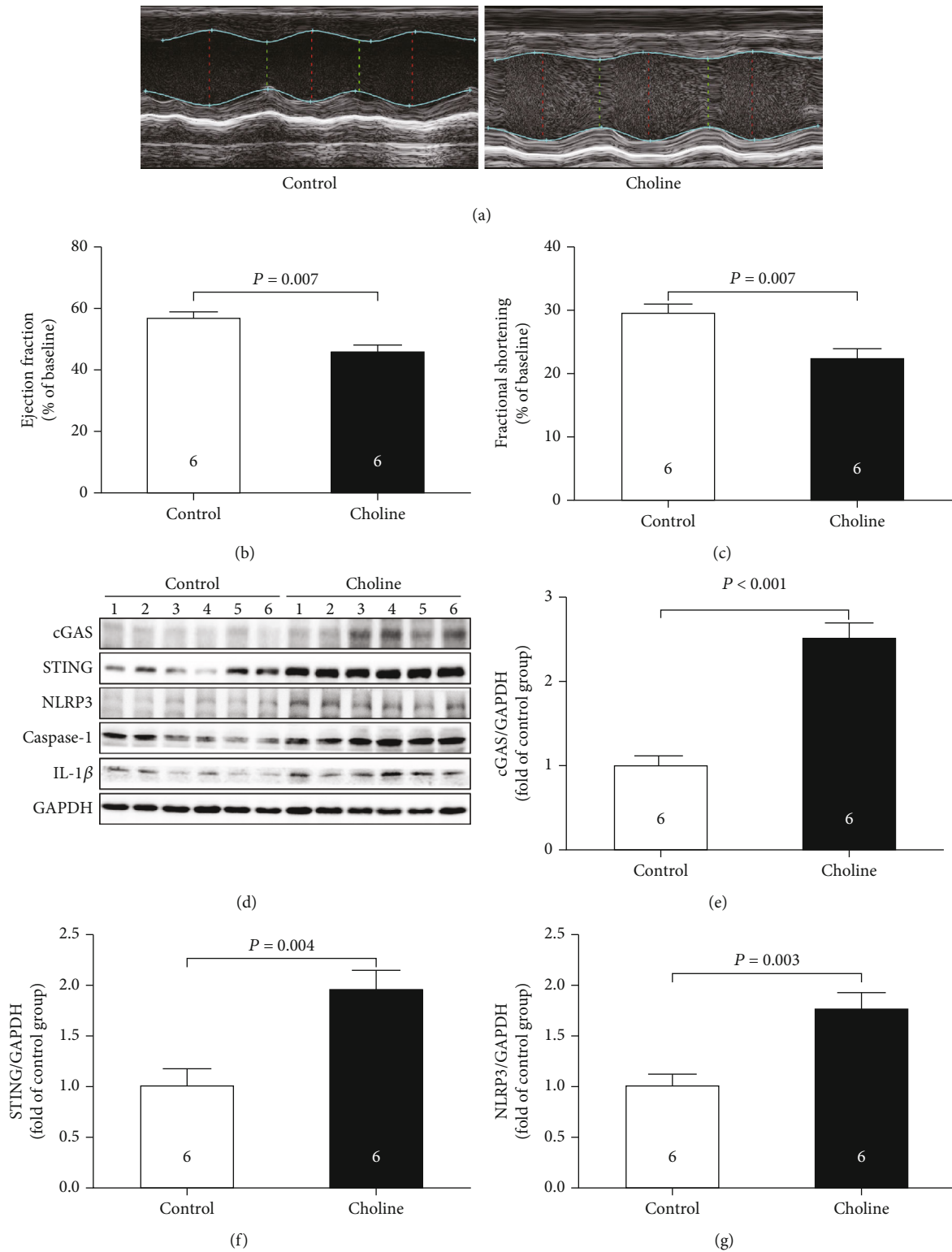


FIGURE 1: Continued.

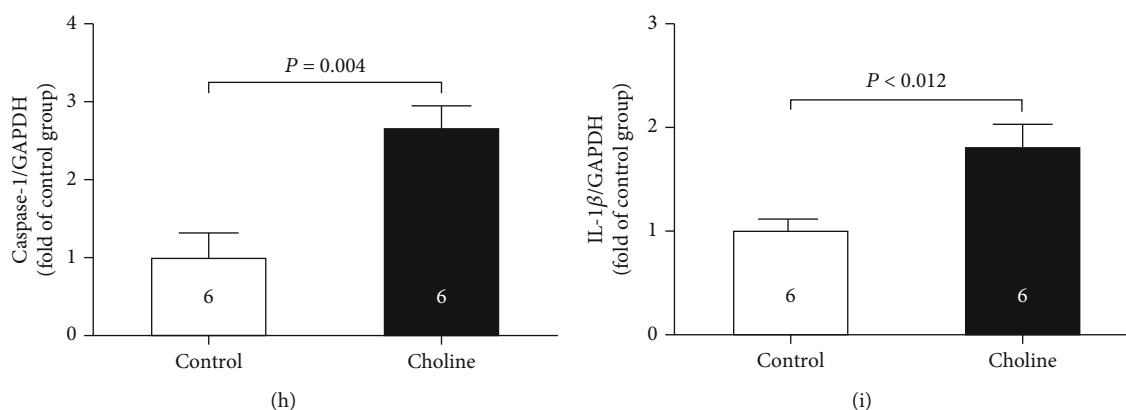


FIGURE 1: Dietary choline induced cardiac dysfunction in mice. (a) Representative M-mode images. (b) The changes of left ventricular ejection fraction (LVEF) after dietary supplementation with choline. (c) The changes of left ventricular fractional shortening (LVFS) after dietary supplementation with choline. (d)–(i) Representative western blots and quantitative analysis for cGAS, STING, NLRP3, caspase-1, and IL-1 β protein expression in heart tissues after dietary supplementation with choline. Results are expressed as mean \pm SEM. A P of <0.05 was considered significant.

cardiovascular disease, which can be cured clinically with choline [2–4]. However, an analysis of a large prospective cohort showed high choline intake was associated with increased risk of cardiometabolic mortality in racially diverse populations [5]. And a growing body of preclinical studies highlighted that high choline intake directly or indirectly via its metabolites such as trimethylamine N-oxide (TMAO) had been to a higher risk of heart disease. For example, Organ et al. reported that choline diet and its derived metabolite, TMAO, exacerbated pressure overload-induced heart failure [6]. Another study found that high-choline diet aggravated cardiac dysfunction, fibrosis, and inflammation in a mouse model of heart failure with preserved ejection fraction [7]. Nevertheless, the potential mechanism of high choline-induced cardiac dysfunction remains to be elucidated.

Hydrogen sulfide (H_2S) is a colorless, water-soluble, and corrosive gas with a characteristic odor of rotten eggs and was traditionally known as an environmental pollutant which is toxic to humans at high concentrations [8]. However, it was not until the pioneering work of Abe and Kimura in 1996 that H_2S was truly considered to be an endogenous gasotransmitter alongside carbon monoxide and nitric oxide [9]. In mammalian cells, H_2S is biosynthesized mainly from L-cysteine and/or L-homocysteine by three endogenous enzymes: cystathionine β -synthase (CBS), cystathionine γ -lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3-MST) coupled with cysteine aminotransferase. The tissue distribution of these H_2S -producing enzymes is different: CBS is predominantly expressed in the central nervous system, whereas CSE is mainly present in the cardiovascular system, and 3-MST is found primarily in the brain and erythrocytes [10, 11]. Numerous studies have shown that physiological concentration of H_2S plays a fundamental role in the cardiovascular system by regulating the biological functions and maintaining homeostasis [12, 13]. Conversely, lack of endogenous H_2S was detrimental and contributed to various cardiovascular diseases including atherosclerosis, hypertension, myocardial infarction, and heart failure

[14–16]. However, whether high choline-induced cardiac dysfunction was associated with the changes in H_2S concentration has not previously been evaluated.

