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# Pde5 Inhibition Reduced Blood Pressure and Alleviated Target Organ Damage in Chronic Intermittent Hypoxia

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*See accompanying Commentary by L.A. Gottlieb on pages 32-33.*

**Abstract:** The role of phosphodiesterase 5 (Pde5) in obstructive sleep apnea–induced damage remains unclear. Our study aimed to investigate the role of Pde5 in the chronic intermittent hypoxia (CIH) model. C57BL/6J wild-type (WT) mice (n = 48) and Pde5 knockout ($Pde5^{-/-}$) mice (n = 24) were randomly assigned to CIH group and room air group. After 6 weeks, some WT mice (n = 24) in CIH group were given sildenafil or saline gavage for another 4 weeks. Blood pressure was regularly measured during the experiment. Echocardiography was used to estimate cardiac function. We collected organs from each group of mice and measured their physical indicators. Histochemical staining was used to explore the size of cardiomyocyte and fibrosis area of various organs. Cyclic guanosine monophosphate and malondialdehyde concentrations in serum were measured by ELISA assay. Compared with the RA-treated group, the 6-week CIH resulted in a significant increase in blood pressure, altered heart structure, and reduced serum cyclic guanosine monophosphate in WT mice. $Pde5^{-/-}$ mice and sildenafil intragastric administration significantly reduced systolic blood pressure in CIH condition and attenuated the damage of target organs. In CIH model, we found that the cardiomyocyte size and fibrosis area of heart and kidney significantly reduced in $Pde5^{-/-}$ groups. Besides, endogenous and exogenous inhibition of Pde5 reduced malondialdehyde level and inflammatory and oxidative stress markers expression in CIH condition. In this study, we found that Pde5 inhibition could reduce blood pressure and alleviate target organ damage in the CIH model, which may be mediated through the oxidative stress pathway.

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**Key Words:** phosphodiesterase 5, chronic intermittent hypoxia, blood pressure, obstructive sleep apnea

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## INTRODUCTION

Obstructive sleep apnea (OSA) is a kind of sleep breathing disorder characterized by recurrent complete or partial upper airway occlusion.[1] A previous study found that more than 1 billion people worldwide may already have OSA, with prevalence rates exceeding 50% in some countries.[2] OSA is an independent risk factor for cardiovascular disease, including arterial hypertension, ischemic heart disease, cardiac hypertrophy, and organ fibrosis.[3–5] Notably, large prospective cohort studies have shown an increased prevalence of hypertension in patients with moderate-to-severe OSA.[6,7] In patients with mild OSA, the prevalence of hypertension ranged from 30% to 50%, while in those with severe OSA, the prevalence increased from 80% to 90%.[8] In addition, OSA is the most common cause of secondary resistant hypertension.[9] There is substantial evidence to suggest that OSA exerts pathophysiological effects on cardiovascular diseases through various mechanisms, including sympathetic activation, inflammation, and oxidative stress.[10]

Phosphodiesterase 5 (Pde5), a cyclic guanosine monophosphate (cGMP)–specific hydrolase, regulates vasodilatation by controlling cGMP levels. CGMP, a common intracellular second messenger, is a regulator of ion channel conductance, glycogenolysis, and smooth muscle tissue relaxation.[11] Jafari et al found reduced cGMP concentrations and decreased vasodilatory capacity in patients with OSA and patients with OSA with hypertension.[12] Reduced cGMP level was also observed in wild-type (WT) mice treated with chronic intermittent hypoxia (CIH).[13] Previous studies have demonstrated that the Pde5 inhibitor tadalafil reduces infarct size and improves cardiac function after ischemia–reperfusion injury in mice.[14] Another study showed that Pde5 inhibitor reversed the hypertrophy caused by transaortic constriction and improved the ejection fraction (EF) in heart failure.[15] Our previous studies confirmed that inhibition of Pde5 can play a protective role in ischemic hearts.[16] However, none of the studies have yet demonstrated the role of Pde5 in cardiovascular diseases caused by OSA. Whether Pde5 is a potential key molecule and a new therapeutic target in cardiovascular diseases caused by CIH exposure is still unknown.

In this study, we aimed to investigate the role and potential mechanism of Pde5 in CIH model in mice.

# METHODS

## Establish Pde5 Knockout Mice

Pde5 knockout (Pde5$^{-/-}$) mice were acquired from GemPharmatech Co., Ltd. We produced Pde5$^{-/-}$ mice using C57BL/6J background mice. Using clustered regularly interspaced short palindromic repeats (CRISPR) / CRISPR-associated protein 9 (Cas9)-facilitated genomic engineering, we established the Pde5$^{-/-}$ mice. The Pde5 gene resided on chromosome 3. We adopted Transcript Pde5-201 (ENSMUST00000066728.9) to delineate our targeting approach, which encompassed 21 exons (adenine-thymine-guanine [ATG] start codon in exon 1, thymine-guanine-adenine [TGA] stop codon in exon 21), a 6939-bp transcript length, and an 865-residue translation length. Customized gRNAs were devised in intron 1–2 and an additional site in intron 2–3, directing Cas9 endonuclease-mediated Pde5 gene cleavage and producing a double-strand break. The subsequent nonhomologous end joining repair yielded exon 2 deletion (589 bp) and Pde5 gene disruption through frame shift mutation. Our animal study adhered to the Guide for the Care and Use of Laboratory Animals provided by the US National Institutes of Health. The Animal Care and Use Committee of Capital Medical University granted approval for all animal experiments.

## Animals and Establish CIH Model

C57BL/6J WT male mice (n = 48) and Pde5$^{-/-}$ male mice (n = 24) were procured from Beijing HFK Bioscience Co., Ltd and GemPharmatech Co., Ltd, correspondingly. Mice were accommodated in standard polypropylene enclosures and subjected to regulated temperature (22 ± 1°C) and humidity (50%–60%) conditions, with a 12-hour light/dark cycle. Unrestricted access to standard nourishment and tap water was granted.

Altogether, 36 C57BL/6J WT mice and 12 Pde5$^{-/-}$ mice were randomly allocated into the CIH group, and the remaining 24 mice (12 WT mice and 12 Pde5$^{-/-}$ mice) were assigned to the room air (RA) group. CIH group mice were housed in custom standard cages and subjected to CIH using an Oxycycler A84 system (BioSpherix). The oxygen fraction cycled from 20.9% to 5.0%–6.0% over a 120-second interval, followed by rapid reoxygenation to normal air concentrations through a 100% oxygen surge in the next 60 seconds. Hypoxic events occurred at a rate of 1 per 180 seconds during the 12-hour dark phase, while CIH mice experienced normoxic conditions throughout the 12-hour light phase. Mice in the RA control group were consistently exposed to normoxic air. After 6 weeks of CIH or RA exposure, all Pde5$^{-/-}$ mice (n = 24) were euthanized and all RA-treated WT mice (n = 12) and a portion of CIH-treated WT mice (n = 12) were euthanized. The residual CIH-treated WT mice (n = 24) were indiscriminately apportioned into the sildenafil-treated group and the saline-treated group, continuing CIH treatments. Sildenafil (5 mg/kg, purity >98%; Sigma-Aldrich) or an equivalent volume of saline was dispensed daily through intragastric administration for 4 weeks (Fig. 1).

