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Authors

Yadav, Pankaj
Mishra, Jay S
Hurt, Mason William
et al.

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Research Article

H_2S donor GYY4137 mitigates sFlt-1-induced hypertension and vascular dysfunction in pregnant rats†

Pankaj Yadav[1], Jay S. Mishra[1], Mason William Hurt[1], Dong-Bao Chen[2] and Sathish Kumar[1,3,*]

[1]Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA
[2]Department of Obstetrics and Gynecology, University of California, Irvine, CA, USA
[3]Department of Obstetrics and Gynecology, School of Medicine and Public Health, University of Wisconsin, Madison, WI, USA

***Correspondence:** Department of Comparative Biosciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI, USA. Tel: +6082651046; E-mail: skumar82@wisc.edu

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Abstract

Gestational hypertension, often associated with elevated soluble Fms-related receptor tyrosine kinase 1 (sFlt-1), poses significant risks to both maternal and fetal health. Hydrogen sulfide (H_2S), a gasotransmitter, has demonstrated blood pressure-lowering effects in hypertensive animals and humans. However, its role in pregnancy-induced hypertension remains unclear. This study investigated the impact of GYY4137, a slow-release H_2S donor, on sFlt-1-induced hypertension in pregnant rats . Pregnant rats were administered sFlt-1 (6 μg/kg/day, intravenously) or vehicle from gestation day (GD) 12–20. A subset of these groups received GYY4137 (50 mg/kg/day, intraperitoneal) from GD 16–20. Serum H_2S levels, mean arterial blood pressure, uterine artery blood flow, and vascular reactivity were assessed. Elevated sFlt-1 reduced both maternal weight gain and serum H_2S levels. GYY4137 treatment restored both weight gain and H_2S levels in sFlt-1 dams. sFlt-1 increased mean arterial pressure and decreased uterine artery blood flow in pregnant rats. However, treatment with GYY4137 normalized blood pressure and restored uterine blood flow in sFlt-1 dams. sFlt-1 dams exhibited heightened vasoconstriction to phenylephrine and GYY4137 significantly mitigated the exaggerated vascular contraction. Notably, sFlt-1 impaired endothelium-dependent relaxation, while GYY4137 attenuated this impairment by upregulating eNOS protein levels and enhancing vasorelaxation in uterine arteries. GYY4137 mitigated sFlt-1-induced fetal growth restriction. In conclusion, sFlt-1 mediated hypertension is associated with decreased H_2S levels. Replenishing H_2S with the donor GYY4137 mitigates hypertension and improves vascular function and fetal growth outcomes. This suggests modulation of H_2S could offer a novel therapeutic strategy for managing gestational hypertension and adverse fetal effects.

Summary Sentence

H_2S levels are reduced in sFlt-1-induced gestational hypertension; replenishing H_2S levels using a slow-release donor alleviates vascular dysfunction in hypertensive pregnant rats, offering a potential avenue for managing gestational vascular dysfunction.

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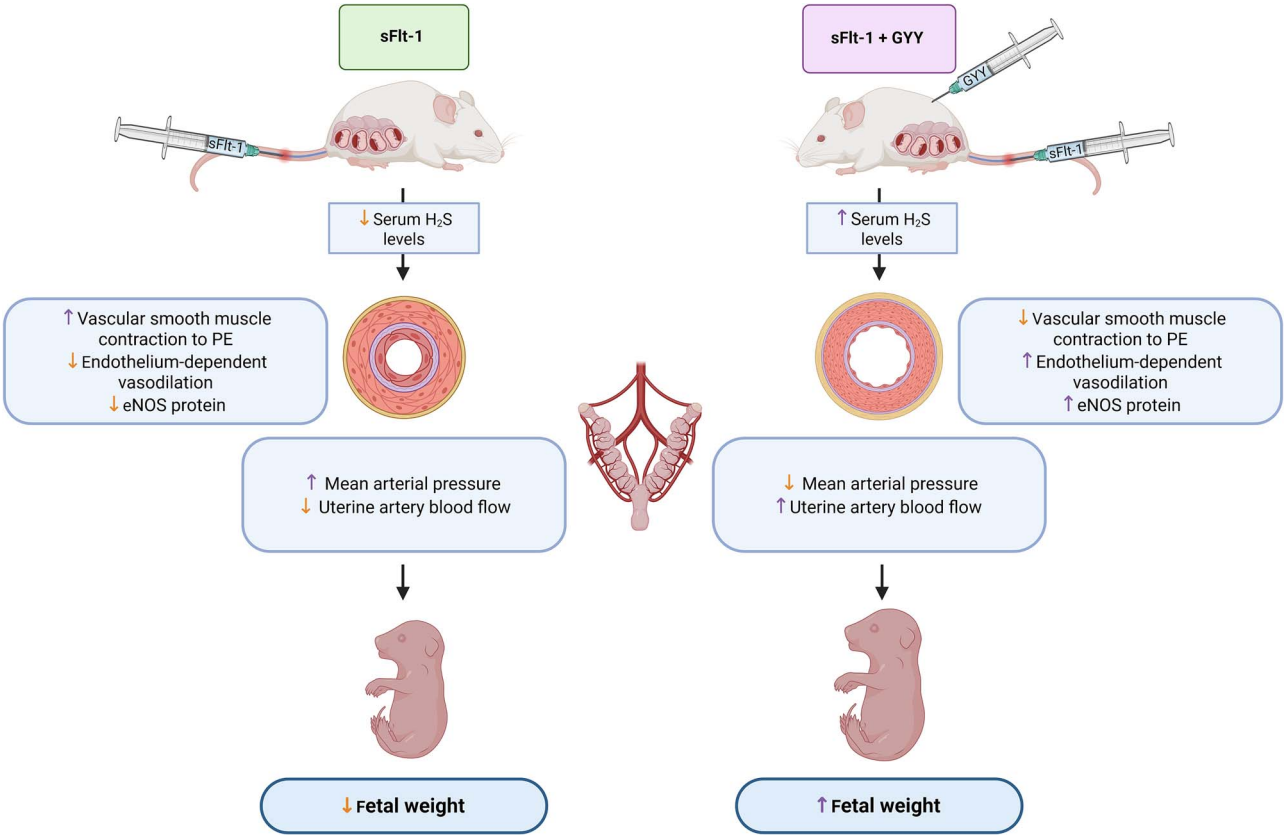
Graphical Abstract



Key words: hypertension, pregnancy, sFlt-1, hydrogen sulfide, GYY4137, uterine artery, endothelium.