With this in mind, the aim of present study was to investigate whether H_2S was involved in high choline-induced cardiac dysfunction and explore the potential mechanisms.

2. Material and Methods

2.1. Animals and Treatments. All animal experimentals were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication, 8th Edition, 2011) and approved by the Ethics Committee for Laboratory Animals Care and Use of Hebei Medical University. Male C57BL/6 J mice were provided from Vital River Laboratories (Beijing, China). CSE knockout (CSE KO) mice with C57BL/6 J genetic bases and its homozygote wild-type (WT) mice were bred from CSE heterozygous mice which were kindly provided as gifts by Professor Yichun Zhu (Fudan University, Shanghai, China). Mice were housed in plastic cages with 12 h light/12 h dark cycles at 22–24°C with 60% humidity and *ad libitum* access to standard rat chow and sterile tap water.

In order to observe the effect of choline, male C57BL/6 J mice were randomly divided into 2 groups: control group and choline group. The mice in the choline group were given a chow diet supplemented with 1.3% choline (Beijing Keao Xieli Feed Co., Ltd., Beijing, China) for 4 months, and the mice in the control group were given a regular chow diet for the same period.

In order to observe the effect of 3,3-dimethyl-1-butanol (DMB, the TMA lyase inhibitors), male C57BL/6 J mice were randomly divided into 3 groups: control group, choline group, and choline + DMB group. The mice in the choline group and choline + DMB group were given a chow diet supplemented with 1.3% choline for 4 months. The mice in the choline + DMB group were fed with 1.3% DMB

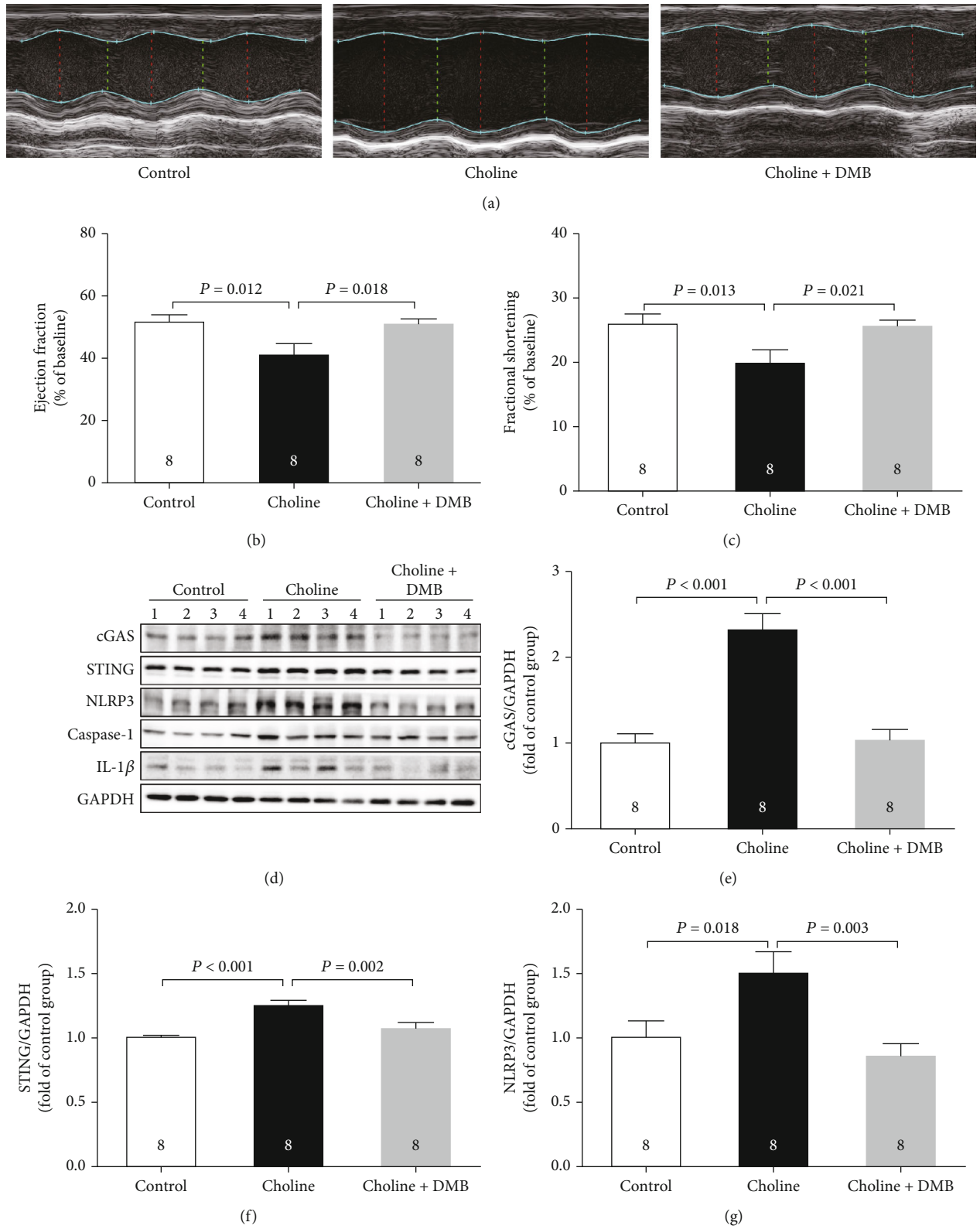


FIGURE 2: Continued.

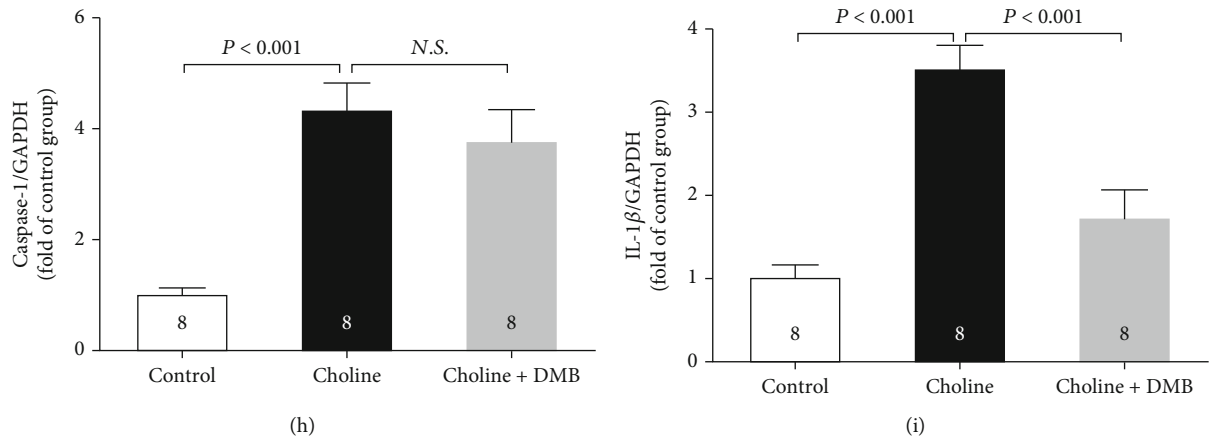


FIGURE 2: Dietary choline induced cardiac dysfunction by generating TMAO in mice. (a) Representative M-mode images. (b) The changes of left ventricular ejection fraction (LVEF) after DMB supplementation. (c) The changes of left ventricular fractional shortening (LVFS) after DMB supplementation. (d)–(i) Representative western blots and quantitative analysis for cGAS, STING, NLRP3, caspase-1, and IL-1 β protein expression in heart tissues after DMB supplementation. Results are expressed as mean \pm SEM. A P of <0.05 was considered significant.

