openheart

# Boosting endogenous production of vasoprotective hydrogen sulfide via supplementation with taurine and N-acetylcysteine: a novel way to promote cardiovascular health

James J DiNicolantonio,[1] James H OKeefe,[1] Mark F McCarty[2]

**To cite:** DiNicolantonio JJ, OKeefe JH, McCarty MF. Boosting endogenous production of vasoprotective hydrogen sulfide via supplementation with taurine and N-acetylcysteine: a novel way to promote cardiovascular health. *Open Heart* 2017;**4**:e000600. doi:10.1136/openhrt-2017-000600

Accepted 25 April 2017

[1]Saint Luke's Mid America Heart Institute, Kansas City, Missouri, USA
[2]Catalytic Longevity, Encinitas, California, USA

**Correspondence to**
Dr James J DiNicolantonio; jjdinicol@gmail.com

## ENDOGENOUS HYDROGEN SULFIDE PRODUCTION CONFERS VERSATILE CARDIOVASCULAR PROTECTION

In recent years, research has established that hydrogen sulfide ($H_2S$) is generated enzymatically within the body, and functions as an important modulator of physiological function—akin in this respect to the physiological gases nitric oxide (NO) and carbon monoxide (CO). Moreover, there is now substantial evidence that physiological levels of $H_2S$ work in a wide range of complementary ways to promote and preserve cardiovascular (CV) health.[1–3] Studies in rodents and in cell cultures—employing molecules which give rise to $H_2S$ in vivo, drugs which inhibit or boost the activity of the enzymes which generate it, and transgenic rodents in which these enzymes are knocked out or upregulated—have established that physiological concentrations of $H_2S$ can oppose atherogenesis, ameliorate systemic and pulmonary hypertension, as well as protect the heart subjected to pressure overload, endoplasmic reticulum (ER) stress or adrenergic overstimulation.[1 2 4–8] With respect to atherogenesis, $H_2S$ has been found to decrease endothelial inflammation, suppress monocyte adhesion, amplify endothelium-dependent vasodilation, decrease the formation and inflammatory activity of foam cells, inhibit smooth muscle migration, oppose intimal hyperplasia, inhibit vascular calcification and oppose thrombogenesis.[1 9–21] Although $H_2S$ does not modulate plasma lipoprotein levels, it has been shown to protect low-density lipoprotein (LDL) from oxidation mediated by the myeloperoxidase product hypochlorous acid.[22] Hypochlorous acid-mediated oxidation of LDL seems likely to play a role in the pathogenesis of atherosclerosis; curiously, alpha-tocopherol, which notoriously failed to confer CV protection in multicentre trials, fails to prevent this oxidation.[23–25]

With respect to regulation of blood pressure (BP), $H_2S$ acts directly as a vasodilator of smooth muscle, via activation of hyperpolarising potassium channels, and also promotes the vasodilatory activity of NO.[26 27] In hearts challenged by pressure overload or adrenergic overstimulation, $H_2S$ opposes cardiomyocyte hypertrophy and cardiac fibrosis, aids angiogenesis, and prevents heart failure.[2 28–33] $H_2S$ also limits the cardiac tissue damage induced by coronary ischaemia reperfusion, and reduces incidence of ischaemic arrhythmias.[34–37]

A bewildering variety of molecular targets have been suggested as mediators of these benefits; it remains to be seen which of these are direct targets that are of physiological importance. $H_2S$ can modify a number of proteins on specific cysteine groups through S-sulfhydration, and this is thought to be the chief basis of its modulatory impact.[38 39] Direct targets reported to date include ATP-sensitive, intermediate conductance, and small conductance potassium channels—the activation of which by $H_2S$ induces membrane hyperpolarisation and smooth muscle relaxation; TRPV1 channels in endothelial cells—leading to endothelial hyperpolarisation and calcium influx; phosphodiesterase-5 (inhibition); Keap1 (leading to induction of phase 2 enzymes); the transcription factor Sp1 (the stabilisation of which modulates expression of many proteins); and endothelial nitric oxide synthase (eNOS)—boosting its activity.[40–46] Under various circumstances,

$H_2S$ has been found to promote antioxidant expression via activation of nrf2, quell oxidative stress, activate haem oxygenase, boost expression of vasoprotective miRNAs, stimulate production of mediators of angiogenesis, activate or suppress ion channels, inhibit nuclear factor-kappaB-mediated inflammation, and suppress or promote apoptosis.[2 26 29 44 47–50] Like NO and CO, $H_2S$ tends to be toxic in relatively high concentrations, but protective in modest physiological concentrations. $H_2S$ is rapidly oxidised, and, again like NO, its chief physiological effects are expected to be exerted within the microenvironment in which it is produced.

