

Taurine Supplementation Lowers Blood Pressure and Improves Vascular Function in Prehypertension Randomized, Double-Blind, Placebo-Controlled Study

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Abstract—Taurine, the most abundant, semiessential, sulfur-containing amino acid, is well known to lower blood pressure (BP) in hypertensive animal models. However, no rigorous clinical trial has validated whether this beneficial effect of taurine occurs in human hypertension or prehypertension, a key stage in the development of hypertension. In this randomized, double-blind, placebo-controlled study, we assessed the effects of taurine intervention on BP and vascular function in prehypertension. We randomly assigned 120 eligible prehypertensive individuals to receive either taurine supplementation (1.6 g per day) or a placebo for 12 weeks. Taurine supplementation significantly decreased the clinic and 24-hour ambulatory BPs, especially in those with high-normal BP. Mean clinic systolic BP reduction for taurine/placebo was 7.2/2.6 mmHg, and diastolic BP was 4.7/1.3 mmHg. Mean ambulatory systolic BP reduction for taurine/placebo was 3.8/0.3 mmHg, and diastolic BP was 3.5/0.6 mmHg. In addition, taurine supplementation significantly improved endothelium-dependent and endothelium-independent vasodilation and increased plasma H₂S and taurine concentrations. Furthermore, changes in BP were negatively correlated with both the plasma H₂S and taurine levels in taurine-treated prehypertensive individuals. To further elucidate the hypotensive mechanism, experimental studies were performed both in vivo and in vitro. The results showed that taurine treatment upregulated the expression of hydrogen sulfide-synthesizing enzymes and reduced agonist-induced vascular reactivity through the inhibition of transient receptor potential channel subtype 3-mediated calcium influx in human and mouse mesenteric arteries. In conclusion, the antihypertensive effect of chronic taurine supplementation shows promise in the treatment of prehypertension through improvement of vascular function. (*Hypertension*. 2016;67:541-549. DOI: 10.1161/HYPERTENSIONAHA.115.06624.)

• [Online Data Supplement](#)

Key Words: blood pressure ■ hydrogen sulfide ■ prehypertension ■ taurine ■ transient receptor potential channels

Prehypertension is highly prevalent worldwide.¹ It is estimated that ≈30% to 50% of the population have this condition. It frequently complicates other cardiometabolic risk factors and is closely associated with coronary heart disease, stroke, and renal dysfunction.² Early intervention in prehypertension substantially prevents the incidence of hypertension and related damage to target organs. Currently, several strategies are used to treat prehypertension, including the incorporation of therapeutic lifestyle changes, such as healthy dietary intake and regular physical activity, as well as the use of antihypertensive drugs, such as an angiotensin II receptor blocker. Although these treatments improve prehypertension, poor compliance and limitations associated with antihypertensive medications prevent their application in the general population. Thus, there

is an urgent need to identify reliable and accurate measures to prevent the development of prehypertension.

Taurine (2-aminoethanesulfonic acid) is the most abundant, semiessential, sulfur-containing amino acid. It can be synthesized in vivo by cysteine in the presence of cysteine dioxygenase,³ but taurine is mainly acquired from dietary sources, such as eggs, meat, and seafood. Hydrogen sulfide (H₂S) is synthesized from 2 sulfur-containing amino acids, L-cysteine and L-methionine, by the 3 enzymes, cystathionine-γ-lyase (CSE), cystathionine-β-synthetase (CBS), and 3-mercaptopyrivate sulfurtransferase.⁴

Taurine has several potentially beneficial cardiovascular effects that involve regulation of the nitric oxide system and endothelial function,^{5,6} the renin-angiotensin-aldosterone

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system,^{7,8} the oxidative stress system and sympathoadrenal activity,⁹ and the endoplasmic reticulum stress system.^{10,11} Epidemiological studies have demonstrated a reduction in plasma sulfur amino acids in hypertensive patients.¹² Several clinical studies have reported that diets rich in taurine can reduce cardiovascular risks regardless of ethnicity and genetic background.^{13,14} In addition, animal experiments have shown that taurine depletion accelerates the development of high salt-induced hypertension.¹⁵ Although taurine has been shown to lower blood pressure (BP) in several hypertensive animal models, few rigorous and long-term clinical trials have confirmed this beneficial effect in human hypertension.⁹

Another key question is what is the mechanism of the antihypertensive effects of taurine supplementation?¹⁶ Recent animal and human studies have shown that taurine supplementation lowers BP and improves vascular function, possibly through suppression of renin-angiotensin-aldosterone system activity,^{17,18} augmentation of kallikrein activity in the blood and peripheral tissues,¹⁹ suppression of the renal sympathetic nervous system,^{9,20} diuretic and natriuretic activities, and vasorelaxant activity.²¹ H₂S can regulate vascular tone through several mechanisms, such as acting on ATP-sensitive potassium channels.^{22–24} A recent study has found that H₂S also affects transient receptor potential channels (TRPCs) in mesenchymal stem cells and regulates calcium homeostasis.⁴ Our previous studies have demonstrated that TRPC3-mediated calcium signaling contributes to the development of hypertension,^{25,26} but it is unclear whether the hypotensive effects of taurine and H₂S are associated with modulation of TRPC3 channels in the vasculature. In this study, we investigated the effects of chronic taurine supplementation on BP and vascular function in prehypertension by performing a randomized, double-blind, placebo-controlled clinical trial.

Methods

Detailed Methods are provided in the online-only Data Supplement.

Study Design and Procedures

This study was a prospective single-center, double-blind, randomized, placebo-controlled trial that was conducted in accordance with the CONSORT (Consolidated Standards of Reporting Trials) guidelines for the presentation of clinical trials (CONSORT 2010 Explanation and Elaboration) and the principles of the Declaration of Helsinki. The protocol was approved by the ethics committee of the Daping Hospital, Third Military Medical University. The protocol is registered in the US National Library of Medicine (<http://www.ClinicalTrials.gov>, identifier: NCT01816698).

Participants were recruited at the Center for Hypertension and Metabolic Diseases of Chongqing from December 2012 to December 2014. They were screened for eligibility after written informed consent was obtained. The prehypertension inclusion criteria for the first visit included the following: an age of between 18 and 75 years and an systolic BP (SBP) of 120 to 139 mmHg or a diastolic BP (DBP) of 80 to 89 mmHg, as determined by performing repeated measurements with a mercury sphygmomanometer. The main exclusion criteria included the following: clinical evidence of recent infection, pregnancy, coronary artery disease, peripheral vascular disease, cerebrovascular disease, renal dysfunction, diabetes mellitus, hypertension, tumor, mental disease, the use of other medications, or being enrolled in another trial within the last 3 months.

In total, 120 untreated participants (51 men and 69 women; age, 56.75±8.26 years) and 58 age-matched normotensive control subjects without taurine supplementation were enrolled only as baseline

comparison in the study. These untreated participants were randomly assigned to either a placebo group or a taurine group (Figure S1 in the online-only Data Supplement). All subjects completed a standardized questionnaire administered by trained personnel on their history of cardiovascular diseases and other illnesses. All subjects were asked not to alter their usual diet over the course of the 12-week study. They all underwent standardized clinical and laboratory examinations. BP was measured by a physician using a mercury sphygmomanometer after each subject had rested for at least 5 minutes in the seated position. Three measurements were obtained at 1-minute intervals, and the average was used to define the SBP and DBP. Laboratory tests were performed after an overnight fast, including measurements of fasting plasma glucose, triglyceride, cholesterol, hepatic enzyme, uric acid, blood urea nitrogen, and serum creatine levels.

Statistical Analysis

For all participants, we analyzed the changes from baseline (randomization) to 12 weeks in BP, vascular functions, biochemical and renal parameters, and other parameters. The sample size was chosen to ensure for 90% power to detect a 3-mmHg difference in our primary outcome, a change in SBP, with a 2-sided significance level of 0.05 and assuming a dropout rate of 20%, according to previous published data and a preliminary trial of prehypertensive participants. All analyses were based on intention-to-treat populations (defined as all patients who took at least 1 dose and had at least 1 efficacy measurement available after randomization), with the last value carried forward for missing values. Comparisons of continuous variables between the placebo and taurine groups were analyzed using the Mann-Whitney test (GraphPad Prism; La Jolla, CA). Comparisons of variables before and after treatments were analyzed using the Wilcoxon signed-rank matched pair test. The χ^2 test was used for categorical variables. Spearman nonparametric correlation analysis was performed to determine the relationships between BP changes and other factors. The immunoblotting results, wire myograph results, and PTI (Photon Technology International) results were compared using the Mann-Whitney test. A 2-tailed $P < 0.05$ was considered statistically significant. The data were expressed as mean±SEM or SD for normally distributed variables and median (25th and 75th percentiles) for non-normally distributed variables, and all the results were analyzed using SPSS 18.0.

Results

Baseline Characteristics of Participants

Compared with the normal controls, the enrolled prehypertensive participants had higher clinic and ambulatory BPs (ABPs) and increased pulse wave velocity and postprandial blood glucose values. In addition, there were no significant differences in the baseline characteristics between the placebo and taurine groups (Table S1). Of 793 participants screened in the study, 120 untreated participants were randomized; of whom, 97 completed the entire study protocol and had complete data, with a loss rate of 19.2% (Figure S1). Both the taurine and placebo interventions were well tolerated, and no serious adverse events were reported by any of the participants.