(Aladdin Biochemical Technology Co., Ltd., Shanghai, China) in the drinking water for 4 months.

In order to observe the effect of TMAO, male C57BL/6 J mice were randomly divided into 2 groups: control group and TMAO group. The mice in the TMAO group were fed with 1.3% TMAO (Aladdin Biochemical Technology Co., Ltd., Shanghai, China) in the drinking water for 2 months.

In order to observe the effect of exogenous H₂S, male C57BL/6 J mice were randomly divided into 3 groups: control group, choline group, and choline + sodium hydrosulfide (NaHS, a H₂S donor) group. The mice in the choline group and choline + NaHS group were given a chow diet supplemented with 1.3% choline for 4 months. The mice in the choline + NaHS group were intraperitoneally injected with NaHS (100 μ mol/kg/day, Sigma-Aldrich Ltd., St. Louis, USA) for 4 months.

In order to observe the effect of endogenous H₂S, male CSE KO and WT mice were randomly divided into 4 groups: WT + control group, WT + choline group, CSE KO + control group, and CSE KO + choline group. The mice in the WT + choline group and CSE KO + choline group were given a chow diet supplemented with 1.3% choline for 4 months.

2.2. Echocardiography. At the end of the experiment, mice were anesthetized with 1% isoflurane, and the cardiac function was evaluated by using a VisualSonics Vevo 2100 system (FUJIFILM VisualSonics Inc., Toronto, Canada) as described in our previous research [17]. M-mode images of the left ventricle were recorded, and three consecutive cardiac cycles were selected to measure left ventricular ejection fraction and fractional shortening (LVEF and LVFS). And then, the heart was harvested and stored at -80°C until assay. Plasma was separated from the blood after centrifugation at 3500 rpm for 10 min and stored at -80°C until assay.

2.3. Measurement of H₂S Concentration in Plasma. The H₂S levels in plasma were measured according to the previously

study [18]. Briefly, 30 μ L of plasma was mixed with 80 μ L monobromobimane (MBB, Sigma-Aldrich Ltd., St. Louis, USA) and 10 μ L 0.1% ammonia with shaking for 1 h at room temperature for derivatization of sulfide, which called sulfide-dibimane. The reaction was then terminated with 10 μ L 20% formic acid and centrifuged at $15000\times g$ for 10 min. The supernatants were stored at -80°C before the measurement of H₂S levels using liquid chromatography coupled with tandem mass spectrometry.

2.4. Western Blot Analysis. The protein expressions in myocardial tissue were evaluated by western blotting according to the previously study [19]. Frozen heart tissues were homogenized with ice-cold radio immunoprecipitation assay (RIPA) lysis buffer. Proteins were extracted and quantified by the bicinchoninic acid (BCA) method. Equal amount of protein samples was separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% nonfat milk for 1 h and incubated with primary antibodies that recognized CSE (1:1000, Santa Cruz Biotechnology, the United States), cyclic GMP-AMP (cGAMP) synthase (cGAS, 1:1000, Proteintech Biotechnology, the United States), stimulator of interferon genes (STING, 1:1000, Proteintech Biotechnology, the United States), NOD-like receptor protein 3 (NLRP3, 1:1000, Proteintech Biotechnology, the United States), caspase-1 (1:1000, Proteintech Biotechnology, the United States), interleukin-1 β (IL-1 β , 1:1000, Proteintech Biotechnology, the United States), and GAPDH (1:5000, Proteintech Biotechnology, the United States) at 4°C overnight. Then, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h after washing with TBST. Specific bands were detected with SuperSignal West Pico Chemiluminescent Substrate (Thermo, Scientific-Pierce, Waltham, the United States). The band intensity was quantified by ImageJ software.