## Blood Pressure Measurement

For the first phase of the experiment, systolic blood pressure (SBP) was measured every 3 days using the tail-cuff method. In brief, conscious animals were placed in a restraining cage, and their tails were placed through a cuff equipped with a photoelectric sensor. Changes in pressure applied to the cuff and arterial pulsations were amplified (IITC, Woodland Hills, CA) and recorded on polygraph tracings. At least 10 measurements were taken for each animal, and the average was calculated as the SBP. For the second phase

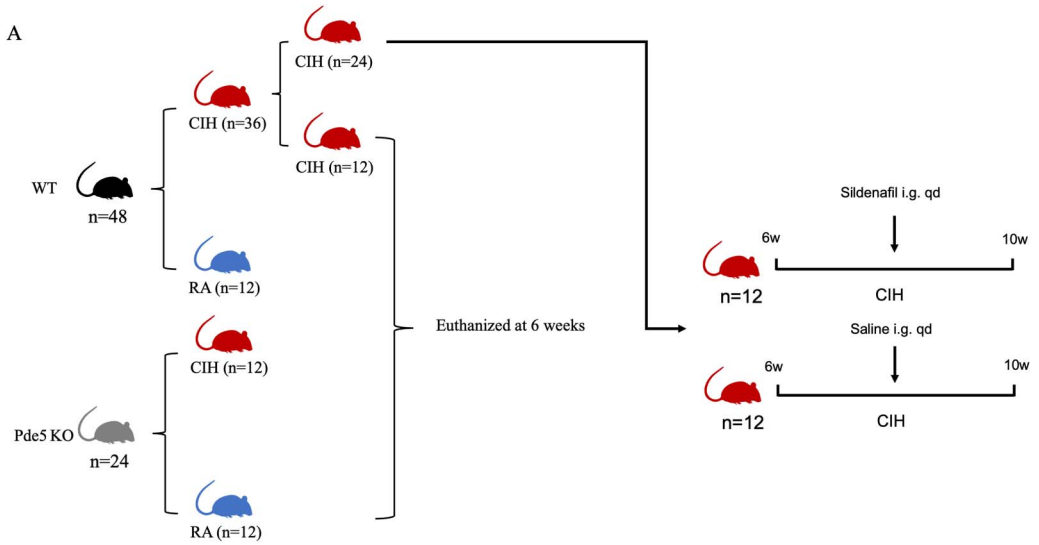


**FIGURE 1.** Study design and experimental groups. WT (n = 48) and Pde5$^{-/-}$ mice (n = 24) were exposed to CIH or RA for 6 weeks. WT mice were divided into CIH exposure (n = 36) and RA control (n = 12) groups. Pde5$^{-/-}$ mice were divided into CIH exposure (n = 12) and RA control (n = 12) groups. All Pde5$^{-/-}$ mice (n = 24) and half WT mice (12 in RA and 12 in CIH) were euthanized after 6 weeks of experiment. Treatment with sildenafil or saline control began after 6 weeks of CIH exposure in WT mice, as indicated by the arrows. The remaining mice were treated with daily oral gavage of sildenafil or saline for the last 4 weeks of the 10-week experimental period.

of the experiment, SBP was measured every 5 days using the same tail-cuff method. At least 10 measurements were taken for each animal, and the average was calculated as the SBP.

## Echocardiography

Vevo 770 High-Resolution Imaging System (Visual Sonics, Inc) with a 15 MHz ultrasound was used to evaluate the cardiac function at weeks 6 and 10 after CIH. Mice were anesthetized with a mixture of 4%–5% isoflurane and oxygen inhalation and maintained with a mixture of 1%–2% isoflurane and oxygen. Parasternal long-axis and midventricular short-axis views were obtained in the 2-dimensional mode, and M-mode tracings at the papillary muscle level were recorded. The relevant parameters were obtained by calculating the average of the 5 cardiac cycles. Vevo 2100 1.6.0 was used to analyze the indicators such as left ventricular posterior wall diastole, left ventricular posterior wall systolic, left ventricular internal dimension diastole (LVID; d), left ventricular internal dimension systolic (LVID; s), EF%, and fractional shortening (%) (FS%).

## Histology Analysis

Heart and kidney tissues from the mice were fixed with 10% paraformaldehyde, dehydrated through a graded series of alcohols, embedded in paraffin, and sectioned to a thickness of 5 μm for Masson's trichrome and hematoxylin and eosin (H&E) staining. Masson's trichrome staining (Solarbio, G1340) was performed following standard protocols as previously described in the literature. The areas of ventricle and renal interstitial fibrosis were quantified using the NISELEMENTS quantitative automatic program (Nikon) in Masson's trichrome-stained heart and renal sections. Fibrosis was expressed as a percentage of the fibrosis area to the total heart area. For HE staining, sections were stained with Harris hematoxylin (Solarbio, G1120) for 5 minutes, washed with 0.6% ammonia in water to restore the blue color, and then washed again before being stained with eosin for 3 minutes. The sections were then mounted and observed under a microscope. Images of the cross-sectional area of stained cardiomyocytes were obtained using a 400× microscope. The cross-sectional area of all cardiomyocytes was then quantified, and the average value was calculated during the late analysis.

## ELISA

After sacrificing the mice, blood was collected to measure cGMP and malondialdehyde (MDA) levels. The level of cGMP was measured using the cGMP ELISA Kit (elabscience, China), following the manufacturer's instructions. Levels of MDA in the serum were determined using the MDA Colorimetric Assay Kit (elabscience, China), according to the manufacturer's protocol. The reaction product was measured spectrophotometrically at 586 nm. Standard curves were constructed with 1,1,3,3-tetraethoxypropane as the standard.

## RNA Extraction and Real-Time Polymerase Chain Reaction Analysis

According to the protocol provided by the manufacturer (Invitrogen), we extracted total RNA from the ventricle by using the Trizol method. RevertAid RT Reverse Transcription Kit (Thermo Fisher Scientific) was used for reverse transcription of mRNA and synthesis of complementary DNA. SYBR Premix Ex Taq (Takara) was used for real-time polymerase chain reaction. All the primers are shown in Table 1. CFX96 Real-Time PCR Detection System was used for real-time polymerase chain reaction (Bio-Rad). The β-actin was used as the internal reference, and the 2 -ΔΔCt method was used for transcriptional change evaluation. The formula was expressed as $\Delta\Delta Ct = \Delta Ct_{\text{experimental group}} - \Delta Ct_{\text{control group}}$ in which $\Delta Ct = Ct_{\text{target gene}} - Ct_{\text{internal reference}}$.

## Statistical Analysis

Data are expressed as means ± SD for normally distributed data, and median ± interquartile range (25th to 75th percentiles) for non-normally distributed data. Data distribution was assessed using the Shapiro–Wilk test. Comparisons between the 2 groups were performed using unpaired Student's *t* tests. Comparisons between multiple groups were analyzed using one-way or two-way analysis of variance followed by Tukey's or Sidak's post hoc tests for multiple comparisons. Normality and equal variance tests were performed to ensure the data met the assumptions of the parametric tests. Non-normally distributed unpaired data were compared using the Mann–Whitney *U* test or the Kruskal–Wallis test. GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA) was used for statistical analyses. $P < 0.05$ were considered significant.