Chronic Taurine Supplementation Reduces BP in Prehypertensive Individuals

Administration of taurine for 12 weeks significantly reduced BP. The clinic SBP and DBP decreased in the taurine group by 7.2 mmHg (95% confidence interval [CI], 3.75–10.55; $P < 0.001$) and 4.7 mmHg (95% CI, 2.16–7.14; $P < 0.001$), respectively, compared with the baseline values; however, these changes were not evident in the placebo group (Figure 1A and 1B; Figure S2A and S2B; Table S2). Similarly, the 24-hour ABP in the taurine group exhibited a similar pattern, with mean

decreases in the SBP and DBP of 3.8 mmHg (95% CI, 1.97–5.56; $P < 0.05$) and 3.5 mmHg (95% CI, 2.14–4.81; $P < 0.05$), respectively, compared with the baseline values; however, no changes were observed in the placebo-treated group (Figure 1C and 1D; Figure S3A and S3B; Table S2; $n = 44$ in the placebo group and $n = 42$ in the taurine group, respectively). Further analysis revealed that taurine treatment reduced the daytime

ambulatory SBP and DBP compared with the baseline values, thereby decreasing the ambulatory SBP by 4.5 mmHg (95% CI, 2.21–6.79 mmHg; $P < 0.05$) and the ambulatory DBP by 4.3 mmHg (95% CI, 2.82–5.80 mmHg; $P < 0.01$; Figure S3C and S3D); however, no significant changes were observed in the placebo group. Meanwhile, the taurine treatment did not influence the nighttime ABP (Figure S3E and S3F).

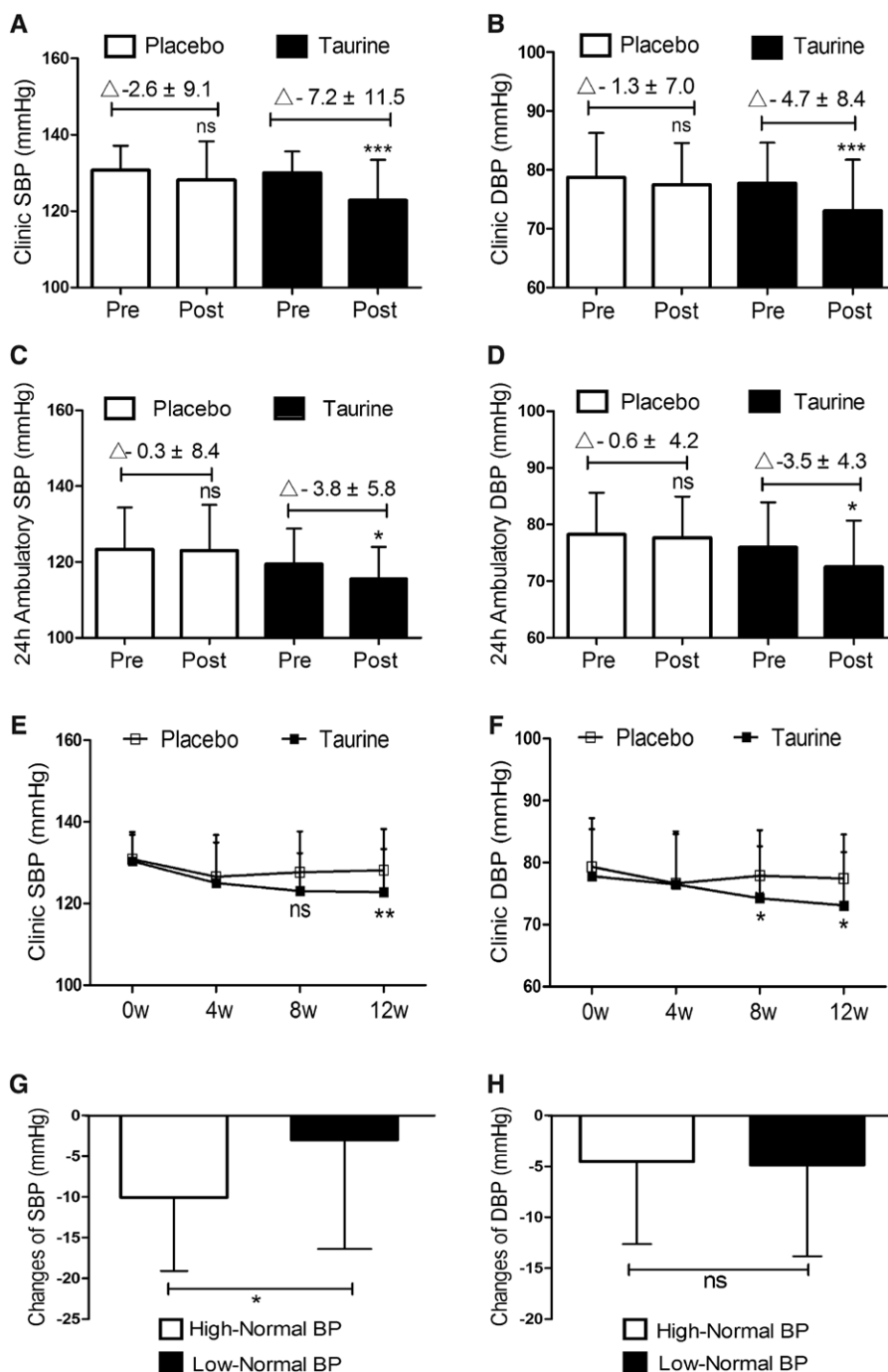


Figure 1. Effect of taurine supplementation on blood pressure (BP). **A** and **B**, Clinic systolic BPs (SBPs; **A**) and diastolic BPs (DBPs; **B**) of participants treated with placebo or taurine at baseline (0 weeks, Pre) and after treatment (12 weeks, Post). The data are presented as the mean \pm SD; *** $P < 0.001$, compared with baseline values. **C** and **D**, Twenty-four-hour average ambulatory BPs of the participants at 0 week and 12 weeks compared with the corresponding baseline values. $n = 44$ in the placebo group and $n = 42$ in the taurine group, respectively; * $P < 0.05$. **E** and **F**, Clinic SBPs and DBPs at 0, 4, 8, and 12 weeks in the 2 groups. * $P < 0.05$ and ** $P < 0.01$ compared with the placebo group. **G** and **H**, Comparisons of BP changes between the prehypertensive participants with high- and low-normal BPs in the taurine group. * $P < 0.05$. ns indicates not significant.

Chronic taurine supplementation time dependently decreased clinic BP. Compared with the placebo group, both the clinic SBP and DBP were significantly reduced at 8 and 12 weeks after taurine administration (Figure 1E and 1F; Figure S2C and S2D). Importantly, taurine supplementation for 12 weeks greatly reduced the BPs of the prehypertensive participants with high-normal BPs (SBP, 130–139/DBP, 85–89 mmHg) compared with the prehypertensive participants with low-normal BPs (SBP, 120–129 mmHg; DBP, 80–84 mmHg). Changes in the SBP of 10.1 mmHg were observed in the high-normal BP group compared with changes of 3.0 mmHg in the low-normal BP group ($P<0.05$; Figure 1G). However, the changes in the DBP were similar between these 2 subgroups (Figure 1H).

Taurine Supplementation Improves Vasodilation in Prehypertensive Individuals

Chronic taurine supplementation significantly improved both endothelium-dependent vasodilation (flow-mediated dilation) and endothelium-independent vasodilation (nitroglycerin-mediated dilation) by 3.2% and 4.4%, respectively, as measured via flow-mediated vasodilation using a sonographer in the prehypertensive individuals. However, the beneficial effect of taurine supplementation on vasodilation was absent in the prehypertensive individuals treated with placebo (Figure 2A–2D).

Taurine Supplementation Elevates Plasma Levels of Taurine and H₂S in Association With BP Changes in Prehypertensive Individuals

After treatment for 12 weeks, the plasma taurine and H₂S levels were significantly higher in the prehypertensive individuals treated with taurine (plasma H₂S level: 43.8±20.82 μmol/L at baseline to 87.0±24.51 μmol/L after treatment; $P<0.001$

and plasma taurine level: 108.3±55.27 μmol/L at baseline to 142.3±62.14 μmol/L after treatment; $P<0.05$); however, these changes were not observed in the participants treated with placebo (Figure 3A and 3B). Furthermore, the changes in BP were negatively correlated with both the plasma H₂S and taurine levels in the taurine-treated prehypertensive individuals (Figure 3C–3F), especially in the prehypertensive participants with a high-normal BP level (Figure S4A–S4C). In contrast, these associations between BP and the plasma levels of H₂S and taurine were not observed in the participants treated with the placebo (Figure S5A–S5D).