Many of $H_2S$'s protective effects may be at least partially attributable to its ability to support effective NO function.[27] $H_2S$ has been shown to promote activating phosphorylations of eNOS.[39 42] It can also directly boost eNOS activity through S-sulfhydration, and by promoting endothelial influx of calcium via activation of TRPV1 channels.[41 46] However, as a countervailing effect, $H_2S$ can inhibit endothelial eNOS activation by certain agonists owing to its ability to suppress inositol-1,4,5-triphosphate-mediated release of calcium from intracellular stores.[51 52] The same mechanism opposes vasoconstriction of smooth muscle and platelet aggregation.[52] Although, unlike NO and CO, $H_2S$ cannot directly activate soluble guanylate cyclase, it functions to reverse an inhibitory oxidation of this enzyme that occurs in oxidatively stressed cells and that renders this enzyme non-responsive to NO and CO.[53] $H_2S$ can also boost cyclic guanosine monophosphate (cGMP) by inhibiting phosphodiesterase 5.[42] Hence, while the impact of $H_2S$ on eNOS activity can vary depending on the circumstances, $H_2S$ tends to amplify the bioactivity of NO. Conversely, suppression of eNOS activity has been found to decrease expression of cystathionine γ-lyase (CSE) and synthesis of $H_2S$ in the rat vaculature.[54–56] Perhaps it is appropriate to view NO and $H_2S$ as teammates that work together in complementary ways to promote CV health.

Case–control studies have found that plasma $H_2S$ levels are lower in patients with coronary disease than with angiographically clean arteries, lower in in those with unstable angina or myocardial infarction than in those with stable angina, and lower in smokers, diabetics and hypertensives.[57 58] While low $H_2S$ production may contribute to progression of these syndromes (aside from smoking), it may also be a marker for loss of NO bioactivity or other metabolic dysfunctions associated with vascular disease. Epidemiologists should now be encouraged to measure plasma $H_2S$ levels in prospective studies focusing on vascular health; such studies might well establish low plasma $H_2S$ as a potent CV risk factor.

Even though we are very far from having a full understanding of how $H_2S$ works at the molecular level to guard the vascular system, it seems highly likely that practical strategies which either boost endogenous enzymatic synthesis of $H_2S$, or that provide an exogenous source of this mediator (eg, drugs that gradually degrade to release $H_2S$), will have a bright future in CV medicine.[59] In regard to the former possibility, a simple nutraceutical protocol can be proposed.

## ENZYMATIC SYNTHESIS OF $H_2S$

At least three enzymes generate $H_2S$ in the human body.[2 60] CSE, better known for its ability to cleave cystathionine to generate cysteine, α-ketobutyrate and ammonia (an essential step in methionine catabolism), can also act on cysteine to yield pyruvate, ammonia and $H_2S$.[61] CSE is the primary source of $H_2S$ in the vasculature; it is also expressed in the liver, kidney, ileum, uterus and placenta.[2] The chief source of $H_2S$ in the central nervous system is the enzyme cystathionine-β-synthase (CBS). Although this is best known for generating cystathionine from homocysteine and serine (likewise participating in methionine catabolism), it can also synthesise cystathionine from homocysteine and cysteine, producing $H_2S$ in the process.[62] A third route to $H_2S$ production involves deamination of cysteine by cysteine aminotransferase; the product 3-mercaptopyruvate can then be acted on by 3-mercaptopyruvate sulfurtransferase (3-MST), an enzyme found in neurons, the retina and vascular endothelium, to yield pyruvate and $H_2S$.[63 64] A recent study indicates that 3-MST may be the chief source of $H_2S$ in human coronary arteries.[65]

## CYSTEINE AVAILABILITY IS RATE LIMITING FOR $H_2S$ SYNTHESIS

From the standpoint of vascular health, CSE appears to be of primary importance. CSE-knockout mice are prone to hypertension, atherogenesis and heart failure.[66–69] It is notable that CSE's $K_m$ for cysteine has been found to be around 3.5 mM—a concentration far higher than ambient levels of free cysteine in cells.[70] It should follow that supplementation with nutraceuticals that can boost cellular levels of cysteine will boost CSE-mediated $H_2S$ production to a commensurate degree. CBS's $K_m$ for cysteine is even higher—around 6 mM.[71] With respect to 3-MST, its $K_m$ for 3-mercaptopyruvate has been measured at over 7 mM, and the $K_m$ of cysteine aminotransferase for cysteine is 22 mM.[63 72] Hence, there is reason to suspect that increasing cellular cysteine levels should proportionately increase $H_2S$ generation by all three enzymatic sources of this gas.

N-acetylcysteine (NAC), a well-tolerated and well-absorbed nutraceutical that is rapidly cleaved in vivo to yield cysteine, has long been employed clinically to enhance cellular levels of glutathione.[73 74] (L-cysteine per se, when administered as a pure chemical, is more reactive, tending to oxidise spontaneously to cysteine; it is less bioavailable and more prone to evoke side effects than NAC.) The rate-limiting enzyme for glutathione synthesis, γ-glutamylcysteine synthetase, also has a rather high $K_m$ for cysteine, which is why NAC supplementation is effective for boosting glutathione levels.[75] The clinical efficacy of NAC in this regard demonstrates that feasible NAC intakes do indeed meaningfully enhance the

cysteine content of cells. There is no evident reason why supplementary NAC should not in a comparable manner stimulate $H_2S$ production by CSE.

