

# Hydrogen Sulfide, Adipose Tissue and Diabetes Mellitus

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Lin Zhu<sup>1</sup>  
Bo Yang<sup>2</sup>  
Dongxia Ma<sup>3</sup>  
Lan Wang<sup>4</sup>  
Wu Duan<sup>5</sup>

<sup>1</sup>Department of Pediatrics, Tongji Hospital, Tongji Medicine College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China; <sup>2</sup>Institute of Organ Transplantation, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China; <sup>3</sup>Department of Allergy, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, People's Republic of China; <sup>4</sup>Reproductive Medicine Center, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, People's Republic of China; <sup>5</sup>Division of Endocrinology, Department of Internal Medicine, Qilu Hospital of Shandong University, Jinan 250012, People's Republic of China

**Abstract:** Hydrogen sulfide (H<sub>2</sub>S) is now increasingly considered to be the third gasotransmitter alongside other gaseous signaling molecules, nitric oxide (NO) and carbon monoxide (CO). H<sub>2</sub>S is produced by a variety of endogenous enzymatic and non-enzymatic pathways and acts as a modulator of the physiological and pathological events of the body. Adipocytes express the cystathionine  $\gamma$  lyase (CSE)/H<sub>2</sub>S system, which modulates a variety of biological activities in adipose tissue (AT), including inflammation, apoptosis, insulin resistance, adipokine secretion and adipocyte differentiation. Abnormalities in the physiological functions of AT play an important role in the process of diabetes mellitus. Therefore, this review provides an overview of the general aspects of H<sub>2</sub>S biochemistry, the effect of H<sub>2</sub>S on AT function and diabetes mellitus and its molecular signalling mechanisms as well as the potential application of H<sub>2</sub>S in pharmacotherapy.

**Keywords:** hydrogen sulfide, adipose tissue, diabetes mellitus, drug therapy

## Introduction

H<sub>2</sub>S, a toxic gas with a rotten egg flavour used as a chemical reagent, was first proposed by Abe and Kimura in 1996 to be an endogenous neuromodulator.<sup>1</sup> Considerable evidence suggests that H<sub>2</sub>S is produced endogenously in many parts of the body and participates in various physiological and pathological functions in mammals.<sup>2</sup> Altered levels of endogenous H<sub>2</sub>S and H<sub>2</sub>S synthetase expression have been observed in diabetic patients and animals. The blood H<sub>2</sub>S levels in diabetic patients were found to be significantly lower than those in age-matched normal control subjects.<sup>3</sup> However, Yusuf<sup>4</sup> investigated the increased H<sub>2</sub>S production rate in the pancreatic islets and livers of diabetic rats. Zucker diabetic fatty (ZDF) rats have significantly higher levels of pancreatic CSE expression and H<sub>2</sub>S production when compared with Zucker fatty (ZF) or Zucker lean (ZL) rats.<sup>5</sup> Increasing numbers of studies have shown that H<sub>2</sub>S is involved in the development of diabetes,<sup>6</sup> however, its impact on diabetes now appears to be contradictory. The dysfunction of AT plays an important role in the development of diabetes. We will briefly review the effect of H<sub>2</sub>S on diabetes by discussing the influence of H<sub>2</sub>S on the function of AT.