# RESULTS

## Endogenous and Exogenous Inhibition of Pde5 can Exert a Protective Effect in Elevated SBP Caused by CIH

Compared with the RA group, WT mice displayed a marked augmentation in SBP on CIH exposure. The SBP of WT mice progressively escalated from the second week, attaining statistical significance in comparison with RA mice by the third week. Thereafter, the SBP steadily rose throughout the following 4 weeks of CIH exposure (Figs. 2A, B). When we continued the experiment in the CIH environment using $Pde5^{-/-}$ mice, we found $Pde5^{-/-}$ mice had lower blood pressure in the RA group and did not show a CIH-mediated blood pressure increase (Figs. 2A, B). Subsequently, we evaluated the effects of exogenous Pde5 inhibitor on WT mice subjected to CIH. WT mice in CIH condition for 6 weeks were divided into 2 groups. One group was given sildenafil gavage, and the other group was given the same volume saline gavage and continued to receive CIH for 4 weeks. After intragastric instillation of sildenafil, the SBP of CIH mice decreased by nearly 20% and remained stable for the remaining time (Fig. 2C). When measuring serum cGMP concentrations, we found that the $Pde5^{-/-}$ mice showed a significant increase in cGMP concentrations after 6 weeks of CIH compared with WT mice, but it was still lower than $Pde5^{-/-}$ mice in RA environment (Fig. 2D). In WT mice treated with sildenafil, plasma cGMP levels were

**TABLE 1.** Primer Sequences

| Primers | Forward | Reverse |
|---|---|---|
| β-actin | 5′-TAAAGACCTCTATGCCAACACAGT-3′ | 5′-CACGATGGAGGGGCCGGACTCATC-3′ |
| CRP | 5′-AGCCTCTCTCATGCTTTTGG-3′ | 5′-TGTCTCTTGGTGGCATACGA-3′ |
| IL-6 | 5′-CCGGAGAGGAGACTTCACAG-3′ | 5′-TCCACGATTTCCCAGAGAAC-3′ |
| G-CSF | 5′-CAGGCTCTATCGGGTATTT-3′ | 5′-GGAAGGCAGAAGTGAAGG-3′ |
| TNF-α | 5′-TCTTCTCATTCCTGCTTGTGG-3′ | 5′-ATGAGAGGGAGGCCATTTG-3′ |
| NOX1 | 5′-TGAACAACAGCACTCACCAATGCC-3′ | 5′-TCATTGTCCCACATTGGTCTCCCA-3′ |
| SOD-1 | 5′-CAGGACCTCATTTTAATCCTCAC-3′ | 5′-TGCCCAGGTCTCCAACAT-3′ |

CRP, C-reactive protein; G-CSF, granulocyte colony–stimulating factor; IL-6, interleukin 6; TNF-α, tumor necrosis factor-alpha. NOX1, NADPH oxidase 1; SOD1, superoxide dismutase type 1.

restored compared with the saline group during CIH period (Fig. 2E).

## Endogenous and Exogenous Inhibition of Pde5 Ameliorated Ventricular Remodeling after CIH

When performed echocardiography to detect the cardiac structure and function of the $Pde5^{-/-}$ mice, no obvious differences in EF% and FS% were observed among the 4 groups (Figs. 3A, B). Furthermore, diminished levels of LVID, d, and LVID, s, were partially restored in $Pde5^{-/-}$ mice treated by CIH compared with WT mice under CIH. However, LVID, d, and LVID, s, of $Pde5^{-/-}$ mice under CIH were still lower than $Pde5^{-/-}$ mice under RA conditions (Fig. 3C). In addition, $Pde5^{-/-}$ mice negated the impact of CIH on left ventricular posterior wall systolic, s, and left ventricular posterior wall diastole, d, yet remained elevated compared with $Pde5^{-/-}$ mice reared under RA conditions (Fig. 3D). The heart weight/body weight in $Pde5^{-/-}$ mice marginally declined, though not reaching statistical significance when compared with the CIH group (Fig. 3E). H&E staining results revealed that CIH induced hypertrophy in mouse cardiomyocytes. This cardiomyocyte hypertrophy was counteracted in $Pde5^{-/-}$ groups, exhibiting no significant variation in cardiomyocyte hypertrophy levels between RA and CIH groups (Figs. 3F–H). Furthermore, intragastric instillation of sildenafil ameliorated cardiac functionality, as evidenced by the appreciable augmentation of EF (%) and FS (%) (Figs. 4A, B). A markedly diminished heart weight/body weight manifested in the sildenafil-administered group relative to the saline-administered group (Fig. 4C). H&E staining demonstrated sildenafil administration moderately attenuated cardiomyocyte hypertrophy (Fig. 4D).

## Endogenous and Exogenous Inhibition of Pde5 Reduced CIH-Induced Fibrosis of Target Organ

$Pde5^{-/-}$ mitigated both cardiac and renal fibrotic manifestations resulting from CIH exposure (Fig. 5A). Masson's trichrome staining unveiled that in WT mice, CIH exposure culminated in augmented levels of cardiac fibrosis relative to the ambient air group. Nevertheless, compared with WT mice, fibrotic manifestations were markedly ameliorated in $Pde5^{-/-}$ mice within the CIH group (Fig. 5A). Analogous outcomes materialized in renal tissue as in cardiac tissue, wherein the CIH group of WT mice displayed substantially elevated levels of renal fibrosis compared with the RA group. Conversely, fibrotic manifestations were notably diminished in $Pde5^{-/-}$ mice than those of WT mice within the CIH condition, implying that $Pde5^{-/-}$ can effectively attenuate the fibrotic response in both cardiac and renal tissues under the CIH exposure (Fig. 5B). In parallel, fibrosis levels of the heart and kidney were found to be substantially reduced in the Pde5 inhibitor group compared with the saline group (Figs. 5C, D). This reduction in fibrotic manifestations highlighted the potential therapeutic efficacy of Pde5 inhibition in mitigating the adverse effects of CIH exposure.

## Endogenous and Exogenous Pde5 Inhibition Reduces the Expression of Inflammatory and Oxidative Stress Markers

Oxidative stress and inflammatory response induced by CIH were important causes of deteriorating SBP response and organ damage. Levels of MDA in the serum and expression of C-reactive protein, tumor necrosis factor-alpha, and NADPH oxidase 1 were significantly increased in the WT mice treated with 6 weeks of hypoxia when compared with the normoxic mice. In addition, CIH did not affect serum MDA levels and ventricular C-reactive protein, tumor necrosis factor-alpha, and NADPH oxidase 1 expression in $Pde5^{-/-}$ mice (Figs. 6A, 7). Simultaneously, the observed decrease in MDA concentrations and expression of inflammatory and oxidative stress markers indicated mitigation of oxidative stress in the sildenafil-treated mice, suggestive of an overall improvement in their cardiovascular health (Figs. 6B, 8).