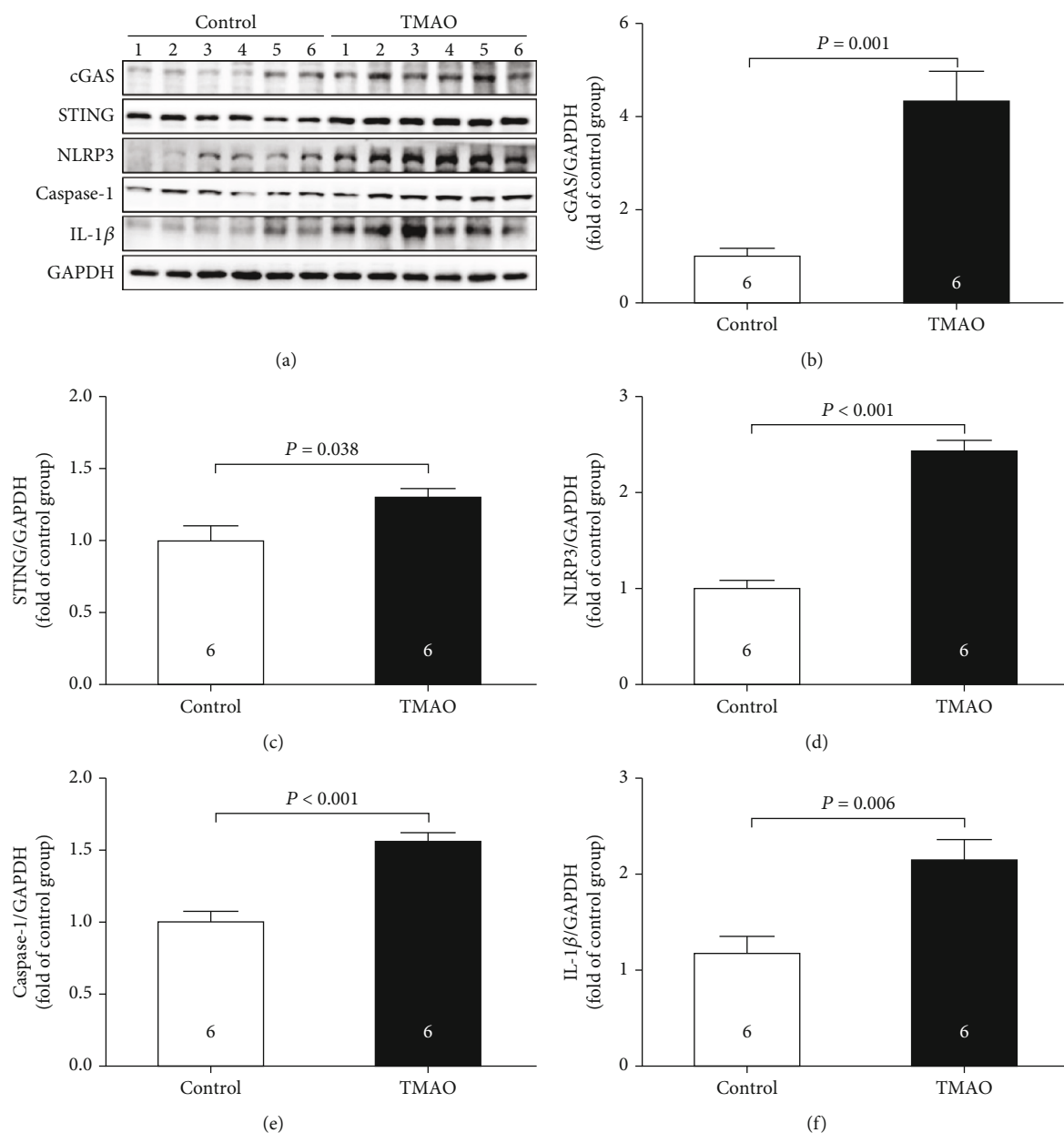


FIGURE 3: Dietary TMAO upregulated the protein expression of cGAS-STING-NLRP3 inflammasome axis. (a)–(f) Representative western blots and quantitative analysis for cGAS, STING, NLRP3, caspase-1, and IL-1 β protein expression in heart tissues. Results are expressed as mean \pm SEM. A P of <0.05 was considered significant.

2.5. Statistical Analysis. The experimental data were presented as mean \pm SEM and statistical significance assessed in SPSS (SPSS 13.0, Inc., Chicago, the United States) using independent t-test to compare values between two groups and one-way ANOVA followed by least significant difference t-test to compare values between multiple groups. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Dietary Choline Induced Cardiac Dysfunction in Mice. As was shown in Figures 1(a)–1(c), EF and FS, the indicators of cardiac function measured by echocardiography, were sig-

nificantly decreased in mice fed a diet containing 1.3% choline as compared to the control. To better understand the mechanism of the action of dietary choline, we quantified the protein expression of cGAS-STING-NLRP3 inflammasome axis in the heart (Figures 1(d)–1(i)) and found that choline significantly increased the protein levels of cGAS, STING, NLRP3, caspase-1, and IL-1 β as compared to the control.

3.2. Dietary Choline Induced Cardiac Dysfunction by Generating TMAO in Mice. To explore whether TMAO produced by choline degradation was involved in choline-induced cardiac dysfunction, DMB, a structural analog of

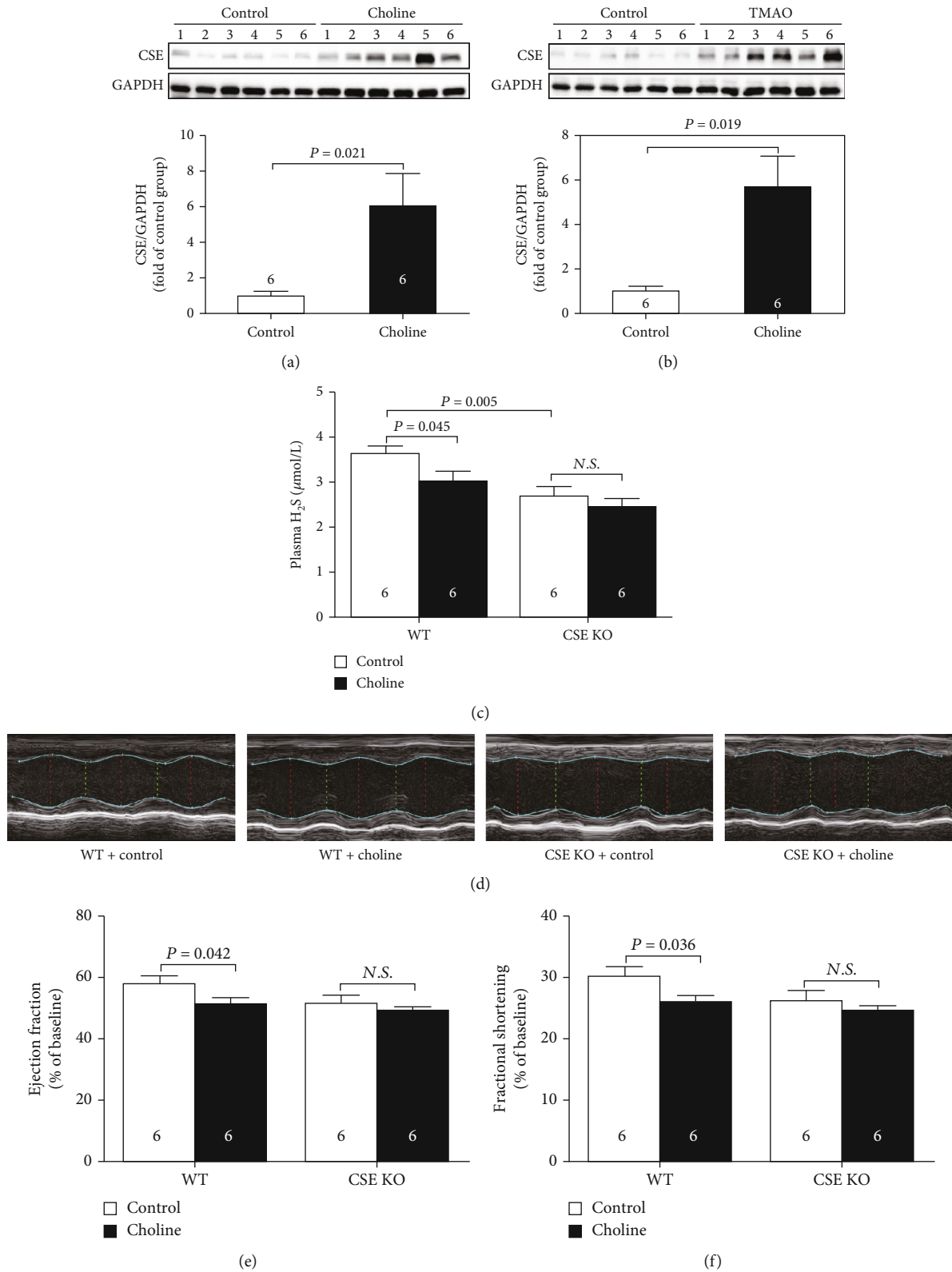
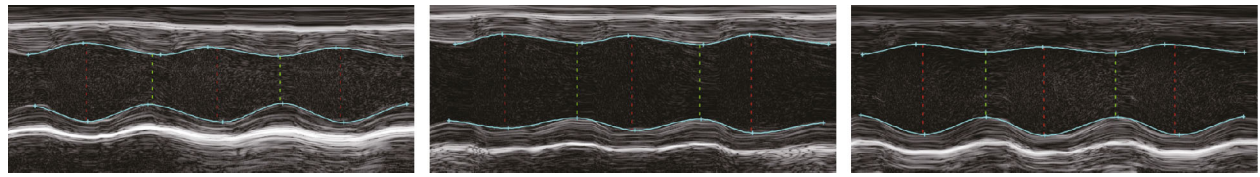
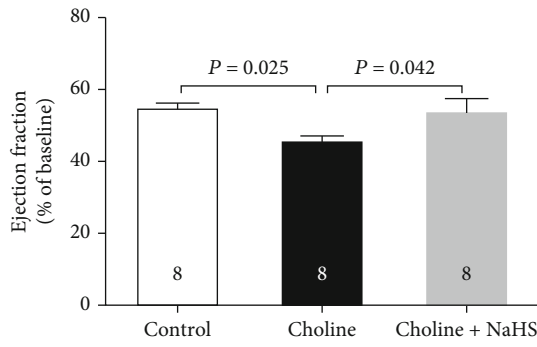