Effects of Taurine on H₂S-Synthesizing Enzymes, TRPC3, and Vascular Relaxation

To elucidate the mechanisms underlying the effects of taurine on BP and vascular functions, we further examined 2 key H₂S-synthesizing enzymes, CBS and CSE. We showed that CBS and CSE were expressed in the endothelia and adventitia of mesenteric arteries (MAs) from human and aortas from mice. However, TRPC3 was mainly expressed in the media of arteries (Figure 4A and 4B). Western blotting also indicated that CBS, CSE, and TRPC3 were coexpressed in MAs from humans and aortas from *Trpc3*^{+/+} wild-type (WT) mice (Figure 4C and 4G). In addition, vascular CBS/CSE expression was upregulated in *Trpc3*^{-/-} mice compared with WT mice (Figure 4C and 4D). Administration of taurine significantly upregulated CBS/CSE expression but inhibited TRPC3 expression in both aortas from spontaneously hypertensive rats treated with taurine and cultured human vascular tissues (Figure 4E–4J). After depletion of intracellular calcium storage using thapsigargin, a sarcoplasmic reticulum Ca²⁺-ATPase inhibitor, KCl-induced vasoconstriction was dose dependently relaxed by NaHS, a H₂S donor; however, this effect was enhanced by a TRPC3 inhibitor, Pyr3, or by

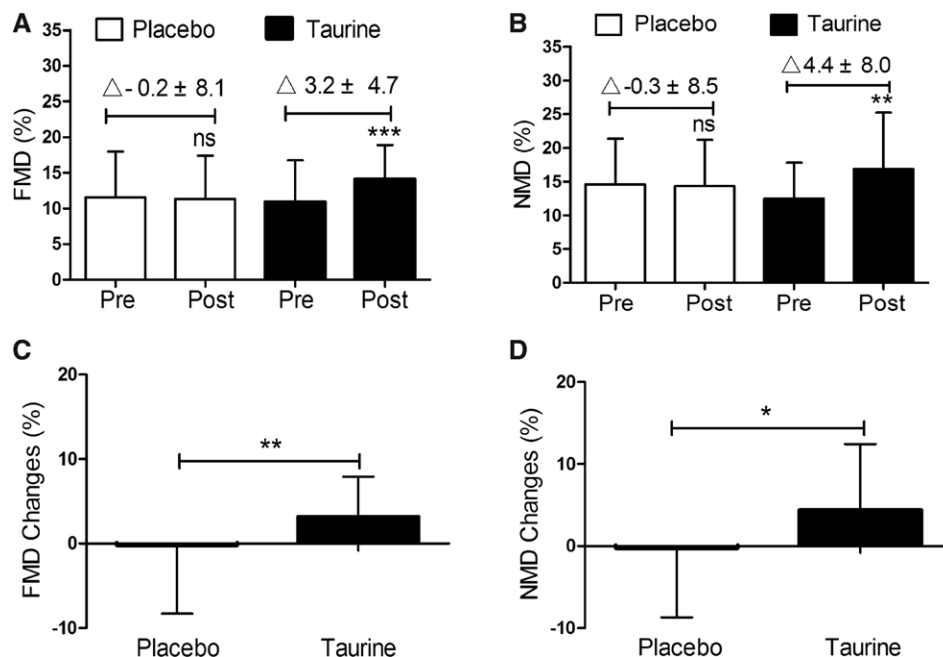


Figure 2. Effect of taurine supplementation on vasodilation. **A** and **B**, Flow-mediated dilation (FMD) and nitroglycerin-mediated dilation (NMD). The data are presented as the mean±SD. ** $P<0.01$ and *** $P<0.001$, compared with pretreatment with taurine (Pre). **C** and **D**, Changes in FMD and NMD in the 2 groups. * $P<0.05$ and ** $P<0.01$, compared with the placebo group. ns indicates not significant.

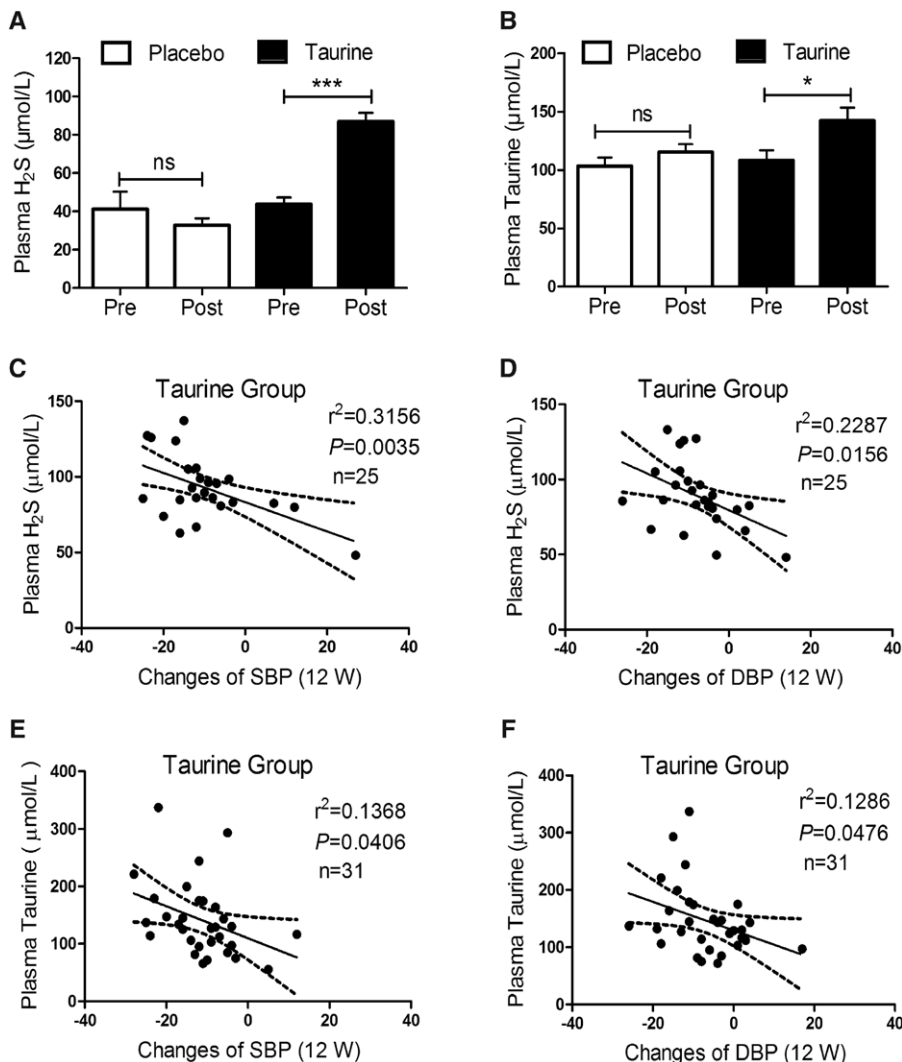


Figure 3. Effects of taurine supplementation on plasma taurine and hydrogen sulfide (H₂S) levels and their associations with blood pressure (BP) changes. **A** and **B**, Changes in the plasma H₂S and taurine levels. The data are presented as the mean±SEM. * $P<0.05$ and *** $P<0.001$, compared with pretreatment with taurine (Pre). **C–F**, Correlations between BP changes and plasma H₂S and taurine levels, respectively. DBP indicates diastolic BP; ns, not significant; and SBP, systolic BP.

Trpc3 gene knockout (Figure 4K and 4L). These findings indicate that TRPC3 might be involved in H₂S-mediated vascular relaxation.

H₂S Exerts Vascular Relaxation by Targeting TRPC3-Mediated Calcium Influx

We further examined H₂S-induced vascular relaxation, which occurs through the targeting of TRPC3. Intact MAs were isolated from WT and *Trpc3*^{-/-} mice. Calcium influx of intact blood vessels was measured using fluorescence techniques after depletion of intracellular calcium storage in the absence of external calcium. The phenylephrine-induced increase in calcium influx in the artery was completely abolished by the TRPC3 inhibitor Pyr3 after thapsigargin treatment (Figure S6A and S6B). Administration of NaHS significantly diminished the thapsigargin- and phenylephrine-induced increase in calcium influx in human MAs (Figure S6C–S6F). Furthermore, NaHS partially inhibited the thapsigargin-induced calcium influx in the *Trpc3*^{+/+} mice, but this effect

was absent in intact arteries isolated from the *Trpc3*^{-/-} mice (Figure S6G–S6N).

Discussion

To the best of our knowledge, this is the first randomized, double-blind, placebo-controlled clinical trial to investigate the effects of taurine supplementation in prehypertensive individuals. Furthermore, we have provided experimental evidence to facilitate elucidation of its mechanism of action. This study has revealed that oral taurine supplementation for 12 weeks significantly reduces the clinic and 24-hour ABPs in prehypertensive individuals, especially in those with high-normal BP. In addition, taurine treatment substantially promotes vasodilation and elevates the plasma taurine and H₂S levels in these individuals. Furthermore, changes in BP were negatively correlated with the plasma taurine and H₂S levels. However, these beneficial effects were absent in the prehypertensive individuals treated with placebo. In experimental studies, administration of taurine has been shown to enhance

