

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

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## ENDOGENOUS HYDROGEN SULFIDE PRODUCTION CONFERS VERSATILE CARDIOVASCULAR PROTECTION

In recent years, research has established that hydrogen sulfide (H<sub>2</sub>S) 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<sub>2</sub>S work in a wide range of complementary ways to promote and preserve cardiovascular (CV) health.<sup>1–3</sup> Studies in rodents and in cell cultures—employing molecules which give rise to H<sub>2</sub>S 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<sub>2</sub>S 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.<sup>1 2 4–8</sup> With respect to atherogenesis, H<sub>2</sub>S 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.<sup>9–21</sup> Although H<sub>2</sub>S 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.<sup>22</sup> 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.<sup>23–25</sup>

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

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<sub>2</sub>S 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.<sup>38 39</sup> Direct targets reported to date include ATP-sensitive, intermediate conductance, and small conductance potassium channels—the activation of which by H<sub>2</sub>S 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.<sup>40–46</sup> Under various circumstances,



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H<sub>2</sub>S 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.<sup>2 26 29 44 47–50</sup> Like NO and CO, H<sub>2</sub>S tends to be toxic in relatively high concentrations, but protective in modest physiological concentrations. H<sub>2</sub>S 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<sub>2</sub>S's protective effects may be at least partially attributable to its ability to support effective NO function.<sup>27</sup> H<sub>2</sub>S has been shown to promote activating phosphorylations of eNOS.<sup>39 42</sup> It can also directly boost eNOS activity through S-sulfhydration, and by promoting endothelial influx of calcium via activation of TRPV1 channels.<sup>41 46</sup> However, as a countervailing effect, H<sub>2</sub>S 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.<sup>51 52</sup> The same mechanism opposes vasoconstriction of smooth muscle and platelet aggregation.<sup>52</sup> Although, unlike NO and CO, H<sub>2</sub>S 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.<sup>53</sup> H<sub>2</sub>S can also boost cyclic guanosine monophosphate (cGMP) by inhibiting phosphodiesterase 5.<sup>42</sup> Hence, while the impact of H<sub>2</sub>S on eNOS activity can vary depending on the circumstances, H<sub>2</sub>S tends to amplify the bioactivity of NO. Conversely, suppression of eNOS activity has been found to decrease expression of cystathionine  $\gamma$ -lyase (CSE) and synthesis of H<sub>2</sub>S in the rat vasculature.<sup>54–56</sup> Perhaps it is appropriate to view NO and H<sub>2</sub>S as teammates that work together in complementary ways to promote CV health.

Case-control studies have found that plasma H<sub>2</sub>S levels are lower in patients with coronary disease than with angiographically clean arteries, lower in those with unstable angina or myocardial infarction than in those with stable angina, and lower in smokers, diabetics and hypertensives.<sup>57 58</sup> While low H<sub>2</sub>S 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<sub>2</sub>S levels in prospective studies focusing on vascular health; such studies might well establish low plasma H<sub>2</sub>S as a potent CV risk factor.

Even though we are very far from having a full understanding of how H<sub>2</sub>S 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<sub>2</sub>S, or that provide an exogenous source of this mediator (eg, drugs that gradually degrade to release H<sub>2</sub>S), will have a bright future in CV medicine.<sup>59</sup>

In regard to the former possibility, a simple nutraceutical protocol can be proposed.

### ENZYMATIC SYNTHESIS OF H<sub>2</sub>S

At least three enzymes generate H<sub>2</sub>S in the human body.<sup>2</sup> CSE, better known for its ability to cleave cystathionine to generate cysteine,  $\alpha$ -ketobutyrate and ammonia (an essential step in methionine catabolism), can also act on cysteine to yield pyruvate, ammonia and H<sub>2</sub>S.<sup>61</sup> CSE is the primary source of H<sub>2</sub>S in the vasculature; it is also expressed in the liver, kidney, ileum, uterus and placenta.<sup>2</sup> The chief source of H<sub>2</sub>S in the central nervous system is the enzyme cystathionine- $\beta$ -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<sub>2</sub>S in the process.<sup>62</sup> A third route to H<sub>2</sub>S 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<sub>2</sub>S.<sup>63 64</sup> A recent study indicates that 3-MST may be the chief source of H<sub>2</sub>S in human coronary arteries.<sup>65</sup>

### CYSTEINE AVAILABILITY IS RATE LIMITING FOR H<sub>2</sub>S 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.<sup>66–69</sup> It is notable that CSE's K<sub>m</sub> for cysteine has been found to be around 3.5 mM—a concentration far higher than ambient levels of free cysteine in cells.<sup>70</sup> It should follow that supplementation with nutraceuticals that can boost cellular levels of cysteine will boost CSE-mediated H<sub>2</sub>S production to a commensurate degree. CBS's K<sub>m</sub> for cysteine is even higher—around 6 mM.<sup>71</sup> With respect to 3-MST, its K<sub>m</sub> for 3-mercaptopyruvate has been measured at over 7 mM, and the K<sub>m</sub> of cysteine aminotransferase for cysteine is 22 mM.<sup>63 72</sup> Hence, there is reason to suspect that increasing cellular cysteine levels should proportionately increase H<sub>2</sub>S 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.<sup>73 74</sup> (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,  $\gamma$ -glutamylcysteine synthetase, also has a rather high K<sub>m</sub> for cysteine, which is why NAC supplementation is effective for boosting glutathione levels.<sup>75</sup> 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<sub>2</sub>S 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<sub>2</sub>S 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.<sup>76–78</sup> 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.<sup>79–80</sup> 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.<sup>81–82</sup> 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 multi-gram doses, highly soluble and so devoid of flavour that it can be added in high amounts to any food or beverage.<sup>83</sup> 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.)<sup>84–85</sup>

Recently, clinical researchers elected to conduct an adequately powered assessment of taurine's ability to lower modestly elevated BP.<sup>86</sup> 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<sub>2</sub>S 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<sub>2</sub>S.

In an effort to determine why H<sub>2</sub>S 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.<sup>65</sup> 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.<sup>87–88</sup> 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<sub>2</sub>S.

The ability of taurine to enhance the expression of CSE is not unprecedented. The drug S-propargyl-cysteine likewise has shown this effect.<sup>89–90</sup> 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.<sup>91–92</sup> 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.<sup>92</sup>

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<sub>2</sub>S. This can be done by noting whether drug-mediated inhibition of H<sub>2</sub>S synthesis, or use of transgenic mice deficient in H<sub>2</sub>S synthesis, eliminates the protective effects of taurine administration. The positive inotropic effect of taurine in heart failure might not be attributable to H<sub>2</sub>S, as the latter is not known to have such an effect.<sup>79–93–96</sup>

### A NUTRACEUTICAL REGIMEN FOR BOOSTING H<sub>2</sub>S SYNTHESIS

Assuming that the recent research linking taurine with H<sub>2</sub>S 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<sub>2</sub>S 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<sub>2</sub>S 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.<sup>74</sup> 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<sub>2</sub>S in the trial in patients with prehypertension.<sup>79–86</sup>

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.<sup>97</sup> This age-related deficit in glutathione can be corrected with supplemental NAC.<sup>98</sup> 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.<sup>99–100</sup> Supplementation with NAC in the elderly may provide health protection by boosting the production of both glutathione and H<sub>2</sub>S, 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.<sup>101–103</sup>

In light of the mutually complementary interactions of NO and H<sub>2</sub>S 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<sup>104–111</sup>—to achieve an ample measure of CV protection.

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