FIGURE 4: Dietary choline inhibited the endogenous production of H_2S . (a) Representative western blots and quantitative analysis for CSE protein expression in heart tissues after dietary supplementation with choline. (b) Representative western blots and quantitative analysis for CSE protein expression in heart tissues after dietary supplementation with TMAO. (c) Plasma H_2S levels in CSE KO mice. (d) Representative M-mode images in CSE KO mice. (e) The changes of left ventricular ejection fraction (LVEF) in CSE KO mice. (f) The changes of left ventricular fractional shortening (LVFS) in CSE KO mice. Results are expressed as mean \pm SEM. A P of <0.05 was considered significant.

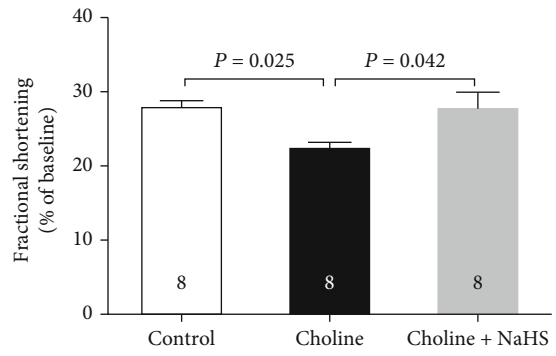


Control Choline Choline + NaHS

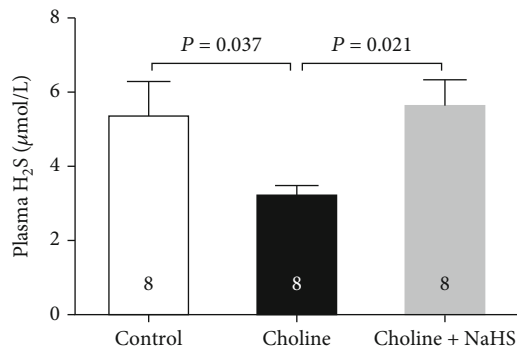
(a)



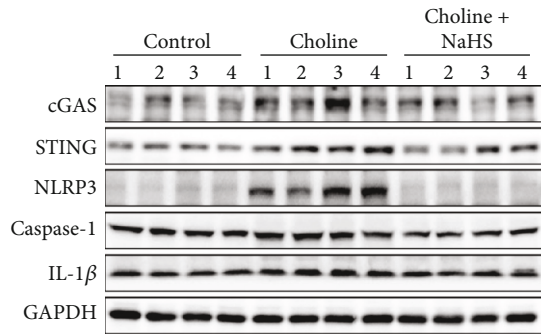
(b)



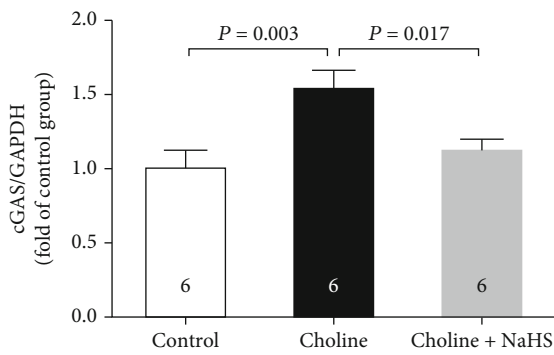
(c)



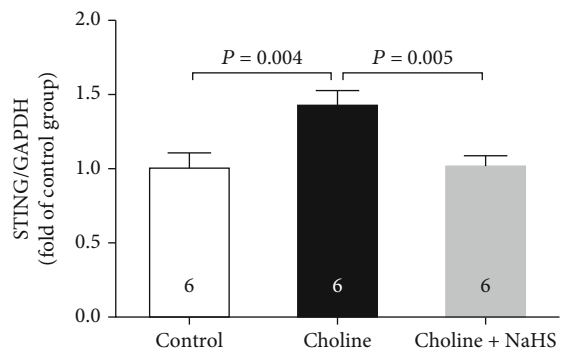
(d)



(e)



(f)



(g)

FIGURE 5: Continued.

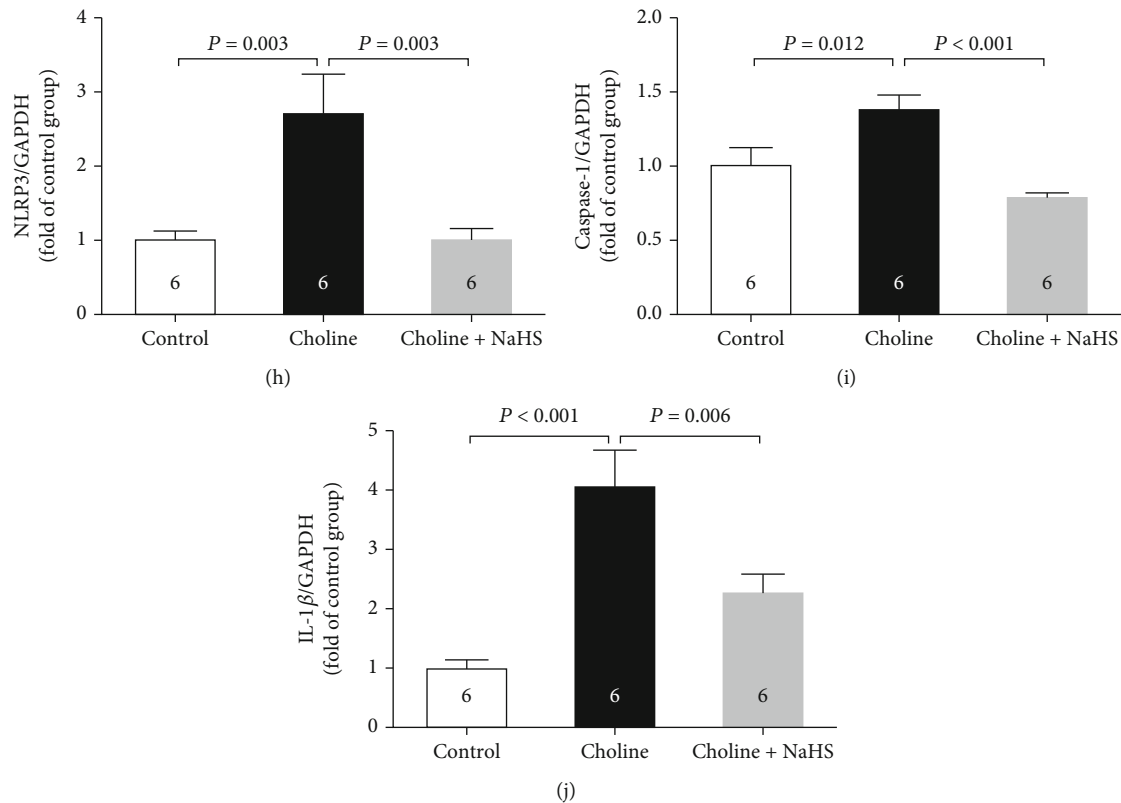


FIGURE 5: Exogenous H_2S improved choline induced-cardiac dysfunction. (a) Representative M-mode images. (b) The changes of left ventricular ejection fraction (LVEF) after NaHS treatment. (c) The changes of left ventricular fractional shortening (LVFS) after NaHS treatment. (d) Plasma H_2S levels after NaHS treatment. (e)–(j) Representative western blots and quantitative analysis for cGAS, STING, NLRP3, caspase-1, and IL-1 β protein expression in heart tissues after NaHS treatment. Results are expressed as mean \pm SEM. A P of <0.05 was considered significant.