# DISCUSSION

As a prevalent global disease, OSA was a risk factor for a multitude of adverse cardiovascular outcomes. In this study, we have ascertained that the suppression of Pde5 expression reduced CIH-induced increases in blood pressure and myocardial hypertrophy, improved cardiac function, and decreased cardiac and renal fibrosis, potentially through the re-establishment of cGMP concentrations and attenuation of oxidative stress.

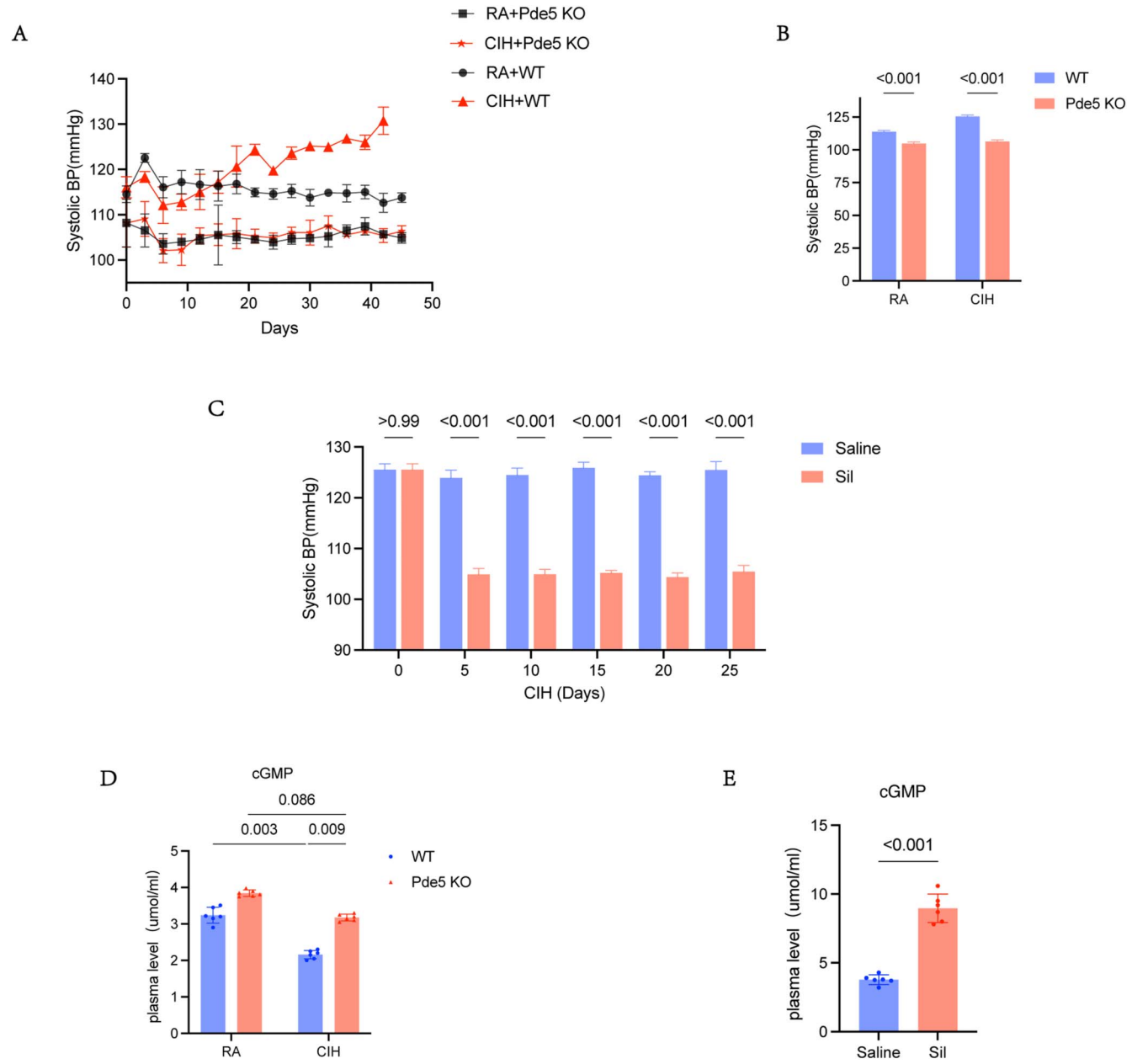


**FIGURE 2.** Pde5$^{-/-}$ and sildenafil treatment attenuated elevated SBP and plasma cGMP caused by CIH. A, Changes in SBP in the Pde5$^{-/-}$ and WT mice exposed to RA or CIH for 6 weeks, n = 12. B, Changes of SBP in WT and Pde5$^{-/-}$ mice after 6 weeks of RA and CIH, n = 12. C, Changes in SBP after intragastric administration of saline or sildenafil under CIH conditions. Time 0 represents the end of the initial 6 weeks of CIH exposure, n = 12. D, Changes of cGMP in WT and Pde5$^{-/-}$ mice after 6 weeks of RA and CIH, n = 6. E, Plasma levels of cGMP after intragastric administration of saline or sildenafil under CIH conditions, n = 6. KO, knock out.

In the current research, we have established that the inhibition of Pde5 expression may counteract CIH-induced hypertension by mitigating MDA levels. CIH-induced oxidative stress has been proven to be a significant contributor to the etiology of hypertension in patients with OSA.[17] MDA, a crucial end product of lipid peroxidation, can inflict tissue and cellular damage by influencing the functionality of vital enzymes in the mitochondrial respiratory chain and serves as a fundamental marker for evaluating oxidative stress–induced harm within the body.[18] Elevated MDA levels have been detected in OSA patients in comparison with control subjects.[19] In an animal model of CIH-induced hypertension, concentrations of MDA, NADPH oxidase 2 and 4 were augmented, and levels of superoxide dismutase (SOD), an antioxidative stress marker, were reduced. The utilization of oxidative stress inhibitors reversed CIH-induced hypertension.[20,21]

Besides, cGMP signaling cascades serve as an integral modulator of vascular tone, water and salt regulation, and