Introduction

Hypertensive disorders in pregnancy (HDPs), including chronic hypertension, preeclampsia, gestational hypertension, and chronic hypertension with superimposed preeclampsia, are serious complications that occur in pregnant women. The prevalence of HDPs in the United States has risen, affecting around 15% of women in their reproductive years [1]. These disorders are significantly associated with severe maternal outcomes such as stroke and heart attack [2] and are the most important cause of pregnancy-related death in the United States [1]. Furthermore, mothers who survive HDPs and their offspring face an increased risk of long-term health issues, including cardiovascular and metabolic diseases [3–10]. The exact mechanism of HDPs is unclear, but it is suggested that impaired endothelial function in the uterine arteries, leading to insufficient blood flow to the feto-placental unit, contributes to the development of HDPs [11]. Because of this unclear mechanism, the current treatments for HDPs are limited.

Several studies have supported the hypothesis that HDPs, especially preeclampsia, result from an unfavorable interaction between placental and maternal factors [12, 13]. The hypoxic placenta in preeclampsia releases proinflammatory cytokines such as tumor necrosis factor-alpha and interleukin-6, along with anti-angiogenic factors like soluble Fms-related receptor tyrosine kinase 1 (sFlt-1) and soluble endoglin [12, 14]. Elevated levels of endogenous sFlt-1 disrupt the beneficial effects of circulating vascular endothelial growth factor, leading to systemic endothelial dysfunction, a hallmark of preeclampsia [12, 13].

Furthermore, dysregulation of the hydrogen sulfide (H_2S) pathway has been implicated in the mechanisms underlying preeclampsia and fetal growth restriction (FGR). H_2S is synthesized in the placenta from L-cysteine by enzymes like cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) [15]. Studies have shown reduced placental H_2S production, CBS and CSE expression, and plasma H_2S levels in pregnancies complicated by preeclampsia and FGR [16, 17]. These reduced H_2S levels have been associated with the anti-angiogenic environment and endothelial dysfunction in preeclampsia [18, 19]. H_2S is known for its ability to promote vasodilation [20], exert cytoprotective anti-inflammatory effects [21], protect against reperfusion injury [22], and stimulate angiogenesis [18], which are crucial for pregnancy-related vascular adaptations [16, 17, 23]. The therapeutic potential of sodium hydrosulfide (NaHS), an H_2S donor, has been explored for its vasodilator and cytoprotective effects on endothelial cell function [24–27]. However, NaHS has limitations, including instability in water solution and the rapid release of H_2S, which does not accurately mimic the physiological process of H_2S release in vivo. Therefore, in this study, we investigated the effects of GYY4137 (GYY), which releases H_2S slowly and consistently over an extended period and within a physiologically relevant concentration range [28, 29], in an sFlt-1-induced rat model of gestational hypertension along with the underlying vascular mechanisms involved.

Materials and methods

Experimental arrangement

All experimental procedures adhered to the guidelines set forth by the National Institutes of Health (NIH Publication No. 85–23, revised 1996) and received approval from the Institutional Animal Care and Use Committee at the University of Wisconsin-Madison (IACUC protocol V005847). Timed-pregnant Sprague–Dawley rats, obtained from Envigo Laboratories (Indianapolis, IN) on gestation day 10 (GD) 10, were housed under controlled conditions with a 12-h light:12-h dark photoperiod in a temperature-regulated room (23°C). These rats had unrestricted access to food and water.

On GD 12, pregnant rats were divided into two groups, each consisting of $n = 12$. The control group received saline (vehicle), while the treatment group received sFlt-1 (recombinant mouse VEGF R1/Flt-1 Fc Chimera, R&D system MN, 6 μg/kg/day, intravenously) from GD 12 to GD 20, as previously described [30]. The chosen dosage and duration of sFlt-1 administration replicate the elevated sFlt-1 levels observed in pregnant women with preeclampsia [31, 32].

Additionally, a subset of control dams ($n = 6$) and sFlt-1-administered dams ($n = 6$) were treated with GYY (GYY4137, MCE NJ, 50 mg/kg/day, intraperitoneal) from GD 16 to GD 20. This specific dose of GYY was based on prior studies demonstrating beneficial effects in some animal models [33–35].

On GD 20, the rats underwent euthanasia using CO_2 asphyxiation. Maternal blood, male and female fetuses, and their placentae were carefully removed and weighed. The serum was separated and utilized for quantifying H_2S levels. Uterine arteries were dissected and isolated for vascular reactivity studies. The remaining uterine artery segments were rapidly frozen in liquid nitrogen for subsequent protein isolation.

H_2S measurement in the maternal serum

The quantification of H_2S concentrations in serum was conducted in accordance with previously established methodologies [36]. Briefly, 75 μL aliquot of serum was combined with 1% zinc acetate (250 μL) and distilled water (425 μL). Subsequently, 150 μL of N-dimethyl-p-phenylenediamine sulfate (20 mmol/L) in 7.2 M HCl and 150 μL of $FeCl_3$ (30 mmol/L) in 1.2 M HCl were introduced to the mixture. Following this, 10% trichloroacetic acid (250 μL) was added and the solution was incubated at ambient temperature for a duration of 10 min. The reaction mixture was then subjected to centrifugation at 12 000 × g for 15 min to facilitate protein removal. The absorbance of the resultant supernatant was measured at a wavelength of 670 nm using a SpectraMax i3x spectrophotometer (Molecular Devices, San Jose, CA). The H_2S concentration in the solution was determined by comparison with a calibration curve generated using NaHS. The H_2S concentration is expressed as nM per mL of serum.

Measurement of mean arterial blood pressure

The mean arterial blood pressure was measured in conscious rats using a CODA computerized noninvasive blood pressure system (Kent Scientific, Litchfield, CT) as described previously [37]. The rats were adapted to a restraint warming chamber for 2 days for 15 min each day, and then blood pressure was measured the following days. Rats were allowed to rest quietly for 10 min in a restrainer placed in a warming chamber at 30°C to stimulate blood flow to the tail. Occlusion and volume pressure-recording cuffs were applied to the base of the tail. The cuff was programmed to inflate and deflate automatically within 90 s. Blood pressure was recorded and analyzed using Kent Scientific software.