choline, was used to inhibit TMAO formation. As was shown in Figure 2(a)–2(c), addition of DMB in the drinking water substantially ameliorated EF and FS as compared to the Choline group. DMB also markedly inhibited the choline diet-induced increase in the protein levels of cGAS, STING, NLRP3, caspase-1, and IL-1 β (Figures 2(d)–2(i)). In addition, the protein levels of cGAS, STING, NLRP3, caspase-1, and IL-1 β were significantly increased in mice receiving dietary TMAO as compared to the control (Figures 3(a)–3(f)).

3.3. Dietary Choline Inhibited the Endogenous Production of H_2S . As was shown in Figures 4(a) and 4(b), the protein expressions of CSE, the main enzyme for H_2S production in the cardiovascular system, were significantly increased after dietary supplementation with choline or TMAO, which indicated that endogenous H_2S was involved in choline-induced cardiac dysfunction. So, WT and CSE KO mice were fed with choline. As was shown in Figure 4(c), the plasma H_2S levels in WT mice were significantly decreased after dietary supplementation with choline, while there was no difference in the plasma H_2S levels between CSE KO + control and CSE KO + choline group. EF and FS were significantly decreased in the WT mice fed with choline, but there was also no significant difference in EF and FS between CSE KO + control and CSE KO + choline group (Figures 4(d)–4(f)).

3.4. Exogenous H_2S Improved Choline-Induced Cardiac Dysfunction. As was shown in Figures 5(a)–5(c), compared with the choline group, both EF and FS were significantly increased in the choline + NaHS group; meanwhile, the plasma H_2S levels were also markedly increased in the choline + NaHS group (Figure 5(d)). In addition, NaHS reduced the protein levels of cGAS, STING, NLRP3, caspase-1, and IL-1 β in mice receiving dietary choline (Figure 5(e)–5(j)).

4. Discussion

In the present study, we found that high choline diet induced cardiac dysfunction via cGAS-STING-NLRP3 inflammasome axis while H_2S treatment could restore the cardiac function by inhibiting cGAS-STING-NLRP3 inflammasome axis.

Although it played vital physiological roles in the development and function of the cardiovascular system as an essential nutrient, emerging evidence implicated that higher dietary intakes of choline were also associated with increased risk of acute myocardial infarction (MI) in patients with stable angina pectoris [20]. Moreover, a high-choline diet was shown to exacerbate the cardiac function and cardiac fibrosis of MI mice through accelerating the transformation of fibroblasts into myofibroblasts [21]. Choline in the diet can be metabolized to trimethylamine (TMA)

by the intestinal microorganisms. After being absorbed into the blood, TMA enters the liver and is oxidized to TMAO which is involved in the onset and development of cardiovascular disease. It was reported that two weeks of TMAO injection significantly induced cardiac hypertrophy and fibrosis in rats [22]. In the present study, the cardiac function represented by EF and FS was significantly decreased in mice after 4 months of 1.3% choline feeding, while applying DMB to suppress TMAO generation improved the cardiac function. Subsequently, we found that feeding choline or TMAO promoted NLRP3 inflammasome formation as well as caspase-1 and IL-1 β activation. The NLRP3 inflammasome is an intracellular protein complex activated upon tissue injury. Once activated, it can trigger and amplify sterile inflammatory responses by activating and releasing IL-1 β , which has been reported to involve in the pathophysiology cardiovascular disease [23, 24]. In line with our findings, one study reported that choline uptake in bone-marrow-derived macrophages regulated activation of the NLRP3 inflammasome, whereas impaired choline uptake and phosphorylation reduced NLRP3 inflammasome activation and inhibited IL-1 β production [25]. Another study reported that TMAO aggravated doxorubicin-induced mouse cardiac fibrosis through activation of the NLRP3 inflammasome [26]. Moreover, Wu et al. reported that either a high-choline diet or TMAO enhanced the allogeneic graft-versus-host (GVH) reaction which was mediated by NLRP3 inflammasome activation-induced macrophage polarization, whereas DMB reversed choline-induced GVH disease severity [27].

Given the chemical and structural diversity of NLRP3-activating stimuli, it is unlikely that those stimuli directly bind to and activated NLRP3. Instead, NLRP3 is likely to sense a common cellular signal induced in response to NLRP3 activators. Multiple molecular or cellular events including K⁺ efflux, Ca²⁺ signaling, reactive oxygen species, mitochondrial dysfunction, and lysosomal damage, were involved in the activation of NLRP3 inflammasome assembly [28, 29]. Recently, it was found that mitochondrial DNA (mtDNA) which was released into the cytoplasm played an important role in the activation of the inflammasome [30]. The DNA sensor cGAS interacted with mtDNA and generated the second messenger cGAMP, which trigger the cGAS-STING-NLRP3 pathway to activate inflammasome response [31]. In the present study, we found that feeding choline or TMAO increased the protein expression of cGAS and STING, while DMB markedly inhibited the choline diet-induced increase in the protein levels of cGAS and STING, which indicated that feeding choline or TMAO activated the cGAS-STING pathway. Although there was no direct evidence that choline or TMAO promoted mtDNA release, it was confirmed that TMAO altered mitochondrial energy metabolism [32] and enhanced the mitochondrial impairments [33], which might induce mtDNA release to trigger the cGAS-STING-NLRP3 pathway [34].

As the third endogenous signaling gasotransmitter, H₂S participates in a wide spectrum of physiological processes in the body including regulating mitochondrial function. Although higher concentrations inhibit the electron trans-

port chain [35], lower concentrations promote mitochondrial biogenesis and function [36, 37]. However, to the best of our knowledge, there are currently no studies exploring the link between H₂S and the cGAS-STING pathway. In the present study, we found that dietary choline significantly decreased the plasma H₂S levels, while application of H₂S donor, NaHS, significantly increased plasma H₂S levels and inhibited cGAS-STING pathway. However, how H₂S inhibited the cGAS-STING pathway still needed to be further elucidated. In addition, we also found that NLRP3 inflammasome activation was inhibited by NaHS, which was generally consistent with our previous studies and others' reports. Our previous studies clarified that H₂S improved hypertension-associated endothelial dysfunction [38] or attenuated lipopolysaccharide-induced acute kidney injury [39] by inhibiting NLRP3 inflammasome. H₂S was also reported to protect against dextran sulfate sodium-induced colitis [40] or paraquat-induced acute liver injury [41] by inhibiting NLRP3 inflammasome.