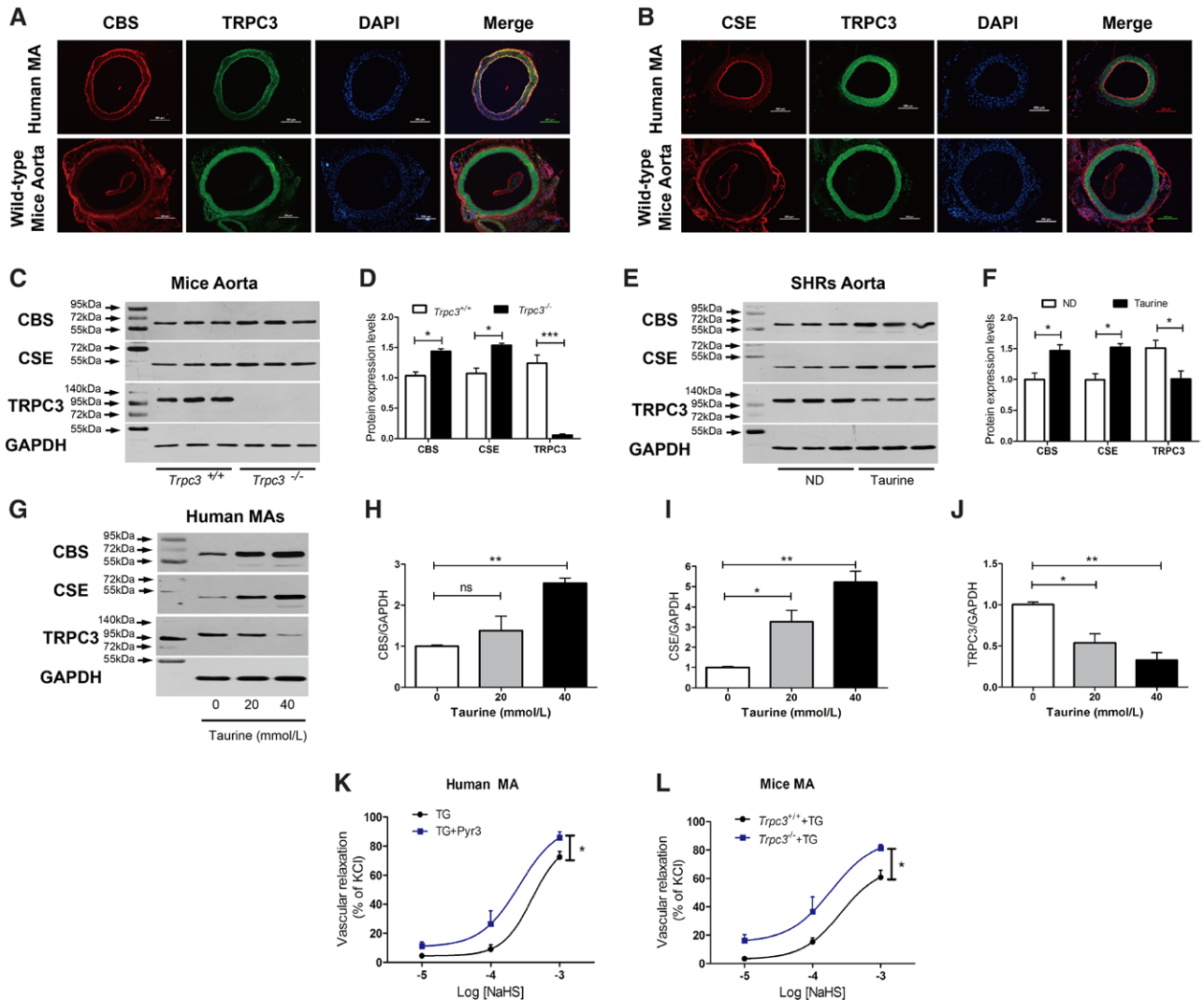


Figure 4. Effects of taurine on cystathionine- β -synthetase (CBS)/cystathionine- γ -lyase (CSE), transient receptor potential channel 3 (TRPC3), and vascular relaxation. **A** and **B**, The immunofluorescence staining results showing that CBS/CSE (red) and TRPC3 (green) were coexpressed in mesenteric arteries (MAs) from humans and in aortas from wild-type mice. 4',6-Diamidino-2-phenylindole (DAPI) was also stained to show the existence of nuclei (blue). **C** and **D**, The expression levels of CBS, CSE, and TRPC3 in aortas from *Trpc3*^{+/+} and *Trpc3*^{-/-} mice were detected by Western blotting. The bands from 3 independent immunoblots were quantified using Image J, and the relative expression levels are shown in **(D)**. The data are presented as the mean \pm SEM; **P*<0.05 and ****P*<0.001, compared with that of *Trpc3*^{+/+} mice. **E** and **F**, Effects of in vivo taurine supplementation on the expression levels of CBS, CSE, and TRPC3. Spontaneously hypertensive rats (SHRs) were fed 2% taurine for 12 weeks from 4-week old, and then aortas were obtained for Western blotting analysis. **P*<0.05, compared with that of the normal diet (ND). **G–J**, Effect of in vitro taurine supplementation on the expression levels of CBS, CSE, and TRPC3. Human MAs were treated with taurine (at doses of 0, 20, and 40 mmol/L) for 24 hours. **P*<0.05 and ***P*<0.01, compared with the vehicles. **K** and **L**, Reactivities of human and mice MAs. After contraction induced using 60 mmol/L KCl, NaHS (10⁻⁵ to 10⁻³ mol/L) was added to promote vasodilation, and then the percentage of vasodilation was plotted. n=4; **P*<0.05. TG indicates thapsigargin.

the expression of H₂S-synthesizing enzymes (CBS/CSE) and to reduce vascular TRPC3 expression in spontaneously hypertensive rats. Furthermore, the vascular relaxation induced by the H₂S donor NaHS is enhanced by TRPC3 antagonist treatment. These findings indicate that taurine intervention improves vascular tone by targeting the H₂S-mediated inhibition of TRPC3-induced calcium influx.

Taurine is a sulfur-containing amino acid that is both cheap and nontoxic, and it is widely used as a functional dietary factor. Seafood containing an abundance of taurine improves cardiovascular and metabolic diseases, such as obesity, diabetes mellitus, and hyperlipidemia. In addition, taurine has multiple

biological effects, such as protection against liver cirrhosis and antioxidative,²⁷ anti-inflammatory, antiatherosclerotic,^{28,29} and antiobesity effects.³⁰ Unfortunately, evidence that taurine supplementation reduces BP in human hypertension is inconclusive despite the fact that multiple experimental studies have demonstrated the hypotensive effect of taurine in different hypertensive animal models.

The antihypertensive effect of taurine in humans has only been confirmed in a few clinical studies with small sample sizes (n=10–12) that were short term (7 days to 6 weeks).³¹ One nonrandomized placebo-controlled trial showed that oral taurine supplementation (6 g per day) for 1 week decreased the

SBP by 9.0 mmHg and the DBP by 4.1 mmHg in borderline hypertensive patients.⁹ In addition, taurine supplementation has been shown to lower BP by ≈ 22 to 49 mmHg in different experimental hypertensive rats.³¹ Therefore, it remains unknown whether oral taurine supplementation is beneficial for prehypertensive individuals. In this randomized, double-blind, placebo-controlled study, we showed that administration of low-dose taurine (1.6 g per day) for 12 weeks can time dependently lower both clinic and ABPs and improve vascular relaxation. In particular, prehypertensive individuals with high-normal BP exhibited a better response to taurine than those with low-normal BP. Our study has provided the first solid evidence of the hypotensive effect of taurine in prehypertensive individuals.

Epidemiological studies have shown that the plasma taurine level is lower in patients with essential hypertension.¹² Nara et al³² have reported that this level is decreased in spontaneously hypertensive rats in relation to the severity of hypertension. The plasma taurine level is negatively correlated with BP in hypertensive patients.¹⁴ Taurine deficiency in rats accelerates high salt intake-induced hypertension through renal dysfunction.¹⁵ Galloway et al³³ reported that acute taurine treatment resulted in a 13-fold increase in the plasma taurine concentration, whereas no significant change in the muscle taurine concentration was observed. In this study, we found that chronic taurine treatment for 12 weeks resulted in an almost 1.5-fold increase of plasma taurine concentration in the prehypertensive individuals and that this increase was correlated with a reduction in BP. In addition, the prehypertensive individuals with a high end point plasma taurine level exhibited a greater hypotensive response to the taurine treatment.

The manner by which taurine exerts its hypotensive effect has been studied for a long time.¹⁶ Previous studies have shown that taurine supplementation improves endothelium-dependent vasodilation through restoration of vascular redox homeostasis and improvement of nitric oxide bioavailability.³⁴ In addition, in human studies, improvement of flow-mediated dilation has been observed in response to dietary taurine supplementation in young smokers.³⁵ The improved vascular function may facilitate the hypotensive effect and provide extra cardiovascular benefits. Available data suggest that the hypotensive effect of taurine does not occur through 1 specific mechanism but rather through multiple mechanisms.

In addition to nitric oxide and carbon monoxide, H₂S, which is another important gas transmitter, has been widely studied in the cardiovascular system in recent years,³⁶ and its vasodilatory effects have also been reported. H₂S is endogenously produced from 2 sulfur-containing amino acids, L-cysteine and L-methionine, by the 2 H₂S-synthesizing enzymes, CBS and CSE.²⁴ Mutant mice lacking CSE display pronounced hypertension and reduced endothelium-dependent vasorelaxation, but this result was not confirmed by other studies.²⁴ However, H₂S replacement has been shown to reduce the SBP in both *Cse*^{-/-} and *Cse*^{+/-} mice,²⁴ suggesting that the H₂S synthases/H₂S pathway confer protection against hypertension. Taurine, as a sulfur-containing amino acid, functions in the methionine cycle and can be converted by cysteine in the presence of cysteine dioxygenase,³ whereas H₂S is synthesized from the 2 sulfur-containing amino acids, L-cysteine

and L-methionine. This study has shown that taurine is probably a substrate for the synthesis of H₂S to increase CBS and CSE expression. Therefore, we assumed that a correlation may exist between taurine and H₂S. Taurine supplementation resulted in a significant elevation in the plasma H₂S level in the prehypertensive individuals, and this elevation was correlated with a decrease in BP in the taurine group. Using MAs from healthy human volunteers and aortas from spontaneously hypertensive rats that were fed taurine for 3 months, we further demonstrated that taurine administration upregulated the expression of vascular CBS/CSE, which caused the increased production of H₂S in blood vessels *in vitro* and *in vivo*. Thus, other mechanisms of the vasorelaxant effect of H₂S have also been identified involving opening of the ATP-sensitive potassium channels,²³ interaction with nitric oxide pathways, functioning as an endothelium-derived hyperpolarizing factor, and direct activation of protein kinase G.³⁷ Recently, Cheang et al³⁸ and Tian et al³⁹ have reported that H₂S dilates blood vessels by opening voltage-gated potassium channels in rat coronary arteries and inhibits calcium channels in rat cerebral arteries, respectively. H₂S has been demonstrated to regulate the activities of TRP channels in bone marrow mesenchymal stem cells through sulfhydration.⁴ Our previous work has demonstrated that TRPC3 upregulation and dysfunction in monocytes and in the vasculature from both genetically hypertensive rats and essential hypertensive patients play important roles in the pathogenesis of hypertension.^{25,26,40,41} In this study, we further verified that the H₂S donor NaHS inhibited phenylephrine- and thapsigargin-induced Ca²⁺ influx and relaxation in MAs from human and *Trpc3*^{+/+} WT mice; however, this effect was absent in intact arteries from *Trpc3*^{-/-} mice. Our work has revealed a novel unrecognized mechanism of taurine- and H₂S-induced vasorelaxation that functions by enhancing the metabolism of sulfur-containing amino acids.