Uterine artery ultrasound

Rats were anesthetized with 2% isoflurane in oxygen and placed in a heated platform for ultrasound imaging. Uterine arteries were examined using a 30-MHz transducer and Vevo 2100 ultrasound system (Visual Sonics, Toronto, ON, Canada), as reported previously [38]. Briefly, the velocities of the main uterine arteries were recorded below the bladder and at the level where the main uterine artery branches from the internal iliac artery. Peak systolic velocity (PSV) and end-diastolic velocity (EDV), the area under the peak velocity-time curve, and the R-R interval were measured from three consecutive cardiac cycles, and the results were averaged. Blood flow velocity distribution was determined using the following formula: $F = {}^1\!/_2\ \mathrm{MV}\pi\ (D/2)^2$ (where MV = mean peak velocity over the cardiac cycle [cm/s], D = diameter [cm], and F = blood flow [mL/min]). Uterine artery Resistance Index (RI = [PSV−EDV]/PSV) and Pulsatility Index (PI = [PSV−EDV]/MV) were calculated to quantify the pulsatility of blood velocity waveforms.

Preparation of uterine arteries

The rats were euthanized by CO_2 inhalation, and uterine vasculature was excised and immersed in ice-cold Krebs physiological salt solution (KPS) (in mM): NaCl, 119; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.17; $NaHCO_3$, 25; KH_2PO_4, 1.18; EDTA, 0.026; and d-glucose, 5.5. Uterine arteries (1.5-mm segments of the first-order branch of the uterine artery) were dissected free of fat and connective tissue and mounted using tungsten wires on a wire myograph (Danish Myo Techniques, Aarhus, Denmark) for the recording of isometric tension. The tissues were incubated for 15 min in KPS at 37°C, which was gassed with 95% O_2 and 5% CO_2 to maintain pH 7.4 and allowed to equilibrate for 30 min before normalization to an internal diameter of 0.9 of L13.3 kPa by using a normalization software package (Myodata; Danish Myotechnologies). For endothelium-intact uterine artery rings, extreme care was taken to avoid injury to the endothelium. For endothelium-denuded rings, the endothelium was removed by gently rubbing the ring interior with tungsten wire. The presence and denudation of the endothelium were verified by relaxation to acetylcholine (ACh) in arterial rings precontracted by a submaximal concentration of phenylephrine (PE).

Assessment of vascular contractile responses

The arterial rings were exposed to 80 mM potassium chloride (KCl) until reproducible depolarization-induced contractions were achieved. After washing and equilibration with KPS, vascular contractile responses to cumulative additions of PE (10^{-9}–10^{-5} M) were determined.

Assessment of vascular relaxation responses

Endothelium-dependent relaxation was assessed by ACh (10^{-9}–10^{-6} M)-induced relaxation in PE-precontracted arteries. The PE concentration used for precontraction

was required to produce 80% of the maximal response. Endothelium-independent relaxation was determined by nitric oxide donor sodium nitroprusside (SNP) (10^{-9}–10^{-6} M)-induced relaxation in PE-precontracted endothelium-denuded arteries.

Western blotting for eNOS protein quantification

The frozen uterine arteries were homogenized in ice-cold RIPA buffer (20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/mL leupeptin; Cell signaling Technology, Danvers, MA) containing a protease inhibitor tablet (Roche, Indianapolis, IN) and phosphatase inhibitor cocktail-2 and -3 (Sigma, St Louis, MO). Tissue lysates were centrifuged (14 000*g* for 15 min at 4°C), and the protein content was measured using the Pierce BCA protein assay kit (Thermo Scientific, Waltham, MA). Then, the supernatant was resuspended in the NuPAGE LDS sample buffer and reducing agent (Invitrogen; Thermo Scientific, Waltham, MA). Proteins (40 μg) alongside Chameleon Duo Pre-stained Protein Ladder (LI-COR Corporate, Lincoln, NE) were resolved on 4%–12% gradient NuPAGE Bis-Tris Gels (Invitrogen) at 100 V for 2.0 h at room temperature and then transferred onto Immobilon-P membranes (Millipore Inc., Billerica, MA) at 20 V for 1 h. The membranes were blocked with 5% BSA for 1 h and then incubated with primary antibodies for 16 h at 4°C. The primary antibodies were rabbit monoclonal eNOS (1:1000; Catalog No. 32027, Cell Signaling, Danvers, MA) and rabbit monoclonal GAPDH (1:2000; Catalog No. 5174, Cell Signaling, Danvers, MA). After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 h and then developed using the Pierce ECL detection kits (Thermo Scientific). The densitometric analysis was done using ImageJ software. Results were expressed as ratios of band intensity to that of GAPDH.

Statistical analysis

Data are represented as the mean ± standard error of the mean (SEM). The data were subjected to analysis using the GraphPad Prism software (GraphPad Software, San Diego, CA). The data derived from multiple vascular rings and fetuses of the same rat were averaged and presented as a single data point, with the "*n*" value denoting the number of dams. Maternal weight gain was quantified as the percentage change relative to the weights recorded on GD 12. Cumulative concentration-response curves were analyzed via computer-assisted fitting to a four-parameter sigmoid curve. Contractile responses to PE were assessed as a percentage of its maximal contraction and as a percentage of KCl contraction. Relaxant responses to ACh were evaluated as the percentage inhibition of the PE-induced contraction. Subsequently, the maximal responses (Emax) and the concentrations that produce 50% of the maximal effect (pD2 values) were determined. The pD2 values were ascertained via regression analysis and expressed as the negative logarithm of the molar concentration. Analysis of variance tests was performed, followed by Dunnets post hoc test for multiple comparisons. Differences were deemed statistically significant at $P \leq 0.05$.

Results

Maternal weight gain and serum H_2S levels

Compared to controls, sFlt-1 dams had reduced maternal weight gain, and GYY treatment effectively counteracted this sFlt-1-induced decrease in weight gain. The non-significant difference between sFlt-1 + GYY and control or sFlt-1 alone groups suggests that GYY treatment successfully preserved maternal weight gain to levels comparable to controls and prevented the suppressive effect of sFlt-1 (Figure 1A). Serum H_2S levels were lower in sFlt-1 dams, but GYY treatment restored the H_2S levels similar to that in control dams (Figure 1B).