## SUPPLEMENTAL TAURINE INCREASES VASCULAR CSE EXPRESSION

An exciting recent research discovery may provide an additional complementary strategy for boosting CSE-mediated $H_2S$ production. In relatively high dietary doses, the physiologically essential amino acid osmolyte taurine has long been known to exert important protective effects in rodent models of atherogenesis, hypertension and heart failure.[76–78] However, with the exception of several early promising clinical studies showing that supplemental taurine can improve cardiac function in heart failure, little effort to date has been made to explore taurine's clinical utility for CV protection.[79 80] This likely reflects the fact that, aside from a few pilot scale clinical studies suggesting a modest favourable impact on elevated BP, supplemental taurine does not seem to influence documented CV risk factors.[81 82] If, for example, taurine notably reduced LDL cholesterol, C-reactive protein or homocysteine, it likely would have received respectful attention from clinical researchers. But to date it has remained a research curiosity that for inexplicable reasons exerts interesting effects on rodents. This is all the more distressing in light of the fact that taurine is essentially free of toxicity (except in severe kidney failure), well absorbed, quite inexpensive in multigram doses, highly soluble and so devoid of flavour that it can be added in high amounts to any food or beverage.[83] Indeed, taurine is currently a standard constituent of so-called 'energy drinks.' (Unjustly, the dangerous side effects of the hypercaffeination which overconsumption of these drinks can induce have led some to question taurine's safety; ironically, the taurine may make these drinks safer.)[84 85]

Recently, clinical researchers elected to conduct an adequately powered assessment of taurine's ability to lower modestly elevated BP.[86] They enrolled 120 prehypertensive subjects, who were randomised to receive 1.6 g taurine daily, or matching placebo, for 12 weeks. BP was assessed both at clinic visits and by 24 hours of ambulatory monitoring. In the taurine group, average BP reductions were significant relative to both placebo and baseline, for both systolic and diastolic pressure (for the clinic, a mean reduction of 7.2/4.7 mm Hg; for ambulatory readings, 3.8/3.5 mm Hg). Both endothelium-dependent and endothelium-independent vasodilation was amplified in the taurine group. But the truly intriguing finding was this: plasma $H_2S$ levels in the taurine group rose from 43.8 µmol/L at baseline to 87.0 µmol/L after 12 weeks ($p<0.001$)—a virtual doubling of plasma $H_2S$.

In an effort to determine why $H_2S$ rose in the taurine-supplemented group, the researchers fed spontaneously hypertensive rats a diet enriched with 2% taurine for 12 weeks, and then measured the protein expression levels of CSE and CBS in the aortas of these rats—each of these levels had risen by about 50%. They also exposed human mesenteric arteries ex vivo to either 20 mM or 40 mM taurine for 24 hours, and found that expressions of both CSE and CBS rose markedly and dose dependently; the increase in CSE expression was over fivefold at 40 mM taurine.

Unfortunately, these researchers did not determine whether taurine supplementation boosts the expression of 3-MST or of cysteine aminotransferase in the vasculature. This could have implications for endothelial function and atherogenesis in human coronary arteries.[65] In this regard, it is interesting to note that some of the first clinical studies evaluating high-dose taurine supplementation found that it conferred symptomatic benefit in angina.[87 88] These Italian studies were open label, and unfortunately were not followed up with a published controlled trial to validate their findings. Nonetheless, if these observations were accurate, they might be rationalised by a taurine-mediated upregulation of 3-MST activity, and a consequent amplification of NO bioactivity via $H_2S$.

The ability of taurine to enhance the expression of CSE is not unprecedented. The drug S-propargyl-cysteine likewise has shown this effect.[89 90] But this drug is not available for clinical use—whereas taurine is a widely available nutraceutical. In light of the increase in CV risk that accompanies menopause, it is intriguing to note that oestrogen administration boosts expression of CSE in the vasculature of ovariectomised mice, an effect dependent on the oestrogen receptor alpha.[91 92] Whereas oestrogen protects ovariectomised mice from diet-induced atherogenesis, it fails to do so in ovariectomised mice in which the CSE gene has been knocked out.[92]

In regard to the multiple protective effects of taurine supplementation documented in rodents—neuroprotective as well as vasoprotective—it will be of interest to determine which of these are mediated by $H_2S$. This can be done by noting whether drug-mediated inhibition of $H_2S$ synthesis, or use of transgenic mice deficient in $H_2S$ synthesis, eliminates the protective effects of taurine administration. The positive inotropic effect of taurine in heart failure might not be attributable to $H_2S$, as the latter is not known to have such an effect.[79 93–96]

## A NUTRACEUTICAL REGIMEN FOR BOOSTING $H_2S$ SYNTHESIS

Assuming that the recent research linking taurine with $H_2S$ can be replicated (one must bear in mind that, to date, only one clinical study has reported the impact of supplemental taurine on plasma $H_2S$ levels), it is logical to propose that a supplementation regimen featuring clinically meaningful doses of both NAC and taurine should boost endogenous production of CSE, and thereby promote vascular health in a number of complementary ways. Clinical studies evaluating the impact of various dose regimens of taurine and NAC on plasma $H_2S$ levels

appear warranted. The dose range in which NAC has shown clinical benefits—and hence presumably achieves a meaningful increase in tissue cysteine levels—is 1200–1800 mg daily, in divided doses.[74] Taurine has been used in daily doses as high as 6 g without any evident adverse effects; 1.6 g daily was sufficient to elevate $H_2S$ in the trial in patients with prehypertension.[79 86]