Study Limitations

The limitation of this study is that it was not performed across multiple centers. Further studies should validate whether this beneficial effect is present in other ethnicities and populations. In addition, the hypotensive effects of taurine have been reported to occur via the central nervous system,^{42,43} attenuation of the overactivity of the sympathetic system and increased urinary norepinephrine, and epinephrine excretion.³¹ An effect of taurine on BP occurring via the central nervous system cannot be excluded.³¹

Perspectives

Prehypertension plays an important role in the development of hypertension. Furthermore, prehypertension is closely associated with the morbidities of stroke, ischemic heart disease, and renal dysfunction. Although lifestyle modifications and an angiotensin II receptor blocker have been used to treat prehypertension, poor compliance and limitations of antihypertensive agents are the main obstacles of treatment. This randomized, double-blind, placebo-controlled clinical trial is the first to demonstrate that taurine supplementation significantly reduces BP and improves vascular function in prehypertensive individuals, especially in those with high-normal BP. Furthermore, changes in BP are correlated with both the

plasma H₂S and taurine levels in the taurine-treated prehypertensive individuals. We further demonstrate that the hypotensive effect of taurine involved the H₂S-mediated inhibition of TRPC3-induced calcium influx. Taurine, as the most abundant, semiessential, sulfur-containing amino acid, is rich in seafood and easily consumed daily. Considering the elevated cardio-metabolic risks of large populations of prehypertensive individuals, consumption of taurine-rich food may be a promising and cost-effective approach to prehypertension treatment.

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Disclosures

None.

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Novelty and Significance

What Is New?

- This randomized, double-blind, placebo-controlled clinical trial is the first to demonstrate the hypotensive effect of taurine supplementation in prehypertensive individuals.
- Taurine treatment remarkably improves blood pressure and vascular function, especially in prehypertensive individuals with high-normal blood pressure.
- The hypotensive mechanism of taurine partially involves the hydrogen sulfide-mediated inhibition of transient receptor potential channel 3-induced calcium influx in the vasculature.

What Is Relevant?

- Taurine supplementation results in a substantial, time-dependent reduction in blood pressure in prehypertensive individuals. Daily taurine supplementation promotes an additional decrease in blood pressure beyond that achieved with conventional lifestyle changes and pharmacotherapy.

Summary

Daily taurine supplementation is a novel strategy for lowering blood pressure in prehypertension, either as a dietary factor or in conjunction with conventional lifestyle changes.

Taurine Supplementation Lowers Blood Pressure and Improves Vascular Function in Prehypertension: Randomized, Double-Blind, Placebo-Controlled Study

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Taurine Supplementation Lowers Blood Pressure and Improves Vascular Function in Prehypertension

A Randomized, Double-Blind, Placebo-Controlled Study

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Supplemental Methods

Randomization and blinding procedures

All eligible participants were randomized at a 1:1 ratio to the taurine or placebo group. Random numbers were generated by Microsoft Excel program, and a randomized block design was used to generate six blocks with 30 patients in each block. All blind codes, including random numbers and allocation sequences, were generated, saved and sealed in opaque envelopes by an independent blind codes manager within the hospital. All patients, study team members, and analysts were masked to the treatment assignments, with the exception of the blind codes manager. The codes could be broken in the case of a serious adverse event if it was deemed essential for the treatment of the patient, but the manager did not assess the patients or participate in the writing of the report. The experimental taurine particles and matching placebo particles were identical in shape, size, and appearance to taurine and were manufactured and packaged by an independent third-party pharmacy, which is where the drugs were produced and then packaged together in cartons that were labeled with random numbers by the pharmacy according to the blind codes and correspondingly allocated as the trial drugs.

Interventions, follow-up and study outcomes

The participants received low-dose taurine (1.6 g/day) or an equivalent amount of placebo. They were told to take the study medication between 08:00 and 09:00 am. All subjects were asked not to alter their usual diet over the course of the 12-week study, and they were all scheduled for four follow-up visits at 4, 8 and 12 weeks after randomization. The primary outcomes were defined as the changes in systolic blood pressure, diastolic blood pressure and 24-hour ambulatory blood pressure. Ambulatory BP monitoring (ABPM) was recorded using Spacelabs 90217 (Spacelabs, Medical Inc., Redmond, Washington, USA). Automatic BP readings were taken every 15 minutes throughout the day (07:00-23:00) and every 30 minutes during the night (23:00-07:00). The other main outcomes were vascular functions (flow-mediated and nitroglycerin-mediated vasodilation).

Flow- and nitroglycerin-mediated vasodilation

Flow-mediated (endothelium-dependent) and nitroglycerin-mediated (endothelium-independent) vasodilation were measured by a single trained sonographer with high-resolution ultrasonography using a 7.5-MHz linear transducer on an HY 6000 system (Wuxi Haiying Electronic Medical System Co. Ltd., Jiangsu, China)¹. In detail, measurements were performed on the brachial artery at 4.5 cm above the antecubital fossa before inflation of a pneumatic cuff on the upper arm to 250 mm Hg for 5 minutes and at 1 minute after cuff release. FMD was expressed as the percentage dilation from the baseline diameter to that observed at 1 minute after cuff release. Then, 0.5 mg nitroglycerin was sublingually administered, and the diameter of the brachial artery was measured. NMD was expressed as the percentage dilation from the baseline diameter to that observed after nitroglycerin administration.

Plasma H₂S measurement

Plasma hydrogen sulfide was measured by reverse-phase high-performance liquid chromatography (RP-HPLC) after derivatization with excess monobromobimane (MBB) to form a stable sulfide-dibimane derivative². Briefly, a total of 30 μ L plasma was mixed with 70 μ L of 100 mmol/L Tris-HCl buffer (pH 9.5, 0.1 mmol/L DTPA), followed by the addition of 50 μ L of 10 mmol/L MBB. The reaction was stopped after 30 minutes by adding 50 μ L of 200 mmol/L 5-sulfosalicylic acid, and then the sample was centrifuged and the supernatant was analyzed using a Shimadzu Prominence HPLC with fluorescence detection (λ_{ex} : 390 nm and λ_{em} : 475 nm) and an Eclipse XDB-C18 column.

Animal treatments

Four-week-old male spontaneously hypertensive rats (SHRs) were obtained from Charles River Laboratories (Bar Harbor, ME, USA). The rats were housed under a 12-hour day/night cycle with free access to normal food and water. The institute's Animal Care and Use Committee approved all animal protocols. A total of six SHRs were randomly assigned to drink water with 2% taurine (w/v) or normal water starting at 8 weeks age and continuing for 12 weeks intervention period. After the intervention, the rats were sacrificed, and aortas were isolated for Western blot analysis.

Immunofluorescence staining

Human mesenteric arteries were obtained from volunteers undergoing laparotomy for other reasons after they signed informed consent forms. Human mesenteric vessels and aortas from *Trpc3* wild-type mice were sectioned to 8 μ m and then fixed with 10% formalin at room temperature for 60 min. The tissues were washed with PBS and then blocked with 5% bovine serum albumin for 20 min and incubated with primary antibodies (CBS from Santa Cruz Biotechnology, TRPC3 from Alomone Labs and CSE from Novus Biologicals) overnight at 4 °C. After incubation, the tissues were washed three times and incubated with fluorescent-labeled secondary antibodies (Abcam) at room temperature for 30 min. Control experiments were performed in the absence of primary antibodies. After being washed three times, the tissues were stained with DAPI staining solution at room temperature. Images were obtained with a TE2000-U Nikon eclipse microscope and analyzed with NIS-Elements imaging software (Nikon, Japan).

Western blotting

Western blotting was conducted as previously described³ using whole protein extracts prepared from human mesenteric vessels and aortas from *Trpc3* wild-type mice. The primary antibodies against CBS, CSE, and TRPC3 were the same ones used for immunofluorescence staining. GAPDH was purchased from Santa Cruz Biotechnology.

Measurement of vascular reactivity

The vascular reactivity of freshly isolated human MAs was examined using a wire myograph (Danish Myo Technology, Denmark). The vascular reactivities of human mesenteric vessels subjected to the different treatments was assessed as previously described³.