Maternal arterial blood pressure and uterine artery blood flow

sFlt-1 dams showed elevated mean arterial blood pressure, and GYY treatment prevented the rise in blood pressure induced by sFlt-1 (Figure 2). However, GYY treatment did not affect blood pressure in the control dams (Figure 2).

In addition, sFlt-1 dams had a significant decrease in uterine artery blood flow, along with increased resistance and pulsatility indices compared to the control dams (Figure 3). GYY treatment significantly reversed the decrease in uterine artery blood flow and normalized resistance and pulsatility indices to control levels (Figure 3). In the control dams, GYY treatment did not alter resistance and pulsatility indices and blood flow (Figure 3).

Vasoconstrictor response

sFlt-1 dams had enhanced PE-induced contraction in endothelium-denuded uterine arteries, with increased sensitivity compared to controls (Figure 4 and Table 1). However, GYY treatment significantly reduced the sFlt-1-induced exaggerated PE contraction (Figure 4A and B, Table 1). GYY treatment did not affect PE contraction in controls (Figure 4A and B, and Table 1).

KCl (80 mM)-induced vascular contraction response was similar in uterine arteries among the different treatment groups (Figure 4C and Table 1).

Vasodilator response

Endothelium-dependent relaxation mediated by ACh was impaired in uterine arteries from sFlt-1 dams, as evidenced by a lower ACh sensitivity than controls (Figure 5A and Table 1). Treatment with GYY ameliorated endothelial dysfunction in sFlt-1 dams by enhancing ACh sensitivity (Figure 5A and Table 1). GYY did not affect ACh-induced relaxation in control uterine arteries (Figure 5A and Table 1).

Endothelium-independent relaxation elicited by SNP, a NO donor, was similar in uterine arteries from control and sFlt-1 dams, regardless of GYY treatment (Figure 5B and Table 1).

eNOS protein levels

The uterine arteries from sFlt-1 dams had reduced eNOS protein expression (Figure 6). However, treatment with GYY restored eNOS protein expression in sFlt-1 dams, while it had no significant impact on the controls (Figure 6).

Litter size and fetal weights

Increased sFlt-1 levels caused a trend of decrease in litter size ($P = 0.14$ vs control) (Figure 7A) and a significant FGR with lower mean male (Figure 7B) and female (Figure 7C) pup weight. Treatment with GYY attenuated the detrimental

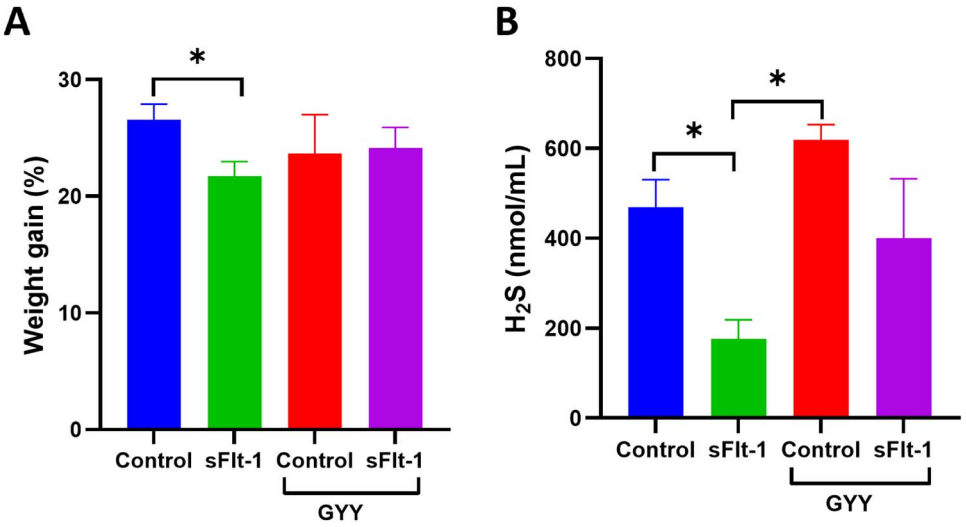


Figure 1. Maternal weight gain and serum H_2S levels. Pregnant rats were exposed to intravenous sFlt-1 (6 μg/kg/day) or vehicle from gestational day (GD) 12 to 20. Both sFlt-1 and control groups received H_2S donor GYY (50 mg/kg, i.p.) from GD 16 to 20. (A) Maternal weight change on GD 20 relative to GD 12 is shown as a percentage. (B) Serum H_2S levels were determined as described in the methods section. Data are means ± SEM of 6 rats per group. Bars with asterisk (*) denote significant differences ($P \leq 0.05$).

Table 1. Vascular response in control and sFlt-1 dams with and without GYY4137

Variable		Control	Control + GYY	sFlt-1	sFlt-1 + GYY
PE	pD_2	5.68 ± 0.01	5.65 ± 0.04	5.84 ± 0.07*	5.73 ± 0.04
	E_{max} (%)	124.3 ± 4.17	136.1 ± 3.41	136.0 ± 7.83	128.5 ± 7.05
ACh	pD_2	7.36 ± 0.08	7.04 ± 0.11	6.32 ± 0.12*	7.31 ± 0.09
	E_{max} (%)	90.60 ± 1.55	85.05 ± 3.15	83.72 ± 3.51	87.24 ± 1.64
SNP	pD_2	7.24 ± 0.14	7.16 ± 0.14	7.05 ± 0.19	7.20 ± 0.05
	E_{max} (%)	97.35 ± 14.82	97.53 ± 12.70	96.17 ± 18.02	92.59 ± 4.56
KCl	E_{max} (mN)	11.87 ± 0.77	11.47 ± 0.93	12.44 ± 0.79	11.36 ± 1.16

pD2 (half-maximal effective concentration) is expressed as −log[mol/L], and E_{max} (maximum effects) is shown as percentage of maximal contraction or relaxation. All acronyms are explained in the text. Asterisk indicates significant differences ($P \leq 0.05$) relative to other groups.