Moreover, we found that the protein expressions of CSE, the main enzyme for H₂S production in the cardiovascular system, were significantly increased after dietary supplementation with choline or TMAO, but the plasma H₂S levels were significantly decreased. Our results were consistent with previous studies in which a significant decrease in H₂S bioavailability was observed in the plasma, aorta, or myocardial tissue but a higher CSE expression in aorta or myocardial tissue [42, 43]. It was reported that the CSE could function as an inducible H₂S generating enzyme, whose expression was upregulated in cells by a range of stimuli including endoplasmic reticulum stress, oxidative stress, nutrient deprivation, and hyperhomocysteinemia [44]. The elevated CSE protein expression could be explained as a compensatory mechanism; although, this compensation did not increase plasma H₂S levels, which was due to the accelerated H₂S metabolism by choline or TMAO induced oxidative stress [45, 46]. On the other hand, CSE produced H₂S at the steady-state low intracellular Ca²⁺ concentrations in cells [47], whereas choline or TMAO could increase Ca²⁺ influx and/or Ca²⁺ release from intracellular stores to inhibit CSE activity and suppress H₂S generation [48, 49]. To further confirm the compensatory increased expression of CSE, CSE KO mice were used, and we found that the plasma H₂S levels in WT mice were significantly decreased after dietary supplementation with choline, while there was no difference in the plasma H₂S levels between CSE KO+control and CSE KO+choline group. Our finding meant that choline or TMAO relied on CSE protein to regulate H₂S levels, but the exact mechanisms remained unclear.

Several limitations of the present study should be noted. Firstly, direct evidence of how choline, TMAO, or H₂S regulated the cGAS-STING pathway needs to be found. Secondly, how choline or TMAO affected the expression of CSE or other H₂S generating enzyme need to be further explored in future studies.

In conclusion, our studies revealed that high choline diet decreased plasma H₂S levels and induced cardiac dysfunction via cGAS-STING-NLRP3 inflammasome axis while

H₂S treatment could restore the cardiac function by inhibiting cGAS-STING-NLRP3 inflammasome axis.

Data Availability

All data supported the findings of this study can be available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors would like to thank Professor Yichun Zhu (Fudan University, Shanghai, China) for kindly providing the CSE heterozygote mice. This study was supported by the National Natural Science Foundation of China (31871154, 91849120, and 31671185), the Natural Science Foundation of Hebei Province of China (H2020206417 and C2020206025), the Key R&D Project of Hebei Province (20277735D), and the Research Foundation for Higher Education of Hebei Province (ZD2019027).

References

- [1] A. M. Wiedeman, S. I. Barr, T. J. Green, Z. Xu, S. M. Innis, and D. D. Kitts, "Dietary choline intake: current state of knowledge across the life cycle," *Nutrients*, vol. 10, no. 10, p. 1513, 2018.
- [2] R. A. Bekdash, "Choline, the brain and neurodegeneration: insights from epigenetics," *Frontiers in Bioscience-Landmark*, vol. 23, no. 3, pp. 1113–1143, 2018.
- [3] A. M. Mahmoud and M. M. Ali, "Methyl donor micronutrients that modify DNA methylation and cancer outcome," *Nutrients*, vol. 11, no. 3, p. 608, 2019.
- [4] M. Xu, R. Q. Xue, Y. Lu et al., "Choline ameliorates cardiac hypertrophy by regulating metabolic remodelling and UPRmt through SIRT3-AMPK pathway," *Cardiovascular Research*, vol. 115, no. 3, pp. 530–545, 2019.
- [5] J. J. Yang, L. P. Lipworth, X. O. Shu et al., "Associations of choline-related nutrients with cardiometabolic and all-cause mortality: results from 3 prospective cohort studies of blacks, whites, and Chinese," *The American Journal of Clinical Nutrition*, vol. 111, no. 3, pp. 644–656, 2020.
- [6] C. L. Organ, H. Otsuka, S. Bhushan et al., "Choline diet and its gut microbe-derived metabolite, trimethylamine N-oxide, exacerbate pressure overload-induced heart failure," *Circulation. Heart Failure*, vol. 9, no. 1, article e002314, 2016.
- [7] W. Shuai, J. Wen, X. Li, D. Wang, Y. Li, and J. Xiang, "High-choline diet exacerbates cardiac dysfunction, fibrosis, and inflammation in a mouse model of heart failure with preserved ejection fraction," *Journal of Cardiac Failure*, vol. 26, no. 8, pp. 694–702, 2020.
- [8] P. Haouzi, T. Sonobe, and A. Judenherc-Haouzi, "Developing effective countermeasures against acute hydrogen sulfide intoxication: challenges and limitations," *Annals of the New York Academy of Sciences*, vol. 1374, no. 1, pp. 29–40, 2016.
- [9] K. Abe and H. Kimura, "The possible role of hydrogen sulfide as an endogenous neuromodulator," *The Journal of Neuroscience*, vol. 16, no. 3, pp. 1066–1071, 1996.
- [10] H. Kimura, "Hydrogen sulfide (H₂S) and polysulfide (H₂Sn) signaling: the first 25 years," *Biomolecules*, vol. 11, no. 6, p. 896, 2021.
- [11] N. Nagahara, "Multiple role of 3-mercaptopyruvate sulfurtransferase: antioxidative function, H₂S and polysulfide production and possible SOx production," *British Journal of Pharmacology*, vol. 175, no. 4, pp. 577–589, 2018.
- [12] G. Meng, S. Zhao, L. Xie, Y. Han, and Y. Ji, "Protein S-sulfhydration by hydrogen sulfide in cardiovascular system," *British Journal of Pharmacology*, vol. 175, no. 8, pp. 1146–1156, 2018.
- [13] S. Yuan, X. Shen, and C. G. Kevil, "Beyond a Gasotransmitter: hydrogen sulfide and polysulfide in cardiovascular health and immune response," *Antioxidants & Redox Signaling*, vol. 27, no. 10, pp. 634–653, 2017.
- [14] S. Mani, H. Li, A. Untereiner et al., "Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis," *Circulation*, vol. 127, no. 25, pp. 2523–2534, 2013.
- [15] G. Yang, L. Wu, B. Jiang et al., "H₂S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase," *Science*, vol. 322, no. 5901, pp. 587–590, 2008.
- [16] K. B. LaPenna, D. J. Polhemus, J. E. Doiron, H. A. Hidalgo, Z. Li, and D. J. Lefer, "Hydrogen sulfide as a potential therapy for heart failure-past, present, and future," *Antioxidants (Basel)*, vol. 10, no. 3, p. 485, 2021.
- [17] H. Zhang, J. Dai, D. Tian et al., "Hydrogen sulfide restored the diurnal variation in cardiac function of aging mice," *Oxidative Medicine and Cellular Longevity*, vol. 2, no. 2021, Article ID 8841575, 2021.
- [18] B. Tan, S. Jin, J. Sun et al., "New method for quantification of gasotransmitter hydrogen sulfide in biological matrices by LC-MS/MS," *Scientific Reports*, vol. 13, no. 7, p. 46278, 2017.
- [19] S. Jin, B. Tan, X. Teng et al., "Diurnal fluctuations in plasma hydrogen sulfide of the mice," *Frontiers in Pharmacology*, vol. 6, no. 8, p. 682, 2017.
- [20] A. Van Parys, V. Lysne, G. F. T. Svingen et al., "Dietary choline is related to increased risk of acute myocardial infarction in patients with stable angina pectoris," *Biochimie*, vol. 173, pp. 68–75, 2020.
- [21] W. Yang, S. Zhang, J. Zhu et al., "Gut microbe-derived metabolite trimethylamine N-oxide accelerates fibroblast-myofibroblast differentiation and induces cardiac fibrosis," *Journal of Molecular and Cellular Cardiology*, vol. 134, pp. 119–130, 2019.
- [22] Z. Li, Z. Wu, J. Yan et al., "Gut microbe-derived metabolite trimethylamine N-oxide induces cardiac hypertrophy and fibrosis," *Laboratory Investigation*, vol. 99, no. 3, pp. 346–357, 2019.
- [23] Y. Wang, X. Liu, H. Shi et al., "NLRP3 inflammasome, an immune-inflammatory target in pathogenesis and treatment of cardiovascular diseases," *Clinical and Translational Medicine*, vol. 10, no. 1, pp. 91–106, 2020.
- [24] M. Takahashi, "NLRP3 inflammasome as a key driver of vascular disease," *Cardiovascular Research*, vol. 118, no. 2, pp. 372–385, 2022.
- [25] E. Sanchez-Lopez, Z. Zhong, A. Stubelius et al., "Choline uptake and metabolism modulate macrophage IL-1 β and IL-18 production," *Cell Metabolism*, vol. 29, no. 6, pp. 1350–1362.e7, 2019.
- [26] X. Li, J. Geng, J. Zhao et al., "Trimethylamine N-oxide exacerbates cardiac fibrosis via activating the NLRP3 Inflammasome," *Frontiers in Physiology*, vol. 10, no. 10, p. 866, 2019.