Intracellular free Ca²⁺ measurement

Measurement of the intracellular Ca²⁺ concentrations in human mesenteric arteries was performed with Fura-2AM using a PTI Fluorescence Master System (Photon Technology International, Birmingham, NJ, USA). Briefly, freshly isolated human and mouse MAs were cut into small pieces and loaded with 5 µmol/L Fura-2-AM (Invitrogen, Thermo Fisher) and 0.025% Pluronic F-127 in physiological saline solution containing 135 mM NaCl, 5 mM KCl, 1.5 mM CaCl₂, 1 mM MgCl₂, 11 mM D-glucose and 10 mM HEPES, pH 7.4, for 45 minutes at 37 °C in the dark. Then, the vessel pieces were washed three times in Ca²⁺-free fluid as outlined above and treated with different reagents. Fluorescence was measured at 510 nm emission and at excitation wavelengths of 340 nm and 380 nm, at baseline and after treatment. Changes in the [Ca²⁺]_i were deduced from the ratios of the transient increases in fluorescence intensity at 340 nm and 380 nm.

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Supplemental Tables

Table S1. Baseline characteristics of the enrolled participants

Characteristics	Normal Controls (n=58)	Placebo (n=55)	Taurine (n=56)
Age (Years±SD)	56.5±10.85	57.1±8.07	55.9±8.49
Male (n, %)	22, 37.93%	23, 41.82%	23, 41.07%
Clinic BP (mmHg)±SD			
SBP	112.5±6.01	130.9±6.65*	130.4±6.47*
DBP	70.4±5.80	79.3±7.89*	77.8±7.63*
Ambulatory BP (mmHg)±SD			
24h ASBP	107.8±7.98	123.5±11.03*	121.6±9.89*
24h ADBP	70.9±5.73	79.0±7.78*	78.0±7.96*
PWV (cm/s) (IQR)			
PWV Right	1317 (1237, 1433)	1478 (1308, 1659)*	1433 (1333, 1584)†
PWV Left	1275 (1201, 1389)	1458 (1311, 1645)*	1430 (1300, 1610)*
Plasma Glucose (mmol/L)			
Fast	5.26 (4.71, 5.75)	5.36 (4.95, 5.85)	5.28 (4.84, 5.99)
Postprandial	6.10 (5.10, 8.01)	6.87 (6.04, 8.55)	8.17 (6.86, 9.46)*
Triglycerides (mmol/L) (IQR)	0.99 (0.80, 1.40)	1.34 (0.94, 1.76)‡	1.59 (1.14, 2.01)*
Cholesterol (mmol/L)±SD			
Total	4.85±1.07	5.11±1.07	5.23±0.97
HDL-C	1.42±0.27	1.43±0.31	1.39±0.32
LDL-C	3.06±0.99	3.15±0.87	3.17±0.85
Hepatic enzymes (IU/l)			
AST	24 (19, 28)	22 (18, 27)	23.5 (19.75, 28.5)
ALT	18 (13, 26)	17 (14, 21)	18 (14, 24)
Uric acid (µmol/L)±SD	358.5±127.0	337.2±102.2	351.0±101.4
BUN (mmol/L)±SD	4.85±1.14	5.12±1.59	5.06±1.38
Serum creatine (µmol/L)±SD	80.49±15.02	80.22±15.83	80.22±14.16
Vascular functions (IQR)			
FMD (%)	10.35 (6.55, 12.48)	10.20 (7.90, 14.40)	9.50 (6.68, 13.40)
NMD (%)	13.75 (9.85, 17.45)	12.61 (10.70, 18.10)	11.65 (9.58, 14.05)

* $P < 0.001$, † $P < 0.01$, ‡ $P < 0.05$, compared with corresponding normal controls.

SD, standard deviation; IQR: Interquartile range; BP, Blood Pressure; SBP, systolic BP; DBP, diastolic BP; ASBP, ambulatory SBP; PWV, Pulse Wave Velocity; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; BUN, Blood Urea Nitrogen; FMD, Flow-mediated Dilation; NMD, Nitroglycerin-Mediated Dilation.

Table S2. BP levels of participants before and after treatment for 12 weeks

Characteristics	Pre-Placebo	Post-Placebo	Pre-Taurine	Post-Taurine
Clinic SBP (mmHg)	130.8±6.30	128.2±10.08	130.0±5.64	122.8±10.57*
Clinic DBP (mmHg)	78.7±7.51	77.4±7.10	77.7±6.87	73.1±8.66*
24-h ASBP (mmHg)	123.3±11.08	123.0±12.06	119.4±9.37	115.6±8.35†
24-h ADBP (mmHg)	78.3±7.38	77.7±7.30	76.0±7.97	72.5±8.22†

* $P < 0.001$, † $P < 0.05$, compared with corresponding pre-taurine group.

Supplemental Figures

Figure S1

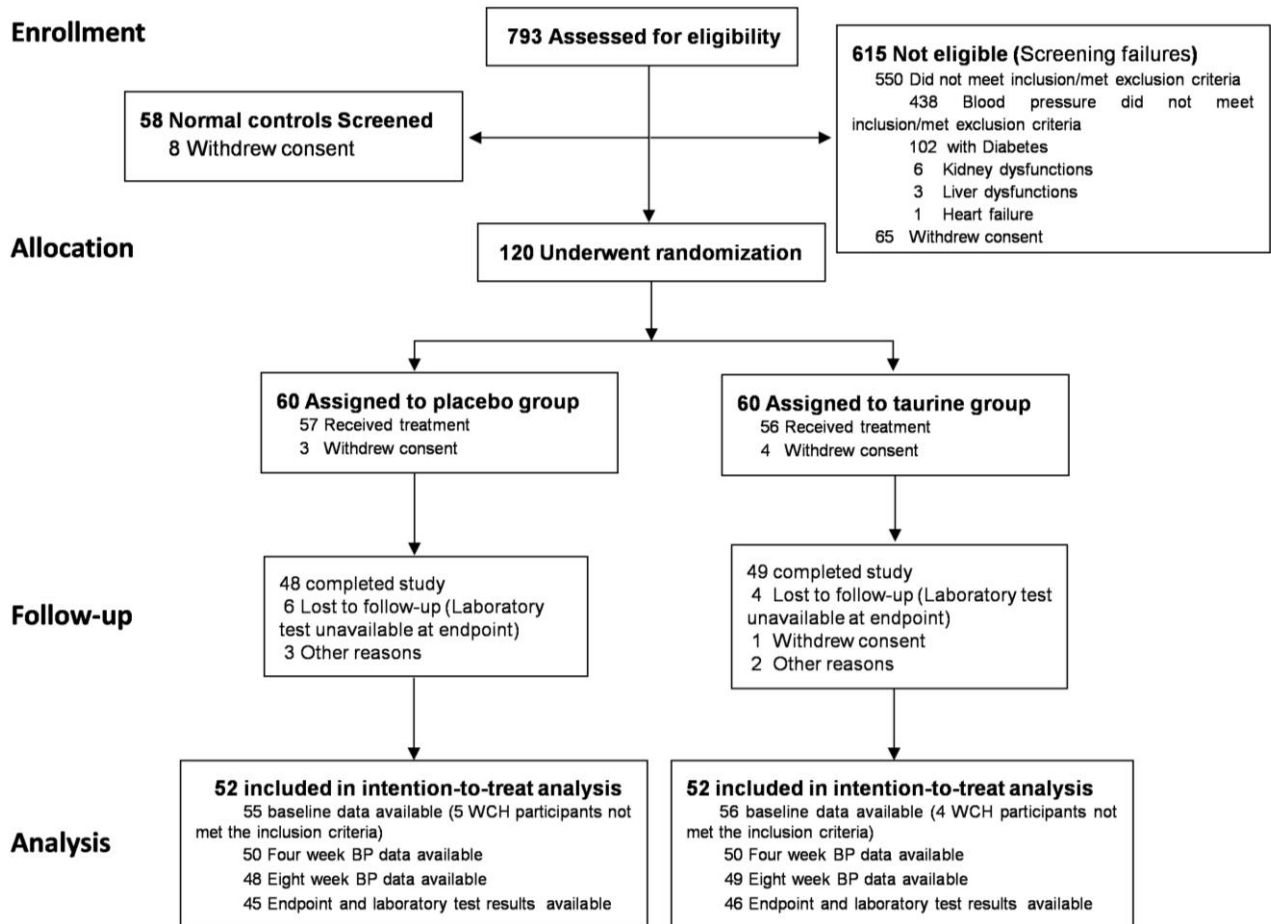


Figure S1. Screening, enrollment, randomization, and follow-up of study participants. Of the 120 randomized participants, 48 (80%) in the placebo group and 49 (81.7%) in the taurine group completed all of the follow-up visits and experimental tests. WCH, White coat hypertension.