In regard to NAC, it has been suggested that the elderly have an increased requirement for cysteine owing to the fact that the efficiency of glutathione synthesis and glutathione tissue levels decline with age.[97] This age-related deficit in glutathione can be corrected with supplemental NAC.[98] This observation may help rationalise epidemiology which concludes that, whereas relatively low dietary protein intakes are associated with lower mortality risk in people under 65 (possibly by downregulating growth factor activities which drive the ageing process), low protein intakes (as a fraction of total calories) predict higher mortality in those over 65.[99 100] Supplementation with NAC in the elderly may provide health protection by boosting the production of both glutathione and $H_2S$, each of which is crucial for optimal physiological function and health promotion. NAC may be of particular merit for 'rejuvenating' immune function in the elderly, and alleviating the symptoms of influenza.[101–103]

In light of the mutually complementary interactions of NO and $H_2S$ in promotion of vascular health, supplementation with taurine and NAC might reasonably be used in conjunction with nutraceutical measures known to support coupled eNOS activity—such as citrulline, high-dose folate and spirulina[104–111] —to achieve an ample measure of CV protection.

**Contributors** All authors contributed to the final manuscript.

**Competing interests** MFM and JHO are owners of nutraceutical companies.

**Provenance and peer review** Not commissioned; externally peer reviewed.



## REFERENCES

1. Xu S, Liu Z, Liu P. Targeting hydrogen sulfide as a promising therapeutic strategy for atherosclerosis. *Int J Cardiol* 2014;172:313–7.
2. Shen Y, Shen Z, Luo S, *et al*. The Cardioprotective Effects of Hydrogen Sulfide in Heart Diseases: From Molecular Mechanisms to Therapeutic Potential. *Oxid Med Cell Longev* 2015;2015:1–13.
3. Polhemus DJ, Lefer DJ. Emergence of hydrogen sulfide as an endogenous gaseous signaling molecule in cardiovascular disease. *Circ Res* 2014;114:730–7.
4. Meng G, Ma Y, Xie L, *et al*. Emerging role of hydrogen sulfide in hypertension and related cardiovascular diseases. *Br J Pharmacol* 2015;172:5501–11.
5. Brampton J, Aaronson PI. Role of Hydrogen Sulfide in Systemic and Pulmonary Hypertension: Cellular Mechanisms and Therapeutic Implications. *Cardiovasc Hematol Agents Med Chem* 2016;14:4–22.
6. Barr LA, Shimizu Y, Lambert JP, *et al*. Hydrogen sulfide attenuates high fat diet-induced cardiac dysfunction via the suppression of endoplasmic reticulum stress. *Nitric Oxide* 2015;46:145–56.
7. Li C, Hu M, Wang Y, *et al*. Hydrogen sulfide preconditioning protects against myocardial ischemia/reperfusion injury in rats through inhibition of endo/sarcoplasmic reticulum stress. *Int J Clin Exp Pathol* 2015;8:7740–51.
8. Li F, Luo J, Wu Z, *et al*. Hydrogen sulfide exhibits cardioprotective effects by decreasing endoplasmic reticulum stress in a diabetic cardiomyopathy rat model. *Mol Med Rep* 2016.
9. Pan LL, Liu XH, Gong QH, *et al*. Hydrogen sulfide attenuated tumor necrosis factor-α-induced inflammatory signaling and dysfunction in vascular endothelial cells. *PLoS One* 2011;6:e19766.
10. Go YM, Lee HR, Park H. H(2)S inhibits oscillatory shear stress-induced monocyte binding to endothelial cells via nitric oxide production. *Mol Cells* 2012;34:56813.