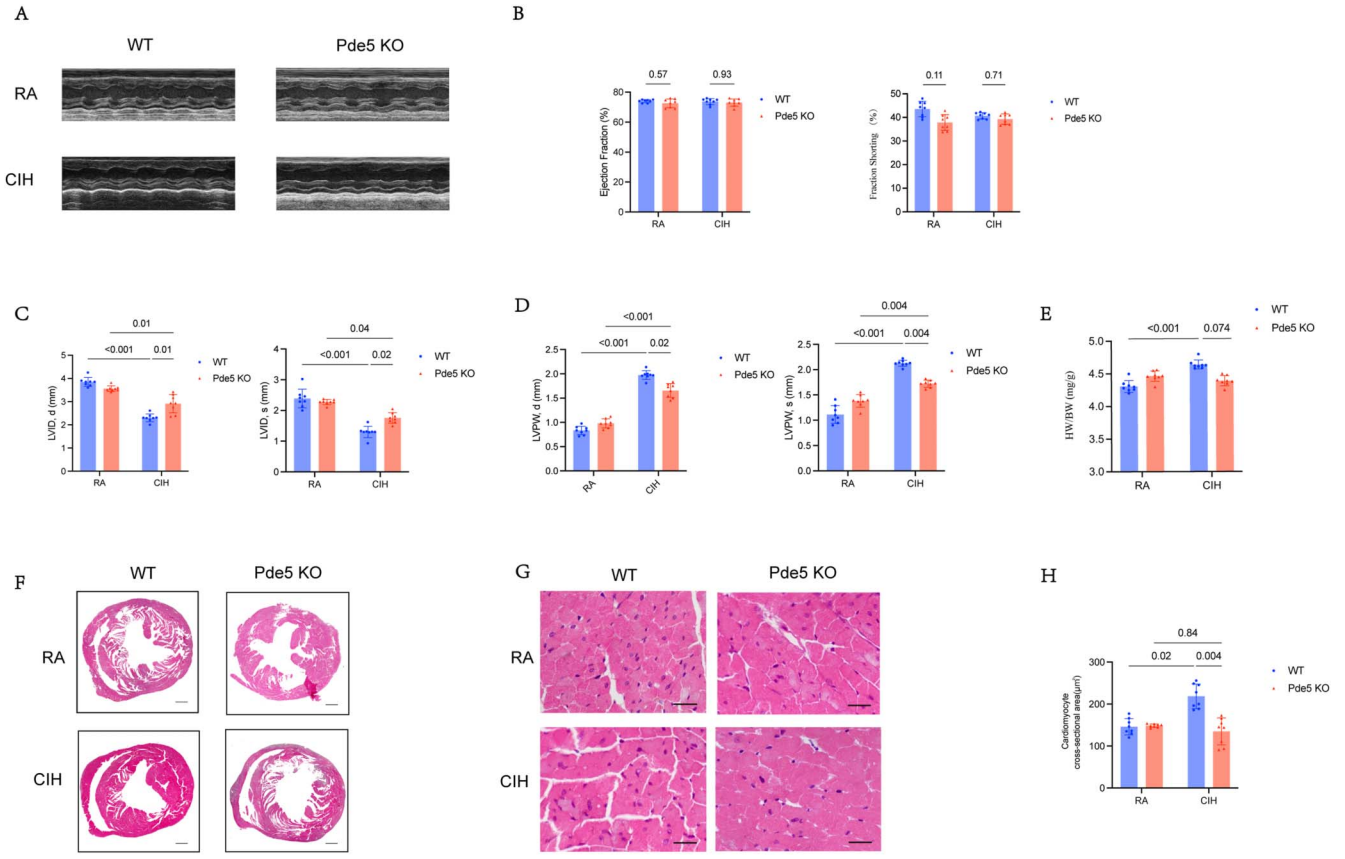


**FIGURE 3.** Pde5$^{-/-}$ mice attenuated cardiac hypertrophy after CIH. A, M-mode echocardiographic images of the left ventricle along the left parasternal long axis. B–D, Quantification of the EF (%), FS (%), LVID, and LVPW from the echocardiography data, n = 12. The interaction *P*-value = 0.024. E, Quantification of the heart weight-to-body weight ratio, n = 12. F–G, HE staining of mouse ventricle. H, Quantification of the cardiomyocyte cross-sectional area, n = 12. Scale bar = 1 mm and 25 μm. KO, knock out; HW/BW, heart weight/body weight; LVPW, d, left ventricular posterior wall, diastole; LVPW, s, left ventricular posterior wall, systolic.

platelet aggregation within the cardiovascular system. Diminished cGMP signaling in the vasculature, central nervous system, or kidneys contributed to the genesis and perpetuation of hypertension. Enhanced degradation of Pde5 played a substantial role in the causes of decreased cGMP availability. Consequently, inhibition of Pde5 resulted in a reduction in SBP through vasodilation.[22] In a spontaneously hypertensive rat model, the Pde5 inhibitor, sildenafil, reinstated pro-oxidant/antioxidant equilibrium and endothelial structure, curtailed oxidative stress, ameliorated cGMP/protein kinase G (PKG) signaling, and significantly reduced SBP.[23] In an animal model of angiotensin II (Ang II)–induced hypertension, sildenafil injection increased plasma cGMP levels compared with controls, improved NO-mediated vasodilation, and reduced SBP after Ang II injection.[24] Our experimental findings revealed that the inhibition of Pde5 expression after either gene or drug treatment salvaged cGMP levels and counteracted CIH-induced hypertension.

In the clinic, Pde5 inhibitors have exhibited a notable capacity for treating cardiac diseases. In a randomized controlled trial encompassing 22 hypertensive patients, a 16-day treatment resulted in an 8 ± 2 mm Hg reduction in SBP and a 6 ± 2 mm Hg decrease in diastolic blood pressure within the sildenafil-administered cohort as opposed to the control group.[25] Another randomized controlled trial that included 133 patients with mild-to-moderate hypertension found that long-acting Pde5 inhibitors in the experimental group increased plasma cGMP concentrations and decreased blood pressure by 7 mm Hg compared with the control group.[26] Furthermore, sildenafil has demonstrated great therapeutic efficacy in addressing refractory hypertension, as evidenced by a clinical trial comprising 26 patients with refractory hypertension, which led to an 8.8 ± 1.4 mm Hg and 5.3 ± 3.3 mm Hg reduction in systolic and diastolic blood pressure, respectively.[27] The aforementioned findings indicated that Pde5 inhibitors may emerge as an innovative pharmacological approach for managing hypertension in clinical practice.

Previous study has found that the use of Pde5 inhibitors mimics intermittent reoxygenation in a hypoxia mice model and exerts a cardioprotection effect in ischemia–reperfusion injury.[13] Salloum et al[14] have found that the use of a Pde5 inhibitor limits myocardial infarction in ischemia–reperfusion injury. Our previous studies confirmed that inhibition of Pde5