Figure S2

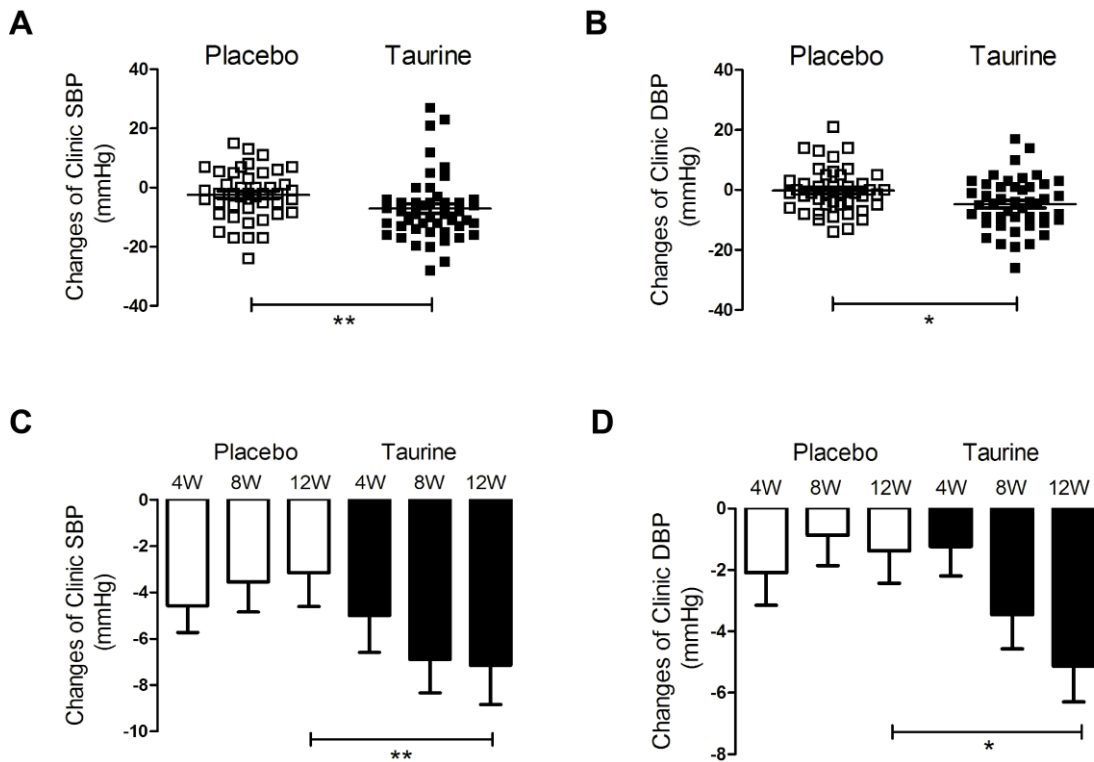


Figure S2. Effects of taurine on clinic BP. **A and B**, Changes in the clinic SBP and DBP in the prehypertensive individuals after treatment with placebo or taurine for 12 weeks. * $P < 0.05$, ** $P < 0.01$. **C and D**, Changes in the clinic SBP and DBP throughout the intervention period in the two groups. The data are presented as the mean \pm SEM. * $P < 0.05$, ** $P < 0.01$.

Figure S3

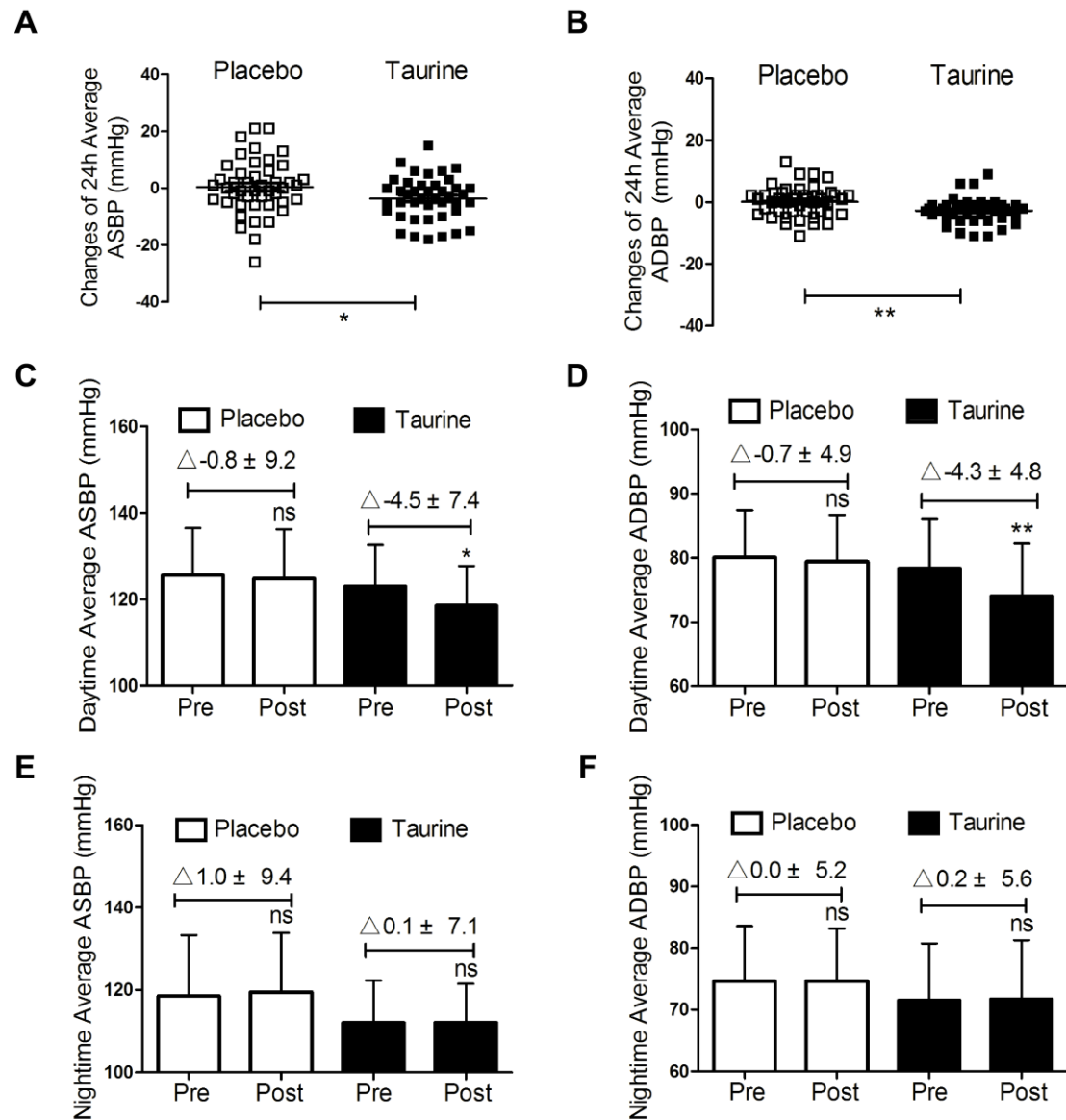


Figure S3. Effects of taurine on 24-h ambulatory BP. **A and B**, Changes in the 24 h ambulatory SBP and DBP after treatment with placebo or taurine for 12 weeks. * $P < 0.05$, ** $P < 0.01$. **C to F**, The daytime (7:00 to 23:00) and nighttime (23:00 to 7:00) average ambulatory BPs before (Pre) and after (Post) treatment with placebo or taurine for 12 weeks. The data are presented as the mean \pm SD. * $P < 0.05$, ** $P < 0.01$. N=44 in the placebo group and n=42 in the taurine group.

Figure S4

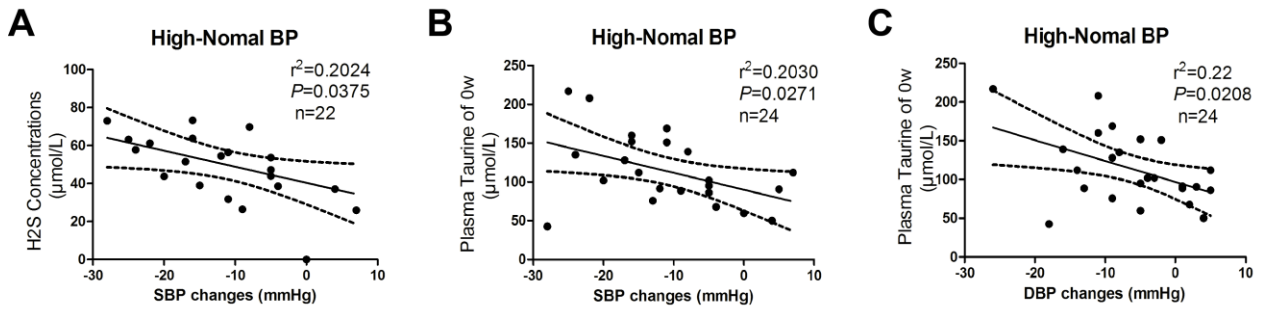


Figure S4. Correlations between the BP changes and plasma H₂S and taurine levels in the prehypertensive participants with high-normal BP after treatment with taurine for 12 weeks.