11. Bełtowski J, Jamroz-Wiśniewska A. Hydrogen sulfide and endothelium-dependent vasorelaxation. *Molecules* 2014;19:21183–99.
12. Coletta C, Papapetropoulos A, Erdelyi K, *et al*. Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. *Proc Natl Acad Sci U S A* 2012;109:9161–6.
13. Zhao ZZ, Wang Z, Li GH, *et al*. Hydrogen sulfide inhibits macrophage-derived foam cell formation. *Exp Biol Med* 2011;236:169–76.
14. Yang G, Li H, Tang G, *et al*. Increased neointimal formation in cystathionine gamma-lyase deficient mice: role of hydrogen sulfide in α5β1-integrin and matrix metalloproteinase-2 expression in smooth muscle cells. *J Mol Cell Cardiol* 2012;52:677–88.
15. Ma B, Liang G, Zhang F, *et al*. Effect of hydrogen sulfide on restenosis of peripheral arteries after angioplasty. *Mol Med Rep* 2012;5:1497–502.
16. Yang R, Teng X, Li H, *et al*. Hydrogen Sulfide Improves Vascular Calcification in Rats by Inhibiting Endoplasmic Reticulum Stress. *Oxid Med Cell Longev* 2016;2016:1–9.
17. Zavaczki E, Jeney V, Agarwal A, *et al*. Hydrogen sulfide inhibits the calcification and osteoblastic differentiation of vascular smooth muscle cells. *Kidney Int* 2011;80:731–9.
18. Shi HQ, Zhang Y, Cheng MH, *et al*. Sodium Sulfide, a Hydrogen Sulfide-Releasing Molecule, Attenuates Acute Cerebral Ischemia in Rats. *CNS Neurosci Ther* 2016;22:625–32.
19. Grambow E, Mueller-Graf F, Delyagina E, *et al*. Effect of the hydrogen sulfide donor GYY4137 on platelet activation and microvascular thrombus formation in mice. *Platelets* 2014;25(3):166–74.
20. Grambow E, Leppin C, Leppin K, *et al*. The effects of hydrogen sulfide on platelet-leukocyte aggregation and microvascular thrombolysis. *Platelets* 2016:1–9.
21. Zhong L, Lv L, Yang J, *et al*. Inhibitory effect of hydrogen sulfide on platelet aggregation and the underlying mechanisms. *J Cardiovasc Pharmacol* 2014;64:481–7.
22. Laggner H, Muellner MK, Schreier S, *et al*. Hydrogen sulphide: a novel physiological inhibitor of LDL atherogenic modification by HOCl. *Free Radic Res* 2007;41:741–7.
23. Hazell LJ, Stocker R. Oxidation of low-density lipoprotein with hypochlorite causes transformation of the lipoprotein into a high-uptake form for macrophages. *Biochem J* 1993;290 (Pt 1):165–72.
24. Hazen SL, Heinecke JW. 3-Chlorotyrosine, a specific marker of myeloperoxidase-catalyzed oxidation, is markedly elevated in low density lipoprotein isolated from human atherosclerotic intima. *J Clin Invest* 1997;99:2075–81.
25. Hazell LJ, Stocker R. Alpha-tocopherol does not inhibit hypochlorite-induced oxidation of apolipoprotein B-100 of low-density lipoprotein. *FEBS Lett* 1997;414:541–4.
26. Zhao W, Zhang J, Lu Y, *et al*. The vasorelaxant effect of H(2)S as a novel endogenous gaseous K(ATP) channel opener. *Embo J* 2001;20:6008–16.
27. Szabo C. Hydrogen sulfide, an enhancer of vascular nitric oxide signalling: mechanisms and implications. *Am J Physiol Cell Physiol* 2016:56750.
28. Katsouda A, Bibli SI, Pyriochou A, *et al*. Regulation and role of endogenously produced hydrogen sulfide in angiogenesis. *Pharmacol Res* 2016;113:175–85.
29. Liu J, Hao DD, Zhang JS, *et al*. Hydrogen sulphide inhibits cardiomyocyte hypertrophy by up-regulating miR-133a. *Biochem Biophys Res Commun* 2011;413:342–7.
30. Huang J, Wang D, Zheng J, *et al*. Hydrogen sulfide attenuates cardiac hypertrophy and fibrosis induced by abdominal aortic coarctation in rats. *Mol Med Rep* 2012;5:923–8.