## Hydrogen Sulfide Biosynthesis and Catabolism

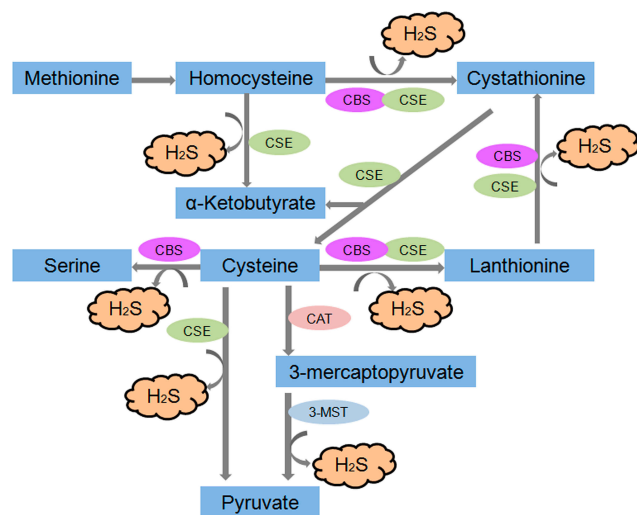
H<sub>2</sub>S is a colourless, flammable gas with a strong odour of rotten eggs. It is produced by cystathionine  $\beta$ -synthetase (CBS) in the mammalian brain, selectively enhances NMDA (N-methyl-D-aspartate) receptor-mediated responses and facilitates the

Correspondence: Wu Duan  
Division of Endocrinology, Department of Internal Medicine, Qilu Hospital of Shandong University, Jinan 250012, People's Republic of China  
Tel/Fax +86-531-8692-7544  
Email duan5wu@qq.com

induction of long-term potentiation in the hippocampus, which was the first proposed biological role of H<sub>2</sub>S as a neuromodulator.<sup>1</sup> Several lines of evidence have suggested that H<sub>2</sub>S is naturally synthesized by four enzymes: CBS, CSE, 3-mercaptopyruvate sulfurtransferase (3-MST) and cysteine aminotransferase (CAT). It is produced in vivo from L-cysteine (LC) by those enzymes.<sup>6,7</sup> Those enzymes are constitutively present and can be found widely in cells and tissues, and their expression can be induced by a number of disease states.<sup>8</sup>

Four enzymatic pathways for H<sub>2</sub>S production have been described thus far. Two of them are catalysed by CBS and CSE, which are pyridoxal 5'-phosphate (vitamin B6)-dependent cytoplasmic enzymes of the trans-sulfuration pathway. The third pathway for H<sub>2</sub>S generation requires two enzymes, CAT and 3-MST. In contrast with CBS and CSE, this pathway operates primarily in the mitochondria<sup>6</sup> (Figure 1). Finally, H<sub>2</sub>S in the cerebellum and the kidney can be synthesized from D-cysteine by the synergism of D-amino acid oxidase (which is highly expressed in the peroxisomes of these organs) and 3-MST.<sup>9</sup> The degradation pathways involved in H<sub>2</sub>S consumption are mediated by ethylmalonic encephalopathy protein 1 (ETHE1), mitochondrial sulfide quinone oxidoreductase (SQR) and cysteine dioxygenase (CDO).<sup>10,11</sup>

Changes in the levels of H<sub>2</sub>S or H<sub>2</sub>S synthetase expression may contribute to the pathogenesis of many pathophysiological processes, such as neurological systems, inflammation, apoptosis, vascular function, energy metabolism and biogenesis, obesity and ageing.<sup>6</sup> The



**Figure 1** Generalised overview of H<sub>2</sub>S production in the cell. The endogenous production of H<sub>2</sub>S occurs via two main pathways—reverse transsulfuration and cysteine oxidation which take place partly inside mitochondria.

pharmacological or genetic inhibition or activation of H<sub>2</sub>S production is protective in some models. Therefore, the application of H<sub>2</sub>S donors and/or the augmentation of endogenous H<sub>2</sub>S or H<sub>2</sub>S inhibitors and/or the reduction in endogenous H<sub>2</sub>S has attracted much attention as possible therapeutic approaches.