Figure S5

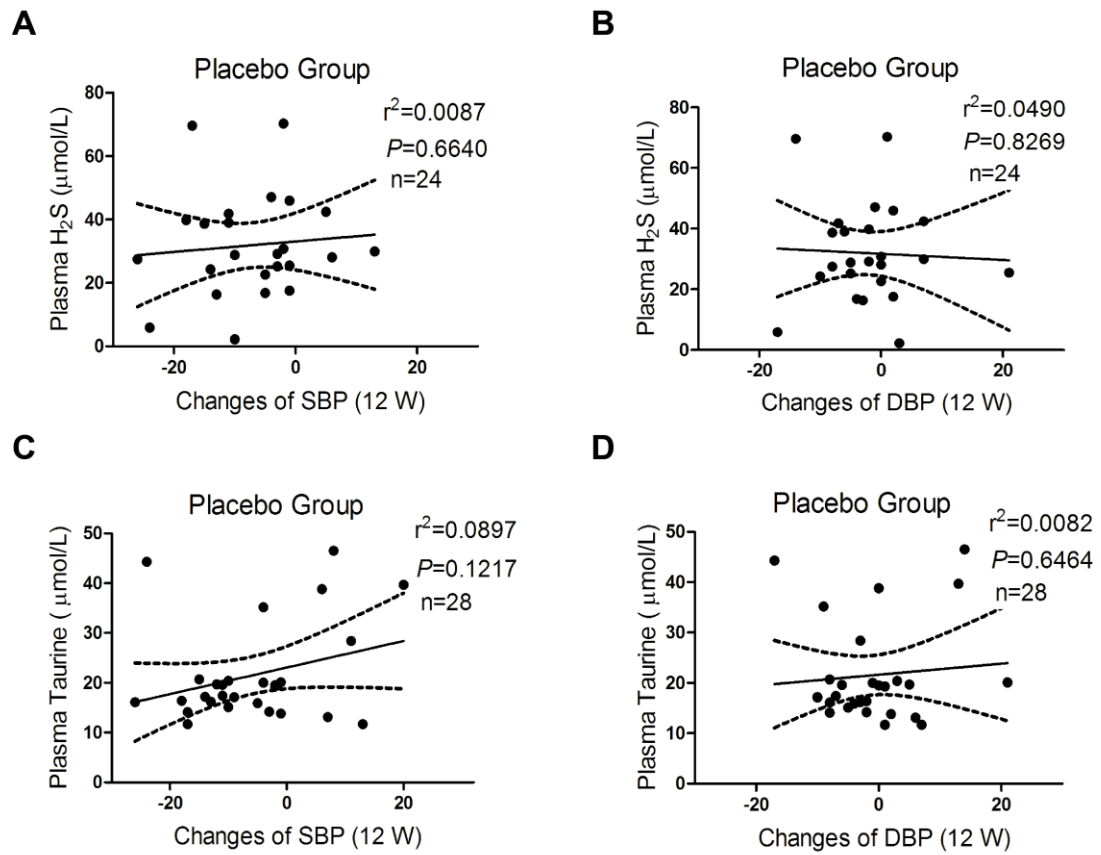


Figure S5. Correlations between the BP changes and plasma H₂S and taurine levels in the prehypertensive participants treated with placebo for 12 weeks.

Figure S6

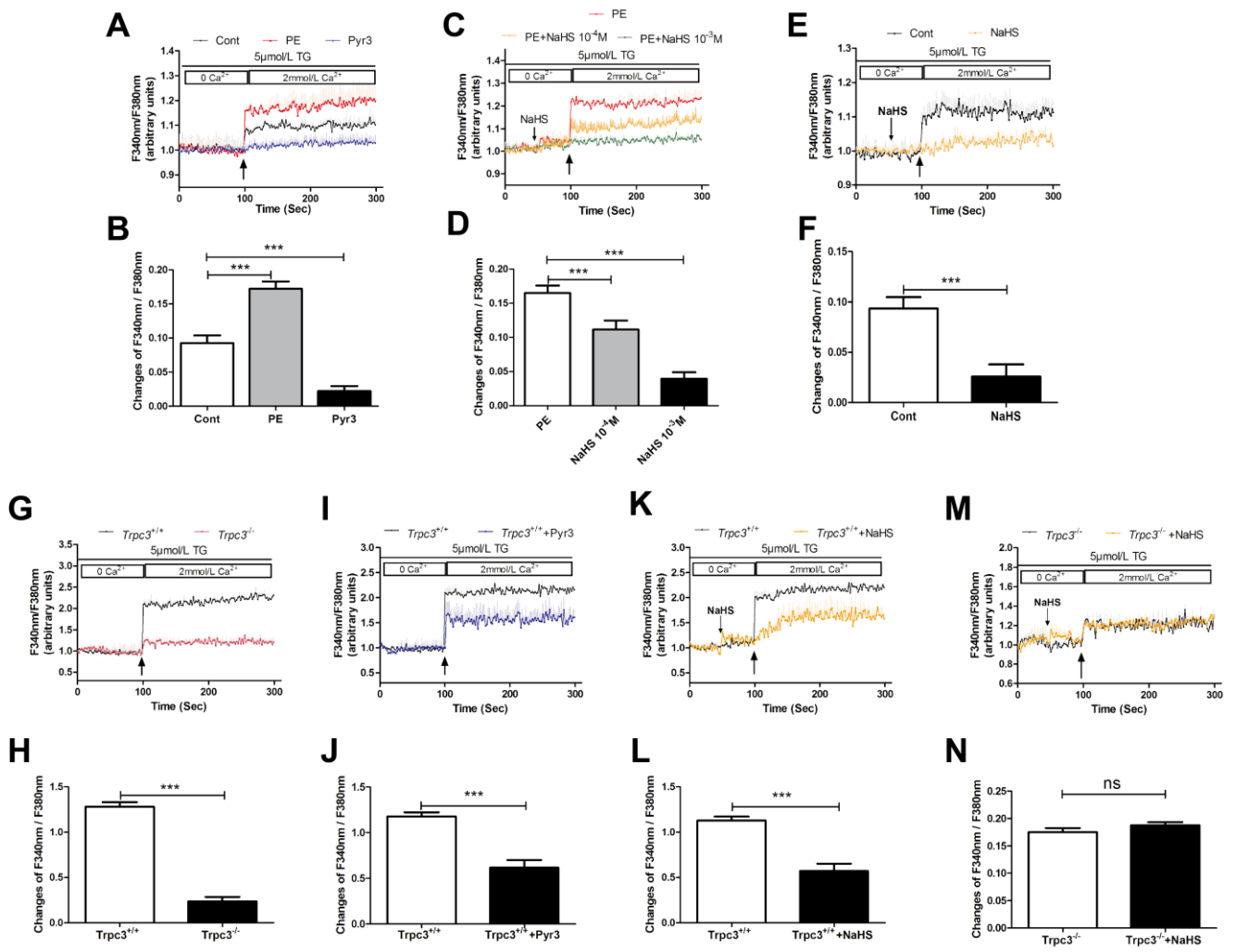


Figure S6. Effects of H₂S on vascular relaxation and its mechanisms. **A, C, E,** F340 nm/F380 nm fluorescence ratios of human mesenteric arteries in response to different treatments were plotted between 0 to 300 seconds (n=4). **B, D, F,** The summary data show the changes in the [Ca²⁺]_i from the baseline, ****P*<0.001 vs. their controls. **G, I, K, and M,** F340 nm/F380 nm ratios of the *Trpc3* mouse mesenteric arteries in response to the different treatments were plotted between 0 to 300 seconds (n=4). **H, J, L, and N,** The summary data show the changes in the [Ca²⁺]_i from the baseline. The data are presented as the mean ± SEM for four independent experiments. ****P*<0.001 vs. their controls.

Figure S7

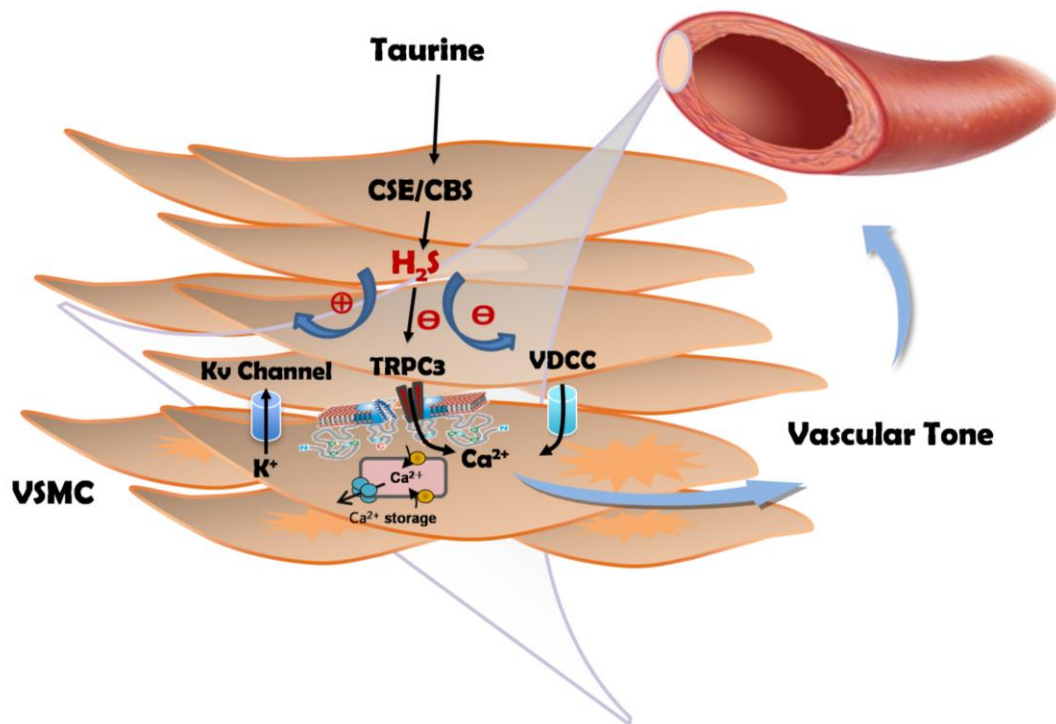


Figure S7. A schematic illustration depicts the hypotensive mechanism of taurine supplementation and H₂S regulation of vascular tone. Administration of taurine supplementation up-regulated H₂S-synthesizing enzymes CBS / CSE expressions and producing H₂S. H₂S regulates the vascular tone through the opening voltage gated potassium channel, inhibits voltage dependent calcium channel and TRPC3-mediates calcium influx.