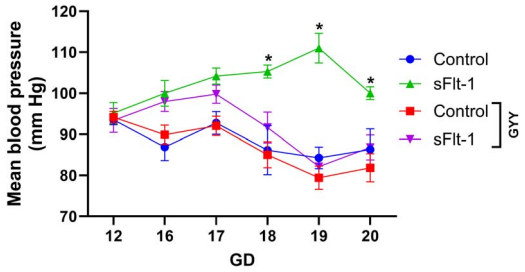


Figure 2. Maternal blood pressure response to H_2S donor GYY. Pregnant rats received sFlt-1 (6 μg/kg/day, i.v.) or vehicle from gestational day (GD) 12–20. GYY (50 mg/kg, i.p.) was administered to both sFlt-1 and control groups from GD 16 to 20. On GD 20, mean arterial blood pressure was noninvasively assessed using the CODA system. Data are means ± SEM of 6 rats per group. *Denote significant differences ($P \leq 0.05$) relative to other groups.

effects of sFlt-1 by improving the litter size and fetal weight (Figure 7A–C). GYY treatment in control dams did not significantly affect litter size and fetal weights (Figure 7A–C). The placental weight was unaffected with sFlt-1 and with or without GYY treatment (Figure 7D and E).

Discussion

The primary outcomes of the present study are as follows: (1) H_2S levels are reduced in pregnant rats with elevated sFlt-1, and GYY administration restored H_2S to control levels. (2) GYY mitigated hypertension induced by sFlt-1 in pregnant rats by enhancing the blood flow in the uterine artery and reducing the vascular contraction mediated by PE. (3) GYY treatment led to an improvement in endothelial-dependent vascular relaxation and associated with increased eNOS protein in the uterine arteries. (4) GYY treatment also enhanced fetal growth in sFlt-1 dams, likely due to improved vasodilation and increased blood flow in the uterine artery. These findings suggest activating the H_2S system with GYY can mitigate hypertension, improve endothelial-mediated vascular function, and enhance fetal growth in pregnant rats with elevated sFlt-1.

The HDPs are hypothesized to involve an imbalance in angiogenic factors, particularly leaning toward antiangiogenic factors [39]. Studies consistently report elevated levels of circulating sFlt-1 in women with preeclampsia [40, 41]. Experimental evidence supports a connection between increased maternal sFlt-1 and adverse maternal outcomes, such as gestational hypertension, preeclampsia [42, 43], and reduced birth weight [44, 45]. Our current observation of decreased serum H_2S levels in pregnant dams with elevated sFlt-1 is consistent with previous findings of low H_2S in preeclamptic women [16]. Our previous study demonstrated that sFlt-1 suppresses VEGF-stimulated CBS expression, leading to reduced H_2S production [46]; however, further investigation is required to determine whether sFlt-1 directly interacts with

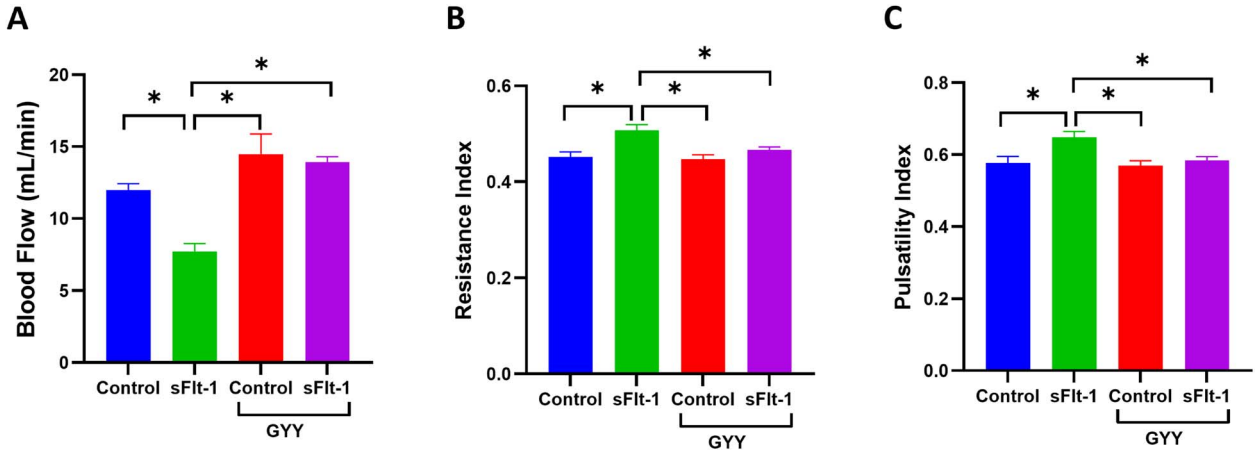


Figure 3. Uterine artery hemodynamics after H_2S donor GYY treatment. Uterine artery (A) blood flow, (B) resistance index, and (C) pulsatility index were assessed using a 30-MHz transducer and Vevo 2100 micro-ultrasound on gestational day 19 in control and sFlt-1 dams with or without GYY. Data are means ± SEM of 6 rats per group. Bars with asterisk (*) denote significant differences ($P \leq 0.05$).