31. Givvimani S, Munjal C, Gargoum R, *et al*. Hydrogen sulfide mitigates transition from compensatory hypertrophy to heart failure. *J Appl Physiol* 2011;110:1093–100.
32. Polhemus DJ, Kondo K, Bhushan S, *et al*. Hydrogen sulfide attenuates cardiac dysfunction after heart failure via induction of angiogenesis. *Circ Heart Fail* 2013;6:1077–86.
33. Lu F, Xing J, Zhang X, *et al*. Exogenous hydrogen sulfide prevents cardiomyocyte apoptosis from cardiac hypertrophy induced by isoproterenol. *Mol Cell Biochem* 2013;381:41–50.
34. Johansen D, Ytrehus K, Baxter GF. Exogenous hydrogen sulfide (H2S) protects against regional myocardial ischemia-reperfusion injury--Evidence for a role of K ATP channels. *Basic Res Cardiol* 2006;101:53–60.
35. Sivarajah A, McDonald MC, Thiemermann C. The production of hydrogen sulfide limits myocardial ischemia and reperfusion injury and contributes to the cardioprotective effects of preconditioning with endotoxin, but not ischemia in the rat. *Shock* 2006;26:154–61.
36. Sun YG, Wang XY, Chen X, *et al*. Hydrogen sulfide improves cardiomyocytes electrical remodeling post ischemia/reperfusion injury in rats. *Int J Clin Exp Pathol* 2015;8:474–81.
37. Zhang Z, Huang H, Liu P, *et al*. Hydrogen sulfide contributes to cardioprotection during ischemia-reperfusion injury by opening K ATP channels. *Can J Physiol Pharmacol* 2007;85:1248–53.
38. Gadalla MM, Snyder SH. Hydrogen sulfide as a gasotransmitter. *J Neurochem* 2010;113:14–26.
39. Altaany Z, Yang G, Wang R. Crosstalk between hydrogen sulfide and nitric oxide in endothelial cells. *J Cell Mol Med* 2013;17:879–88.
40. Mustafa AK, Sikka G, Gazi SK, *et al*. Hydrogen sulfide as endothelium-derived hyperpolarizing factor sulfhydrates potassium channels. *Circ Res* 2011;109:1259–68.
41. Naik JS, Osmond JM, Walker BR, *et al*. Hydrogen sulfide-induced vasodilation mediated by endothelial TRPV4 channels. *Am J Physiol Heart Circ Physiol* 2016;311:H1437–H1444.
42. Coletta C, Papapetropoulos A, Erdelyi K, *et al*. Hydrogen sulfide and nitric oxide are mutually dependent in the regulation of angiogenesis and endothelium-dependent vasorelaxation. *Proc Natl Acad Sci U S A* 2012;109:9161–6.
43. Yang G, Zhao K, Ju Y, *et al*. Hydrogen sulfide protects against cellular senescence via S-sulfhydration of Keap1 and activation of Nrf2. *Antioxid Redox Signal* 2013;18:1906–19.
44. Xie L, Gu Y, Wen M, *et al*. Hydrogen Sulfide Induces Keap1 S-sulfhydration and Suppresses Diabetes-Accelerated Atherosclerosis via Nrf2 Activation. *Diabetes* 2016;65:3171–84.
45. Meng G, Xiao Y, Ma Y, *et al*. Hydrogen Sulfide Regulates Krüppel-Like Factor 5 Transcription Activity via Specificity Protein 1 S-Sulfhydration at Cys664 to Prevent Myocardial Hypertrophy. *J Am Heart Assoc* 2016;5:e004160.
46. Altaany Z, Ju Y, Yang G, *et al*. The coordination of S-sulfhydration, S-nitrosylation, and phosphorylation of endothelial nitric oxide synthase by hydrogen sulfide. *Sci Signal* 2014;7:ra87.
47. Toldo S, Das A, Mezzaroma E, *et al*. Induction of microRNA-21 with exogenous hydrogen sulfide attenuates myocardial ischemic and inflammatory injury in mice. *Circ Cardiovasc Genet* 2014;7:311–20.
48. Gao C, Dq X, Gao CJ, *et al*. An exogenous hydrogen sulphide donor, NaHS, inhibits the nuclear factor kappaB inhibitor kinase/nuclear factor kappab inhibitor/nuclear factor-kappaB signaling pathway and exerts cardioprotective effects in a rat hemorrhagic shock model. *Biol Pharm Bull* 2012:6245–54.
49. Wang JF, Li Y, Song JN, *et al*. Role of hydrogen sulfide in secondary neuronal injury. *Neurochem Int* 2014;64–37–47.
50. Yang G, Wu L, Wang R. Pro-apoptotic effect of endogenous H2S on human aorta smooth muscle cells. *Faseb J* 2006;20:553–5.
51. Kloesch B, Steiner G, Mayer B, *et al*. Hydrogen sulfide inhibits endothelial nitric oxide formation and receptor ligand-mediated Ca(2+) release in endothelial and smooth muscle cells. *Pharmacol Rep* 2016;68:37–43.
52. Castro-Piedras I, Perez-Zoghbi JF. Hydrogen sulphide inhibits Ca2+ release through InsP3 receptors and relaxes airway smooth muscle. *J Physiol* 2013;591:5999–6015.
53. Zhou Z, Martin E, Sharina I, *et al*. Regulation of soluble guanylyl cyclase redox state by hydrogen sulfide. *Pharmacol Res* 2016;111–556–62.
54. Zhong G, Chen F, Cheng Y, *et al*. The role of hydrogen sulfide generation in the pathogenesis of hypertension in rats induced by inhibition of nitric oxide synthase. *J Hypertens* 2003;21–1879–85.
55. Zhao W, Ndisang JF, Wang R. Modulation of endogenous production of H2S in rat tissues. *Can J Physiol Pharmacol* 2003;81–848–53.