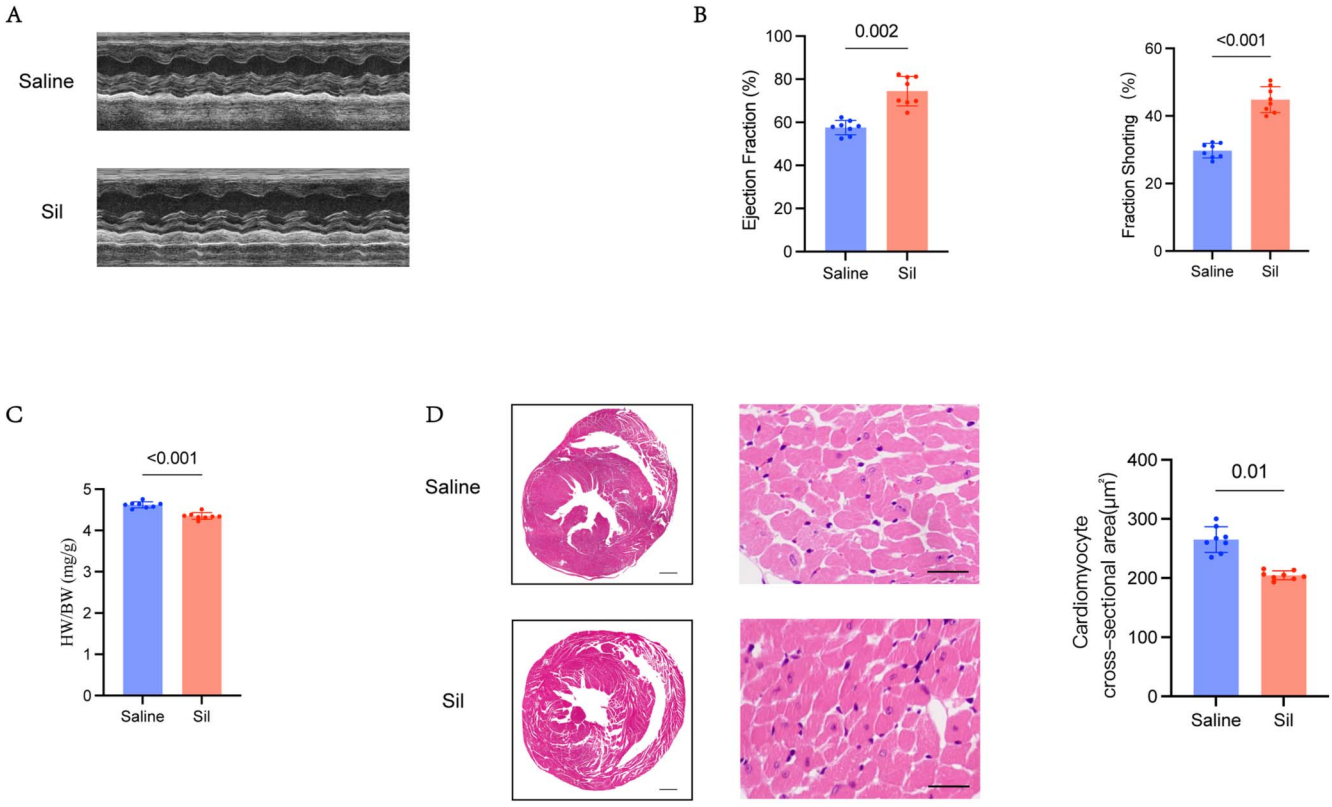


**FIGURE 4.** Sildenafil intragastric administration reversed cardiac hypertrophy in CIH condition. A, M-mode echocardiographic images of the left ventricle along the left parasternal long axis. B, Quantification of the EF (%), FS (%), n = 12. C, Quantification of the heart weight-to-body weight ratio, n = 12. D, The cardiomyocyte cross-sectional area of hearts in H&E staining. Scale bar = 1 mm and 25 μm, n = 8. Sil, sildenafil treatment; HW/BW, heart weight/body weight.

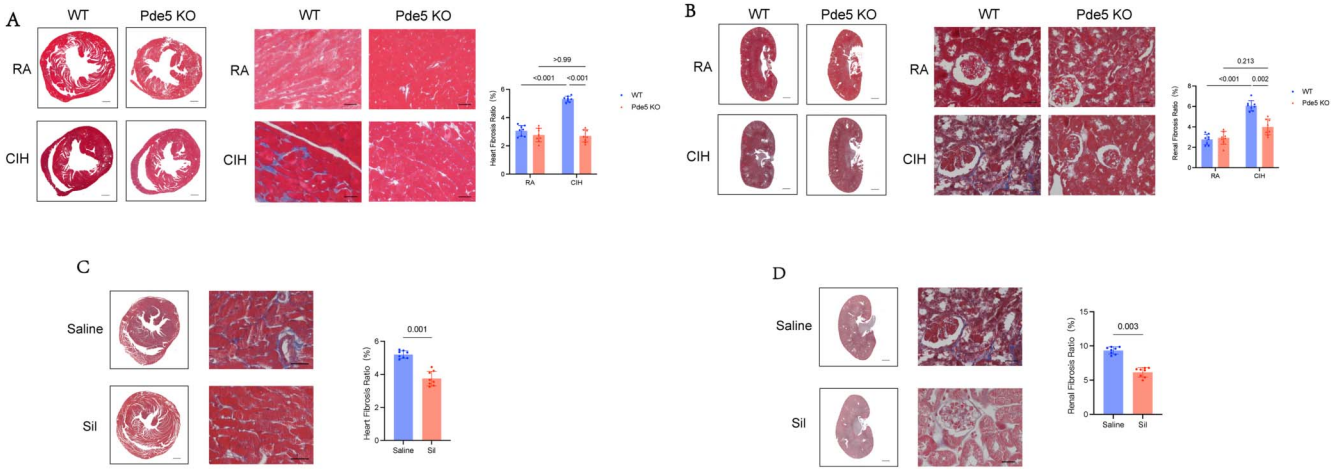


**FIGURE 5.** Pde5$^{-/-}$ and sildenafil intragastric administration reversed cardiac and renal fibrosis caused by CIH exposure. A, Pde5$^{-/-}$ mice Masson staining of heart cross-section images and representative interstitial fibrosis images magnified in boxes; the fibrosis area ratio was quantified, n = 8. Scale bar = 1 mm and 25 μm. B, Pde5$^{-/-}$ mice Masson staining of renal cross-section images and representative interstitial fibrosis images magnified in boxes; the fibrosis area ratio was quantified, n = 8. Scale bar = 1 mm and 25 μm. C, Sil WT mice Masson staining of heart cross-section images and representative interstitial fibrosis images magnified in boxes; the fibrosis area ratio was quantified, n = 8. Scale bar = 1 mm and 25 μm. D, Sil WT mice Masson staining of renal cross-section images and representative interstitial fibrosis images magnified in boxes; the fibrosis area ratio was quantified, n = 8. Scale bar = 1 mm and 25 μm. KO, knock out; Sil, sildenafil.

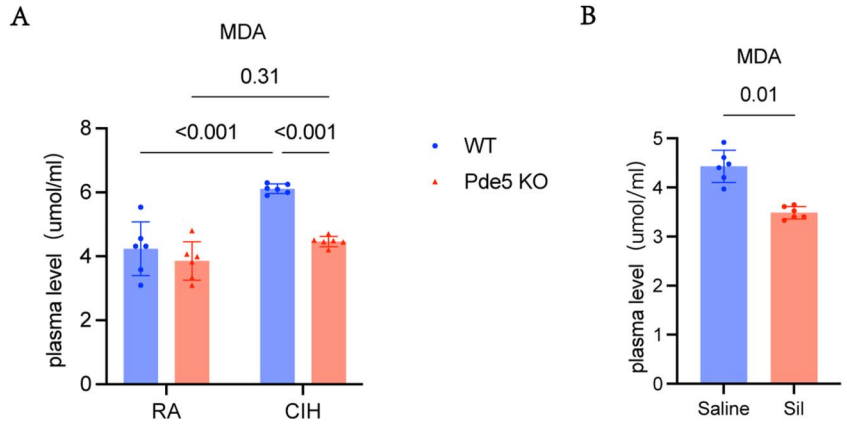


**FIGURE 6.** Pde5$^{-/-}$ and sildenafil intragastric administration antagonism of the CIH-induced increase in MDA levels. A, Serum levels of MDA of WT and Pde5$^{-/-}$ mice in RA and CIH condition, n = 6. B, Serum levels of MDA in saline or Sil WT mice after CIH exposure, n = 6. KO, knock out; Sil, sildenafil.