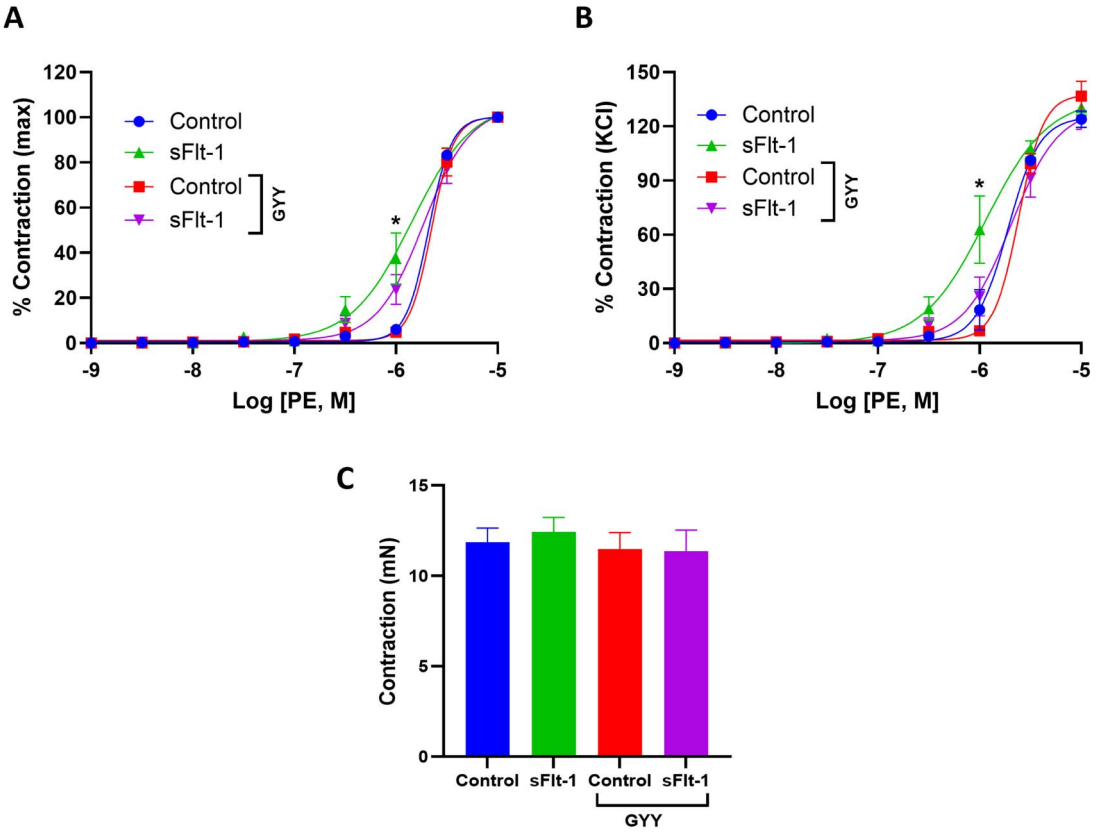


Figure 4. Uterine artery contractility following H_2S donor GYY treatment. Uterine artery rings were obtained from pregnant rats on gestational day 20 after exposure to control or sFlt-1, with or without GYY treatment. Vascular contractile responses to cumulative phenylephrine (PE) additions were assessed in endothelium-removed rings and shown as (A) percentage of maximal contraction and (B) percentage of contraction elicited by 80 mM potassium chloride (KCl). (C) Contractile responses to 80 mM KCl. Data are means ± SEM of 6 rats per group. *$P \leq 0.05$ compared to all other groups.

other H_2S synthesizing or metabolizing enzymes. GYY treatment restored serum H_2S levels in sFlt-1 dams as expected. However, it is interesting to note that GYY treatment did not affect serum H_2S levels in control dams, suggesting a possible feedback regulation to maintain H_2S homeostasis either by downregulating endogenous CBS and CSE activity or upregulating H_2S metabolism. This notion needs to be investigated in future studies.

In this study, pregnant rats exposed to sFlt-1 exhibited elevated blood pressure, consistent with previous findings [32]. However, administration of the H_2S donor GYY to sFlt-1-exposed dams effectively prevented the increase in blood pressure, indicating that GYY may have the potential to attenuate sFlt-1-induced hypertension. This is consistent with previous studies demonstrating the blood pressure-lowering effects of other H_2S donors in various hypertensive models, including RUPP, heme oxygenase-1 (HO-1) deficiency, deoxycorticosterone (DOCA)-salt, inhibition of CSE, and LPS models [16, 24, 25, 47, 48]. Future studies should investigate multiple donors concurrently, such as GYY and others,

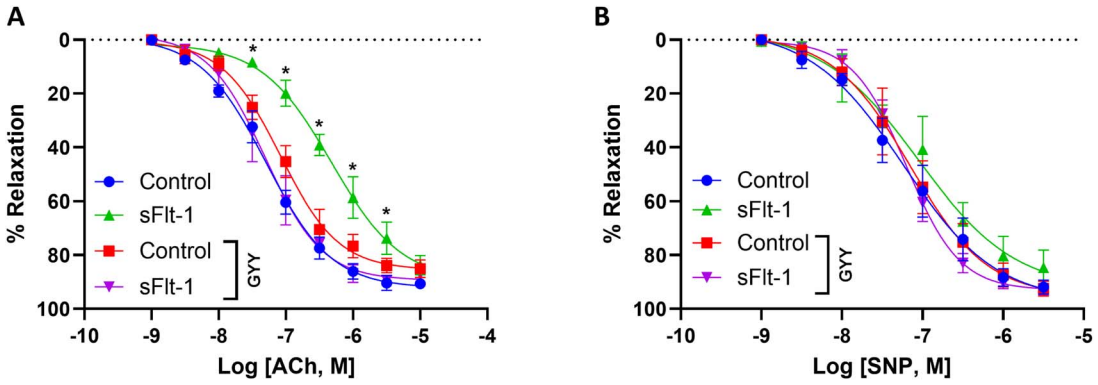


Figure 5. Uterine artery relaxation after H_2S donor GYY treatment. Uterine artery rings were obtained from pregnant rats on gestational day 20 after exposure to control or sFlt-1, with or without GYY treatment. Rings were pre-contracted with submaximal phenylephrine and then the relaxation responses to cumulative doses of (A) acetylcholine (ACh) in endothelium-intact rings and (B) sodium nitroprusside (SNP) in endothelium-removed rings were assessed. Data are means ± SEM of 6 rats per group. $^*P \leq 0.05$ compared to other groups.