- [27] K. Wu, Y. Yuan, H. Yu et al., "The gut microbial metabolite trimethylamine N-oxide aggravates GVHD by inducing M1 macrophage polarization in mice," *Blood*, vol. 136, no. 4, pp. 501–515, 2020.
- [28] Y. Yang, H. Wang, M. Kouadir, H. Song, and F. Shi, "Recent advances in the mechanisms of NLRP3 inflammasome activation and its inhibitors," *Cell Death & Disease*, vol. 10, no. 2, p. 128, 2019.
- [29] K. V. Swanson, M. Deng, and J. P. Ting, "The NLRP3 inflammasome: molecular activation and regulation to therapeutics," *Nature Reviews. Immunology*, vol. 19, no. 8, pp. 477–489, 2019.
- [30] Z. Zhong, S. Liang, E. Sanchez-Lopez et al., "New mitochondrial DNA synthesis enables NLRP3 inflammasome activation," *Nature*, vol. 560, no. 7717, pp. 198–203, 2018.
- [31] M. M. Gaidt, T. S. Ebert, D. Chauhan et al., "The DNA inflammasome in human myeloid cells is initiated by a STING-cell death program upstream of NLRP3," *Cell*, vol. 171, no. 5, pp. 1110–1124.e18, 2017.
- [32] M. Makrecka-Kuka, K. Volska, U. Antone et al., "Trimethylamine N-oxide impairs pyruvate and fatty acid oxidation in cardiac mitochondria," *Toxicology Letters*, vol. 267, pp. 32–38, 2017.
- [33] D. Li, Y. Ke, R. Zhan et al., "Trimethylamine-N-oxide promotes brain aging and cognitive impairment in mice," *Aging Cell*, vol. 17, no. 4, article e12768, 2018.
- [34] C. H. Yu, S. Davidson, C. R. Harapas et al., "TDP-43 triggers mitochondrial DNA release via mPTP to activate cGAS/STING in ALS," *Cell*, vol. 183, no. 3, pp. 636–649.e18, 2020.
- [35] E. Blackstone, M. Morrison, and M. B. Roth, "H₂S induces a suspended animation-like state in mice," *Science*, vol. 308, no. 5721, p. 518, 2005.
- [36] B. D. Paul, S. H. Snyder, and K. Kashfi, "Effects of hydrogen sulfide on mitochondrial function and cellular bioenergetics," *Redox Biology*, vol. 38, article 101772, 2021.
- [37] B. Murphy, R. Bhattacharya, and P. Mukherjee, "Hydrogen sulfide signaling in mitochondria and disease," *The FASEB Journal*, vol. 33, no. 12, pp. 13098–13125, 2019.
- [38] J. Li, X. Teng, S. Jin et al., "Hydrogen sulfide improves endothelial dysfunction by inhibiting the vicious cycle of NLRP3 inflammasome and oxidative stress in spontaneously hypertensive rats," *Journal of Hypertension*, vol. 37, no. 8, pp. 1633–1643, 2019.
- [39] Y. Chen, S. Jin, X. Teng et al., "Hydrogen sulfide attenuates LPS-induced acute kidney injury by inhibiting inflammation and oxidative stress," *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 6717212, 2018.
- [40] M. Qin, F. Long, W. Wu et al., "Hydrogen sulfide protects against DSS-induced colitis by inhibiting NLRP3 inflammasome," *Free Radical Biology & Medicine*, vol. 137, pp. 99–109, 2019.
- [41] Z. Liu, X. Wang, L. Li, G. Wei, and M. Zhao, "Hydrogen sulfide protects against Paraquat-induced acute liver injury in rats by regulating oxidative stress, mitochondrial function, and inflammation," *Oxidative Medicine and Cellular Longevity*, vol. 2020, Article ID 6325378, 2020.
- [42] Y. Wang, X. Zhao, H. Jin et al., "Role of hydrogen sulfide in the development of atherosclerotic lesions in apolipoprotein E knockout mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 2, pp. 173–179, 2009.
- [43] L. Chang, B. Geng, F. Yu et al., "Hydrogen sulfide inhibits myocardial injury induced by homocysteine in rats," *Amino Acids*, vol. 34, no. 4, pp. 573–585, 2008.
- [44] J. I. Sbdio, S. H. Snyder, and B. D. Paul, "Regulators of the transsulfuration pathway," *British Journal of Pharmacology*, vol. 176, no. 4, pp. 583–593, 2019.
- [45] C. Y. Chen, H. B. Leu, S. C. Wang et al., "Inhibition of trimethylamine N-oxide attenuates neointimal formation through reduction of inflammasome and oxidative stress in a mouse model of carotid artery ligation," *Antioxidants & Redox Signaling*, vol. 2022, 2022.
- [46] Y. Ke, D. Li, M. Zhao et al., "Gut flora-dependent metabolite trimethylamine-N-oxide accelerates endothelial cell senescence and vascular aging through oxidative stress," *Free Radical Biology & Medicine*, vol. 20, no. 116, pp. 88–100, 2018.
- [47] Y. Mikami, N. Shibuya, Y. Ogasawara, and H. Kimura, "Hydrogen sulfide is produced by cystathionine γ -lyase at the steady-state low intracellular Ca²⁺ concentrations," *Biochemical and Biophysical Research Communications*, vol. 431, no. 2, pp. 131–135, 2013.
- [48] R. Seddik, A. Bradaia, and J. Trouslard, "Choline induces Ca²⁺ entry in cultured sympathetic neurones isolated from rat superior cervical ganglion," *European Journal of Pharmacology*, vol. 471, no. 3, pp. 165–176, 2003.
- [49] W. Zhu, J. C. Gregory, E. Org et al., "Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk," *Cell*, vol. 165, no. 1, pp. 111–124, 2016.