56. Łowicka E, Bełtowski J. Hydrogen sulfide (H2S) - the third gas of interest for pharmacologists. *Pharmacol Rep* 2007;59:4-24.
57. Jiang HL, Hc W, Zl L, *et al*. Changes of the new gaseous transmitter H2S in patients with coronary heart disease]. *Di Yi Jun Yi Da Xue Xue Bao* 2005.
58. Jain SK, Bull R, Rains JL, *et al*. Low levels of hydrogen sulfide in the blood of diabetes patients and streptozotocin-treated rats causes vascular inflammation? *Antioxid Redox Signal* 2010;12–1333–7.
59. Zheng Y, Ji X, Ji K, *et al*. Hydrogen sulfide prodrugs-a review. *Acta Pharm Sin B* 2015;5–367–77.
60. Pan LL, Liu XH, Gong QH, *et al*. Role of cystathionine γ-lyase/hydrogen sulfide pathway in cardiovascular disease: a novel therapeutic strategy? *Antioxid Redox Signal* 2012;17:106–18.
61. Yang G, Cao K, Wu L, *et al*. Cystathionine gamma-lyase overexpression inhibits cell proliferation via a H2S-dependent modulation of ERK1/2 phosphorylation and p21Cip/WAK-1. *J Biol Chem* 2004.
62. Chen X, Jhee KH, Kruger WD. Production of the neuromodulator H2S by cystathionine beta-synthase via the condensation of cysteine and homocysteine. *J Biol Chem* 2004;279:52082–6.
63. Shibuya N, Mikami Y, Kimura Y, *et al*. Vascular endothelium expresses 3-mercaptopyruvate sulfurtransferase and produces hydrogen sulfide. *J Biochem* 2009;146–623–6.
64. Shibuya N, Tanaka M, Yoshida M, *et al*. 3-Mercaptopyruvate sulfurtransferase produces hydrogen sulfide and bound sulfane sulfur in the brain. *Antioxid Redox Signal* 2009;11:703–14.
65. Kuo MM, Kim DH, Jandu S, *et al*. MPST but not CSE is the primary regulator of hydrogen sulfide production and function in the coronary artery. *Am J Physiol Heart Circ Physiol* 2016;310:H71–H79.
66. Yang G, Wu L, Jiang B, *et al*. H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. *Science* 2008;322:587–90.
67. Mani S, Li H, Untereiner A, *et al*. Decreased endogenous production of hydrogen sulfide accelerates atherosclerosis. *Circulation* 2013;127:2523–34.
68. Kondo K, Bhushan S, King AL, *et al*. H□S protects against pressure overload-induced heart failure via upregulation of endothelial nitric oxide synthase. *Circulation* 2013;127:1116–27.
69. Yang G, Li H, Tang G, *et al*. Increased neointimal formation in cystathionine gamma-lyase deficient mice: role of hydrogen sulfide in α5β1-integrin and matrix metalloproteinase-2 expression in smooth muscle cells. *J Mol Cell Cardiol* 2012;52–677–88.
70. Zhu W, Lin A, Banerjee R. Kinetic properties of polymorphic variants and pathogenic mutants in human cystathionine gamma-lyase. *Biochemistry* 2008;47:6226–32.
71. Chen X, Jhee KH, Kruger WD. Production of the neuromodulator H2S by cystathionine beta-synthase via the condensation of cysteine and homocysteine. *J Biol Chem* 2004;279:52082–6.
72. Akagi R. Purification and characterization of cysteine aminotransferase from rat liver cytosol. *Acta Med Okayama* 1982;36:187-97.
73. Atkuri KR, Mantovani JJ, Herzenberg LA, *et al*. N-Acetylcysteine--a safe antidote for cysteine/glutathione deficiency. *Curr Opin Pharmacol* 2007;7–355–9.
74. Dodd S, Dean O, Copolov DL, *et al*. N-acetylcysteine for antioxidant therapy: pharmacology and clinical utility. *Expert Opin Biol Ther* 2008;8:1955–62.
75. Richman PG, Meister A. Regulation of gamma-glutamyl-cysteine synthetase by nonallosteric feedback inhibition by glutathione. *J Biol Chem* 1975;250:1422-6.
76. Yamori Y, Taguchi T, Hamada A, *et al*. Taurine in health and diseases: consistent evidence from experimental and epidemiological studies. *J Biomed Sci* 2010;17 Suppl 1:S6.
77. Abebe W, Mozaffari MS. Role of taurine in the vasculature: an overview of experimental and human studies. *Am J Cardiovasc Dis* 2011;1.
78. Murakami S. Taurine and atherosclerosis. *Amino Acids* 2014;46:73–80.
79. Azuma J, Sawamura A, Awata N, *et al*. Therapeutic effect of taurine in congestive heart failure: a double-blind crossover trial. *Clin Cardiol* 1985;8–276–82.
80. Azuma J. Long-term effect of taurine in congestive heart failure: preliminary report. Heart Failure Research with Taurine Group. *Adv Exp Med Biol* 1994;359:425-33.
81. Fujita T, Ando K, Noda H, *et al*. Effects of increased adrenomedullary activity and taurine in young patients with borderline hypertension. *Circulation* 1987;75:525–32.
82. Militante JD, Lombardini JB. Treatment of hypertension with oral taurine: experimental and clinical studies. *Amino Acids* 2002;23:381–93.
83. Shao A, Hathcock JN. Risk assessment for the amino acids taurine, L-glutamine and L-arginine. *Regul Toxicol Pharmacol* 2008;50–376–99.