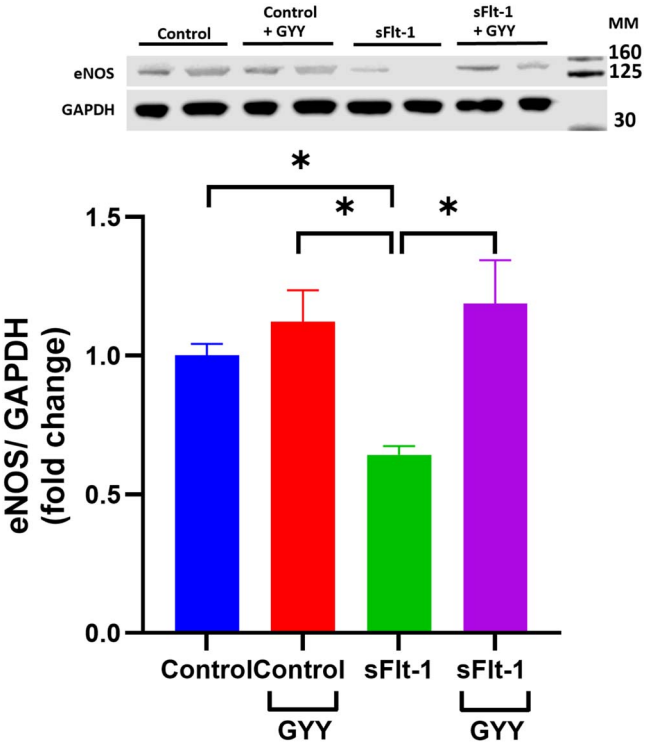


Figure 6. Uterine artery endothelial nitric oxide synthase (eNOS) protein expression after H_2S donor GYY treatment. Uterine artery samples from pregnant rats on gestational day 20 after exposure to control or sFlt-1, with or without GYY treatment, were subjected to Western blotting for eNOS expression. The top panel shows representative blots for eNOS and glyceraldehyde 3-phosphate dehydrogenase (GAPDH), while the bottom panel presents normalized densitometry data. Data are means ± SEM of 6 rats per group. Bars with asterisk (∗) denote significant differences ($P \leq 0.05$).

within the same model to determine if GYY exhibits superior or more consistent effects compared to other H_2S-releasing compounds.

Maternal vascular adaptations during pregnancy are essential for enhancing uterine artery blood flow to meet the metabolic demands of the growing placenta and fetus [49, 50]. Uterine vascular remodeling and placental angiogenesis play crucial roles in forming a "low resistance, high capacitance vessel" capable of augmenting uterine blood flow [51, 52]. Significant changes occur in uterine spiral and placental arteries, including increased branching, diameter, and total area, contributing to enhanced uterine blood flow [53]. In line with previous studies [54], we found that elevated sFlt-1 reduced uterine artery blood flow and increased resistance and pulsatility indices. Such alterations in uterine artery blood flow and vascular resistance have been linked to adverse outcomes like preeclampsia and FGR [55, 56]. However, in our study, GYY administration to sFlt-1-exposed dams ameliorated these changes, improving uterine artery blood flow and restoring the resistance and pulsatility indices. This aligns with other studies where H_2S donors enhanced endothelial function in the mesenteric arteries of nonpregnant streptozotocin-induced diabetic rats [34] and uterine blood flow in pregnant Dahl salt-sensitive rats [27]. While the blockade of H_2S production has been reported to inhibit angiogenesis [57], H_2S has been shown to promote angiogenesis and neovascularization [18, 58, 59]. The mechanism underlying GYY-induced improvement in uterine artery blood flow could involve enhancement in angiogenesis and placental vascularization. Further investigations are warranted to elucidate the role of GYY in vascular remodeling and placental vascularization during elevated sFlt-1 levels.

We investigated uterine artery function to understand the vascular mechanisms underlying the observed blood pressure and uterine artery blood flow changes associated with GYY treatment in sFlt-1 dams. The increased blood pressure in sFlt-1 dams was accompanied by enhanced vasoconstriction in response to PE, resembling the adrenergic hyperreactivity observed in hypertensive pregnancies [60, 61]. However, GYY administration effectively reversed the exaggerated vasoconstriction, suggesting adrenergic suppression in restoring vascular function in sFlt-1-elevated dams. GYY treatment did not significantly alter nonreceptor-mediated (KCl-induced) contraction, indicating that the reduced PE vasoconstriction in sFlt-1 dams given GYY is likely due to modifications in adrenergic receptors or their downstream signaling rather than broad nonreceptor-mediated changes, such as vascular smooth muscle cell hypertrophy or hyperplasia. In line with this, GYY has been found to attenuate the excessive norepinephrine-induced vasoconstriction in the mesenteric arteries of diabetic rats [34] and reduce