can play a protective role in ischemic hearts.[16] This study found that Pde5 inhibition can exert antihypertensive effects and antagonize target organ damage in CIH condition, a key feature of OSA. Moreover, OSA independently affected left ventricular diastolic function, leading to the development of ventricular hypertrophy.[28,29] Previous study suggested that Pde5 overexpression mice exhibited impaired cardiac function, diminished cGMP levels, and more pronounced adverse ventricular remodeling.[30] In addition, in the transverse aortic constriction (TAC)-induced cardiac hypertrophy model, Pde5

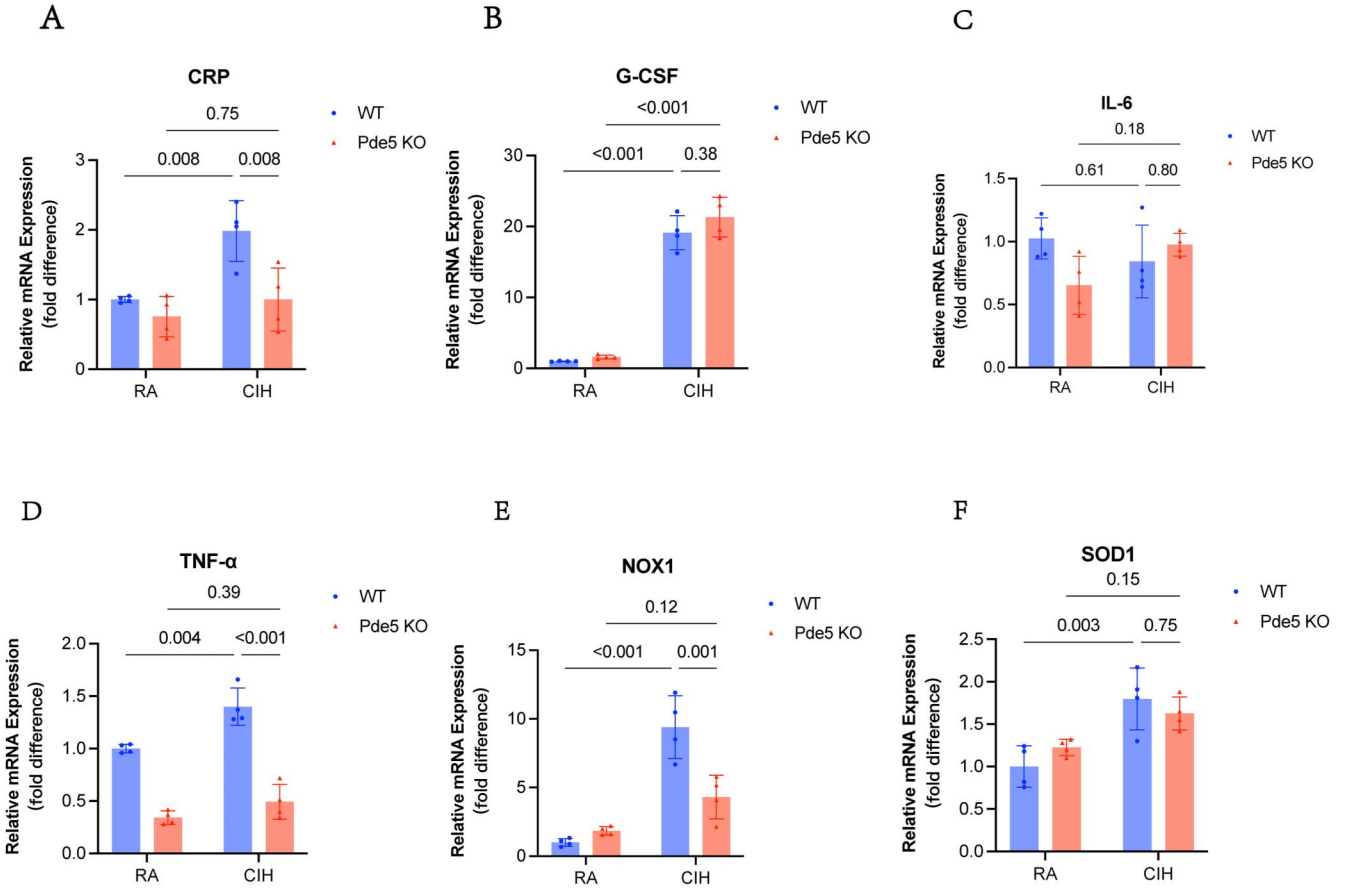


**FIGURE 7.** RT-PCR analysis of relative inflammation and oxidative markers levels in Pde5$^{-/-}$ mice heart tissue. A, Relative mRNA expression in CRP, n = 4. B, Relative mRNA expression in granulocyte colony–stimulating factor, n = 4. C, Relative mRNA expression in interleukin 6, n = 4. D, Relative mRNA expression in TNF-α, n = 4. E, Relative mRNA expression in NOX1, n = 4. F, Relative mRNA expression in superoxide dismutase type 1, n = 4. CRP, C-reactive protein; TNF-α, tumor necrosis factor-alpha; NOX1, NADPH oxidase 1; RT-PCR, real-time polymerase chain reaction.