84. Wolk BJ, Ganetsky M, Babu KM. Toxicity of energy drinks. *Curr Opin Pediatr* 2012;24:243–51.
85. Schaffer SW, Shimada K, Jong CJ, *et al*. Effect of taurine and potential interactions with caffeine on cardiovascular function. *Amino Acids* 2014;46:1147–57.
86. Sun Q, Wang B, Li Y, *et al*. Taurine Supplementation Lowers Blood Pressure and Improves Vascular Function in Prehypertension: Randomized, Double-Blind, Placebo-Controlled Study. *Hypertension* 2016;67.
87. Sicuteri F, Franchi G, Fanciullacci M, *et al*. [Anti-anginous effect of a non-coronaro-dilator sulfurated amino acid]. *Clin Ter* 1969;49:205-19.
88. McCarty MF. The reported clinical utility of taurine in ischemic disorders may reflect a down-regulation of neutrophil activation and adhesion. *Med Hypotheses* 1999;53:290–9.
89. Wang Q, Liu HR, Mu Q, *et al*. S-propargyl-cysteine protects both adult rat hearts and neonatal cardiomyocytes from ischemia/hypoxia injury: the contribution of the hydrogen sulfide-mediated pathway. *J Cardiovasc Pharmacol* 2009;54–139–46.
90. Ma K, Liu Y, Zhu Q, *et al*. H2S donor, S-propargyl-cysteine, increases CSE in SGC-7901 and cancer-induced mice: evidence for a novel anti-cancer effect of endogenous H2S? *PLoS One* 2011;6:e20525.
91. Zhou K, Gao Q, Zheng S, *et al*. 17β-estradiol induces vasorelaxation by stimulating endothelial hydrogen sulfide release. *Mol Hum Reprod* 2013;19:169–76.
92. Li H, Mani S, Wu L, *et al*. The interaction of estrogen and CSE/H2S pathway in the development of atherosclerosis. *Am J Physiol Heart Circ Physiol* 2017;312:H406–H414.
93. Iwata HI, Fujimoto S. Potentiation by taurine of the inotropic effect of ouabain and the content of intracellular Ca++ and taurine in the heart. *Experientia* 1976;32:1559–61.
94. Azuma J, Takihara K, Awata N, *et al*. Beneficial effect of taurine on congestive heart failure induced by chronic aortic regurgitation in rabbits. *Res Commun Chem Pathol Pharmacol* 1984;45:261.
95. Sitdikova GF, Khaertdinov NN, Zefirov AL. Role of calcium and potassium channels in effects of hydrogen sulfide on frog myocardial contractility. *Bull Exp Biol Med* 2011;151–163–6.
96. Porokhya MV, Abramochkin DV, Abramov AA, *et al*. Inotropic effects of gaseous transmitters in isolated rat heart preparation. *Bull Exp Biol Med* 2012;153–856–8.
97. Dröge W. Oxidative stress and ageing: is ageing a cysteine deficiency syndrome? *Philos Trans R Soc Lond B Biol Sci* 2005;360–2355–72.
98. Sekhar RV, Patel SG, Guthikonda AP, *et al*. Deficient synthesis of glutathione underlies oxidative stress in aging and can be corrected by dietary cysteine and glycine supplementation. *Am J Clin Nutr* 2011;94–847–53.
99. Levine ME, Suarez JA, Brandhorst S, *et al*. Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. *Cell Metab* 2014;19:407–17.
100. McCarty MF, DiNicolantonio JJ. An increased need for dietary cysteine in support of glutathione synthesis may underlie the increased risk for mortality associated with low protein intake in the elderly. *Age* 2015;37:96.
101. Arranz L, Fernández C, Rodríguez A, *et al*. The glutathione precursor N-acetylcysteine improves immune function in postmenopausal women. *Free Radic Biol Med* 2008;45:1252–62.
102. De La Fuente M, Miquel J, Catalán MP, *et al*. The amount of thiolic antioxidant ingestion needed to improve several immune functions is higher in aged than in adult mice. *Free Radic Res* 2002;36:119–26.
103. De Flora S, Grassi C, Carati L. Attenuation of influenza-like symptomatology and improvement of cell-mediated immunity with long-term N-acetylcysteine treatment. *Eur Respir J* 1997;10:1535–41.
104. Romero MJ, Platt DH, Caldwell RB, *et al*. Therapeutic use of citrulline in cardiovascular disease. *Cardiovasc Drug Rev* 2006;24:275–90.
105. Schwedhelm E, Maas R, Freese R, *et al*. Pharmacokinetic and pharmacodynamic properties of oral L-citrulline and L-arginine: impact on nitric oxide metabolism. *Br J Clin Pharmacol* 2008;65:51–9.
106. McCarty MF. Asymmetric Dimethylarginine Is a Well Established Mediating Risk Factor for Cardiovascular Morbidity and Mortality-Should Patients with Elevated Levels Be Supplemented with Citrulline? *Healthcare* 2016;4:40.
107. Siu KL, Miao XN, Cai H. Recoupling of eNOS with folic acid prevents abdominal aortic aneurysm formation in angiotensin II-infused apolipoprotein E null mice. *PLoS One* 2014;9:e88899.
108. Chalupsky K, Kračun D, Kanchev I, *et al*. Folic Acid Promotes Recycling of Tetrahydrobiopterin and Protects Against Hypoxia-Induced Pulmonary Hypertension by Recoupling Endothelial Nitric Oxide Synthase. *Antioxid Redox Signal* 2015;23:1076–91.
109. McCarty MF. Oster rediscovered--mega-dose folate for symptomatic atherosclerosis. *Med Hypotheses* 2007;69:325–32.
110. Riss J, Décordé K, Sutra T, *et al*. Phycobiliprotein C-phycocyanin from Spirulina platensis is powerfully responsible for reducing oxidative stress and NADPH oxidase expression induced by an atherogenic diet in hamsters. *J Agric Food Chem* 2007;55:7962–7.
111. McCarty MF, Barroso-Aranda J, Contreras F. Potential complementarity of high-flavanol cocoa powder and spirulina for health protection. *Med Hypotheses* 2010;74:370–3.