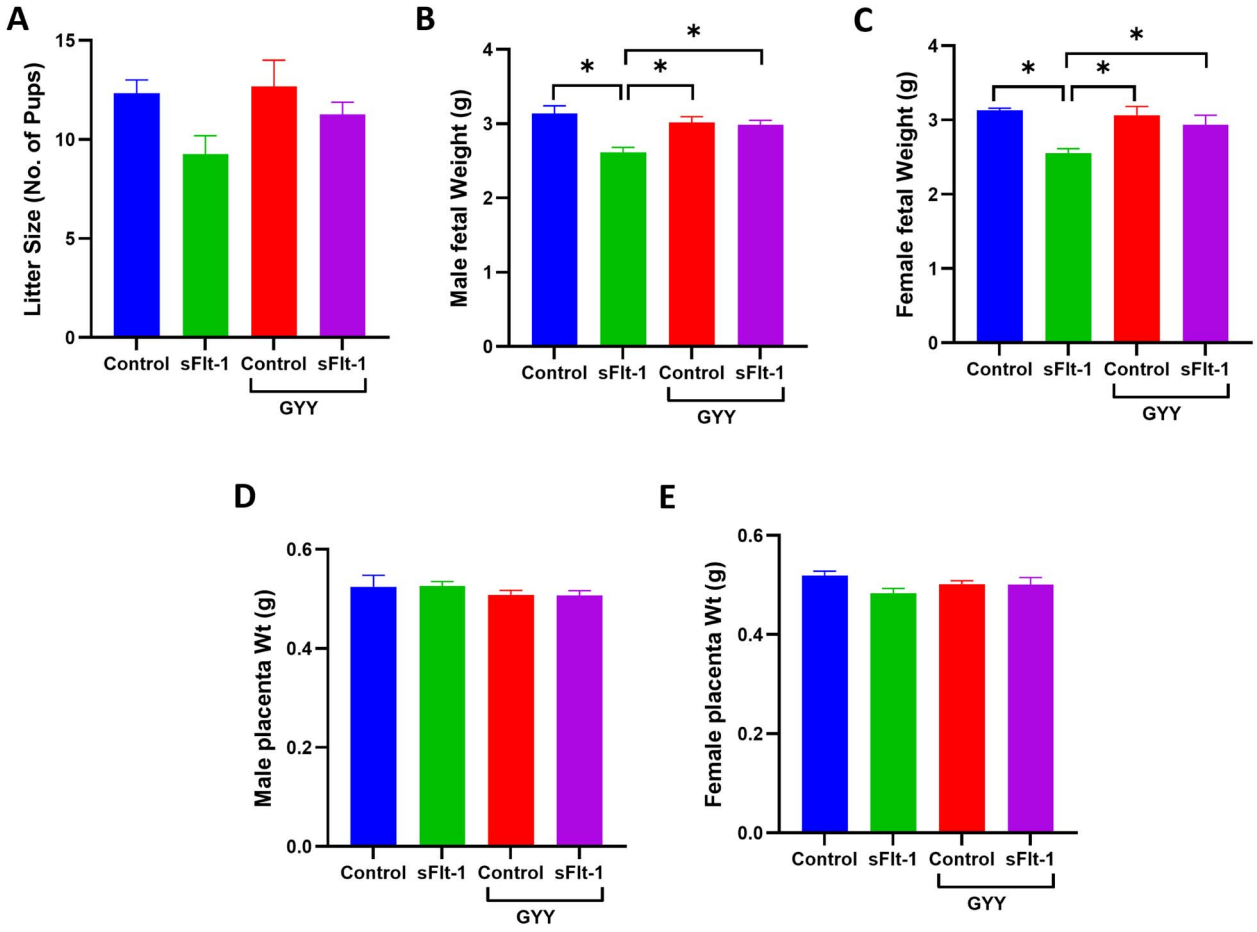


Figure 7. Litter size, fetal, and placental weights after H_2S donor GYY treatment. Pregnant rats were exposed to control or sFlt-1, with or without GYY treatment. Litter size, fetal and placental weights were recorded on gestational day 20. (A) Litter size (B) male fetal weights (C) female fetal weights (D) male placental weights, and (E) female placental weights were calculated as the mean data per dam/litter. Data are means ± SEM of 6 rats per group. Bars with asterisk (*) denote significant differences ($P \leq 0.05$).

the responsiveness of adrenergic receptors to norepinephrine in Wistar rats [62]. Further studies should investigate how GYY mitigates adrenergic signaling in uterine arteries during gestation.

To investigate the impact of GYY on endothelial function, we evaluated the endothelium-dependent relaxation response to ACh. Notably, ACh-induced relaxation was diminished in the uterine arteries of dams exposed to sFlt-1, indicating impaired endothelial control of vascular tone, aligning with prior studies [63]. GYY treatment enhanced ACh-induced relaxation in the uterine arteries of sFlt-1-exposed dams while having minimal effects in control dams. These findings suggest that GYY treatment preserves vascular relaxation responses to ACh. Importantly, SNP (NO donor)-induced relaxation response showed no significant difference between the sFlt-1 dams with and without GYY, indicating that the observed differences were unrelated to the smooth muscle vasodilatory capacity but more related to endothelial function.

In agreement with this, earlier studies have shown that GYY enhances endothelium-dependent NO-mediated relaxations in porcine coronary arteries exposed to hypochlorous acid [64], mesenteric arteries of spontaneously hypertensive rats [65], and aorta of diabetic rats [66], suggesting that GYY may enhance NO synthesis in endothelial cells. This notion is supported by the observation that the levels of eNOS protein were increased in the uterine arteries of GYY-treated sFlt-1 dams. While the precise mechanism by which GYY induces eNOS expression remains unclear, previous studies have proposed that GYY can directly stimulate eNOS expression in cultured human umbilical vein endothelial cells [67]. Furthermore, H_2S was shown to preserve eNOS protein stability in endothelial cells by promoting microRNA-455-3p expression [68]. Overall, these findings provide evidence supporting the role of H_2S in augmenting eNOS function and preserving endothelium-dependent vasodilation in sFlt-1-elevated dams. Future studies should aim to elucidate the precise molecular mechanism underlying GYY-mediated upregulation of eNOS expression.

The link between sFlt-1 and negative outcomes, including FGR and low birth weight, has been consistently noted in human [27, 44] and animal studies [60, 63]. In our current study, GYY administration significantly improved maternal weight gain and weights of both male and female fetuses in sFlt-1-exposed dams. Thus, the beneficial effect of GYY treatment in sFlt-1 dams maybe attributed to the improvement in vascular function and enhanced uterine artery blood flow. Interestingly sFlt-1 and GYY did not affect placental weights in line with previous reports [69]. Studies indicate that sFlt-1 decreases placental nutrient transporters [70], raising the possibility of GYY improving placental function such as nutrient availability to the fetus.

Although our preclinical investigations demonstrate the efficacy of GYY in restoring maternal vascular function in the sFlt-1 rodent model, the translatability of these findings to human preeclampsia warrants cautious interpretation. Since there are inherent discrepancies in sFlt-1/VEGF dynamics between rodents and humans [71], further research is necessary to determine the clinical relevance of our findings. The use of alternative models that more accurately recapitulate the human pregnancy milieu, such as non-human primate models or humanized rodent models, may help bridge the translational gap between preclinical models and human preeclampsia.

In conclusion, this study aligns with the previous report that increased maternal sFlt-1 during pregnancy disrupts endothelial function, leading to hypertension and FGR [72]. We provide evidence that supplementation with H_2S using the pharmacological agent GYY in dams with elevated sFlt-1 restores blood pressure, enhances uterine artery blood flow, reduces exaggerated vasoconstriction, improves endothelial-mediated relaxation, and enhances fetal growth. It is important to note that our findings specifically highlight the mitigatory effect of H_2S on vascular hemodynamics in dams with elevated sFlt-1, and caution should be exercised in generalizing these results to other non-vascular sFlt-1-induced adverse outcomes or other models of gestational hypertension. Nonetheless, our results suggest that augmenting H_2S activity through pharmacological agonists holds promise as a preventive or therapeutic strategy for managing gestational hypertension and FGR associated with elevated sFlt-1.

Author contributions

PY performed research, data analysis, and drafted the manuscript. JM performed research, contributed to the study design and drafting the manuscript. MH performed experiments and data analysis. DC designed research and revised the manuscript. SK designed research, performed data analysis, and revised the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of Interest: The authors have declared that no conflict of interest exists.

Data availability

The underlying data are available in the article.

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