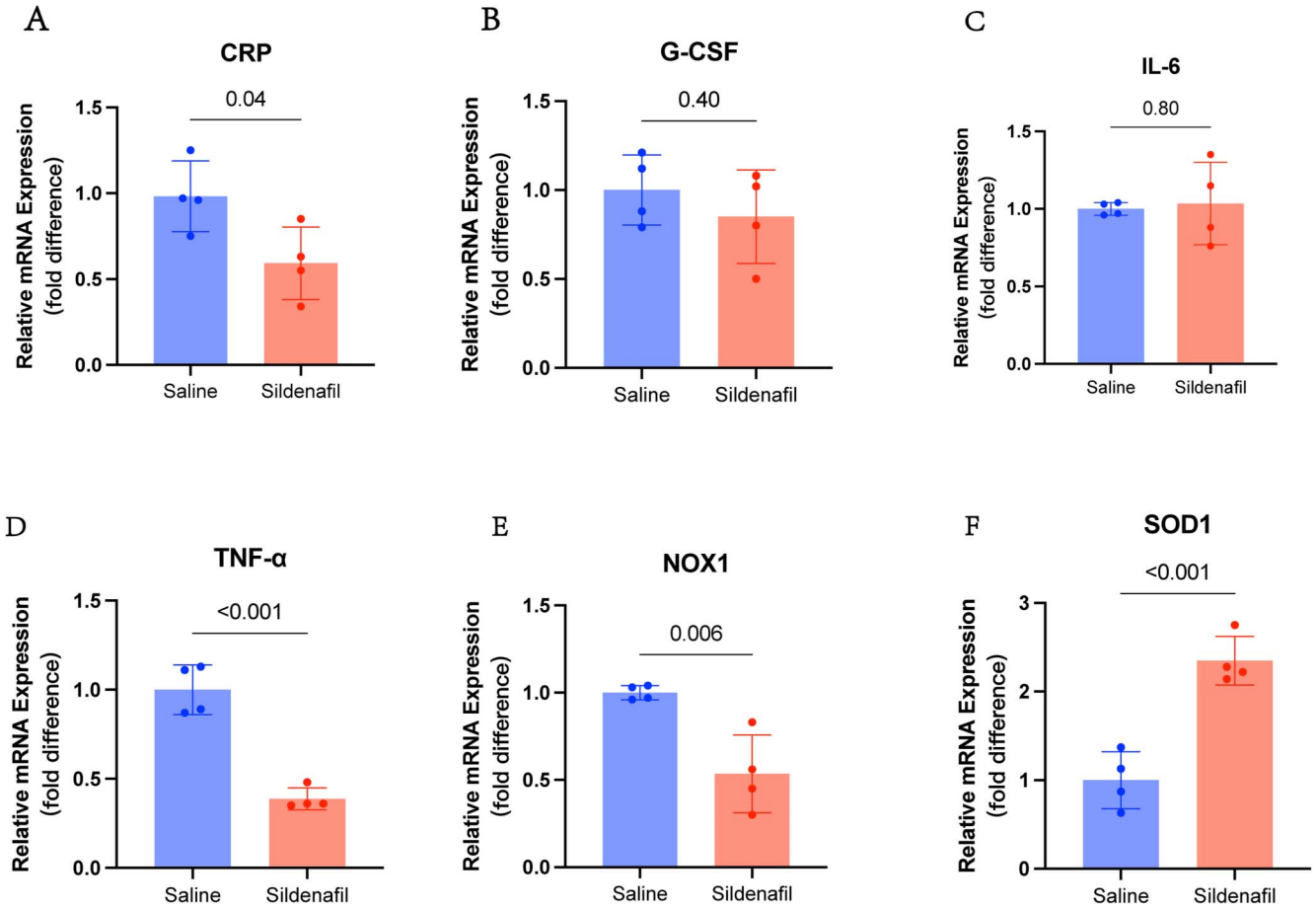


**FIGURE 8.** RT-PCR analysis of relative inflammation and oxidative markers levels in heart tissue in WT mice treated with sildenafil or saline. A, Relative mRNA expression in CRP, n = 4. B, Relative mRNA expression in granulocyte colony–stimulating factor, n = 4. C, Relative mRNA expression in interleukin 6, n = 4. D, Relative mRNA expression in TNF-α, n = 4. E, Relative mRNA expression in NOX1, n = 4. F, Relative mRNA expression in superoxide dismutase type 1, n = 4. CRP, C-reactive protein; TNF-α, tumor necrosis factor-alpha; NOX1, NADPH oxidase 1; RT-PCR, real-time polymerase chain reaction.

overexpression mice developed more severe cardiac dysfunction and ventricular remodeling, while Pde5 inhibitor administration reversed the established maladaptive remodeling.[31] Our previous study also discovered that Pde5 inhibitor therapy significantly mitigated isoproterenol-induced and TAC-induced cardiac hypertrophy and ventricular dysfunction by inhibiting cardiac endoplasmic reticulum stress and apoptosis.[32] Our study found that both endogenous and exogenous inhibition of Pde5 levels can ameliorate cardiac hypertrophy resulting from CIH, even when ventricular hypertrophy is already established. However, it is worth noting that studies have found that the use of low-dose sildenafil in patients with severe OSA exerted a negative effect on the disease,[33] and although there are differences between this study and the present study regarding experimental model, sildenafil regimen, etc., this still suggests that we should be aware of the potential risks of sildenafil therapy.

The decrease in LVID, indicating ventricular chamber shrinkage, coupled with an increase in LVPW, indicating concentric hypertrophy, suggests CIH led to a concentric remodeling pattern in our model. This concentric change counterbalanced to preserve cardiac output, despite altered geometry. Previous studies have reported similar concentric remodeling and equivocal impacts on EF in response to intermittent hypoxia exposure.[34] The mechanistic basis for this distinct remodeling phenotype is not fully understood but may relate to the differential effects of hypoxia on cardiomyocytes versus cardiac fibroblasts. In addition, increased cGMP catabolism by Pde5 in pressure-loaded hearts triggered the activation of cGMP-dependent protein kinases, which in turn exerted inhibitory and antihypertrophic effects.[35] Pde5 inhibitors suppressed adverse cardiac hypertrophy and exerted cardioprotective actions in a TAC model through activation of the cGMP/PKG pathway through the G protein signaling regulator 2 (RGS2) and in a calcineurin-independent manner.[36,37] Sildenafil partially counteracted the decline in cardiac output in L-NAME–treated hypertensive rats by reinstating circulating plasma cGMP levels, reducing the total area of myocardial lesions, and, to some extent, attenuating cardiomyocyte and vascular smooth muscle remodeling.[38] Therefore, Pde5 inhibition may restore the decreased cGMP level, exerted antihypertrophy effects.

Pde5 is closely associated with fibrosis. Pde5 overexpression exacerbated cardiac fibrosis in TAC-treated mice, and cardiac fibrosis diminished in response to Pde5 inhibitors, a process that might be mediated by the Pde5/cGMP-PKG pathway.[31] Sildenafil administration in rats with catecholamine-induced heart failure mitigated type I collagen expression and alleviated diastolic dysfunction and myocardial fibrosis.[39] Patrucco et al discovered that the activation of cGMP and cGMP-dependent protein kinase I cascades with sildenafil reduced fibrosis levels but did not affect cardiomyocyte hypertrophy in the Ang II–induced cardiomyocyte hypertrophy and fibrosis model.[40] In the CIH rat model, CIH mediated macrophage infiltration and macrophage–fibroblast transformation in the kidney through saline corticosteroids, intensifying the extent of renal fibrosis.[41] Subsequent investigations revealed that elevated renal cGMP concentrations significantly inhibited chronic renal fibrosis, suggesting that increasing cGMP maybe an efficacious antifibrotic approach.[42] Our research revealed that Pde5 was intimately associated with CIH-mediated cardiac and renal fibrosis. Pde5 inhibition can elevate plasma cGMP levels and ameliorate the severity of CIH-mediated cardiac and renal fibrosis.

## LIMITATION

Our article presents several limitations. First, human intervention may have led to biased results in the tail-cuff method, unlike the more reliable radio-telemetry method. Second, the article used the CIH model to represent OSA, which simulated only periodic hypoxia without upper airway stenosis; however, previous articles confirm the use of the CIH model to represent the OSA is feasible.[43] Furthermore, the Vevo 2100 software calculated the value of EF% from M-mode scans; however, this represents a raw estimation and must be considered carefully. Finally, it is essential to use conditional $Pde5^{-/-}$ mice in future research to unravel the specific underlying mechanisms influenced by Pde5.

## CONCLUSION

In our study, Pde5 inhibition reduced blood pressure and alleviated target organ damage by suppressing oxidative stress in CIH. Pde5 may have cardiovascular protective effects in the CIH model. Pde5 inhibitors may be a new therapeutic agent to improve the adverse outcomes of OSA.

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