## Hydrogen Sulfide and Diabetes

Endogenous H<sub>2</sub>S can be synthesized by multiple systems, with the highest rates occurring in the brain, cardiovascular system, liver and kidney.<sup>11</sup> At present, studies have shown that almost every mammalian tissue cell can produce a certain amount of H<sub>2</sub>S. Under physiological conditions, the H<sub>2</sub>S concentration in rat brain tissue is 50–160 μmol/L, and the serum H<sub>2</sub>S concentration is approximately 46 μmol/L.<sup>12</sup> Human blood contains a significant amount (10–100 μmol/L) of H<sub>2</sub>S.<sup>13</sup>

Altered endogenous H<sub>2</sub>S levels, and the expression of H<sub>2</sub>S synthetases have been observed in diabetic animals and patients.<sup>6</sup> Yusuf<sup>4</sup> first reported that a marked increase in H<sub>2</sub>S synthesis from exogenous LC in the pancreas and liver is associated with streptozotocin (STZ)-induced diabetes in rat, and the liver pancreatic CBS and CSE mRNA levels were concomitantly increased, with no change in the pancreatic CSE mRNA levels in diabetic rats. However, the plasma H<sub>2</sub>S concentrations were similar in diabetic and non-diabetic animals. Therefore, the enhanced formation of H<sub>2</sub>S is likely to be a local tissue response to diabetes, which is not reflected in increased circulating plasma levels. Other researchers<sup>5</sup> have also indicated that the expression of CSE and the production rate of H<sub>2</sub>S in pancreatic islets were significantly higher in ZDF rats than in ZL or ZF rats. The serum H<sub>2</sub>S levels of ZDF rats were also significantly higher than those of ZF or ZL rats. Increased pancreatic H<sub>2</sub>S production underlies the reduced insulin release from the pancreatic β cells of ZDF rats. The activation of K<sup>+</sup><sub>ATP</sub> channels by high levels of endogenous H<sub>2</sub>S in pancreatic β cells may be the molecular basis underlying H<sub>2</sub>S-suppressed insulin release.<sup>5</sup> It is well-known that H<sub>2</sub>S functions as an endogenous activator of K<sup>+</sup><sub>ATP</sub> channels in β cells.<sup>14</sup> Further research<sup>15</sup> has found that the CSE/H<sub>2</sub>S system plays a critical role in regulating cell functions by stimulating β cell apoptosis and inducing K<sup>+</sup><sub>ATP</sub> channel activity and that STZ-induced diabetes is largely mediated by the effects of pancreas-produced H<sub>2</sub>S on β cell mass and K<sup>+</sup><sub>ATP</sub> channel activity. A CSE deficiency significantly delayed the development of T1DM induced by STZ.<sup>16</sup>

Interestingly, the results of other studies are diametrically opposite. Brancaleone et al<sup>17</sup> found that the plasma H<sub>2</sub>S value in NDR (non-obese resistant) mice was approximately 50 μmol/L, whereas NOD (non-obese diabetic) mice exhibited a progressive decrease in plasma H<sub>2</sub>S levels that paralleled the disease severity, reaching a 50% reduction in NOD III mice and indicating that endogenous H<sub>2</sub>S production is significantly impaired under hyperglycaemic conditions. A further study found that the blood H<sub>2</sub>S levels of diabetic patients were significantly decreased when compared with age-matched normal control subjects, which is in accordance with their study of rats.<sup>3</sup> Recently, Kunihiro et al<sup>13</sup> investigated the plasma H<sub>2</sub>S levels in Japanese individuals with type 2 diabetes mellitus and found that the patients showed a progressive decrease in the plasma H<sub>2</sub>S levels (45.1±15.5 μmol/L versus 54.0±26.4 μmol/L), which is in accordance with poor glycaemic control. There was a significant correlation between the reduction in plasma H<sub>2</sub>S levels and the level of HbA1c. They also found that the reduction in plasma H<sub>2</sub>S levels was associated with a history of cardiovascular disease in type 2 diabetic patients.

The above study shows that H<sub>2</sub>S may play a role in the development of diabetes. Researchers have observed different changes in H<sub>2</sub>S levels in different diabetic patients and animals, possibly due to the different stages of diabetes in the diabetic patients studied by different researchers, the different methods of inducing diabetic animals and the use of different animal species.

## Hydrogen Sulfide and Adipose Tissue

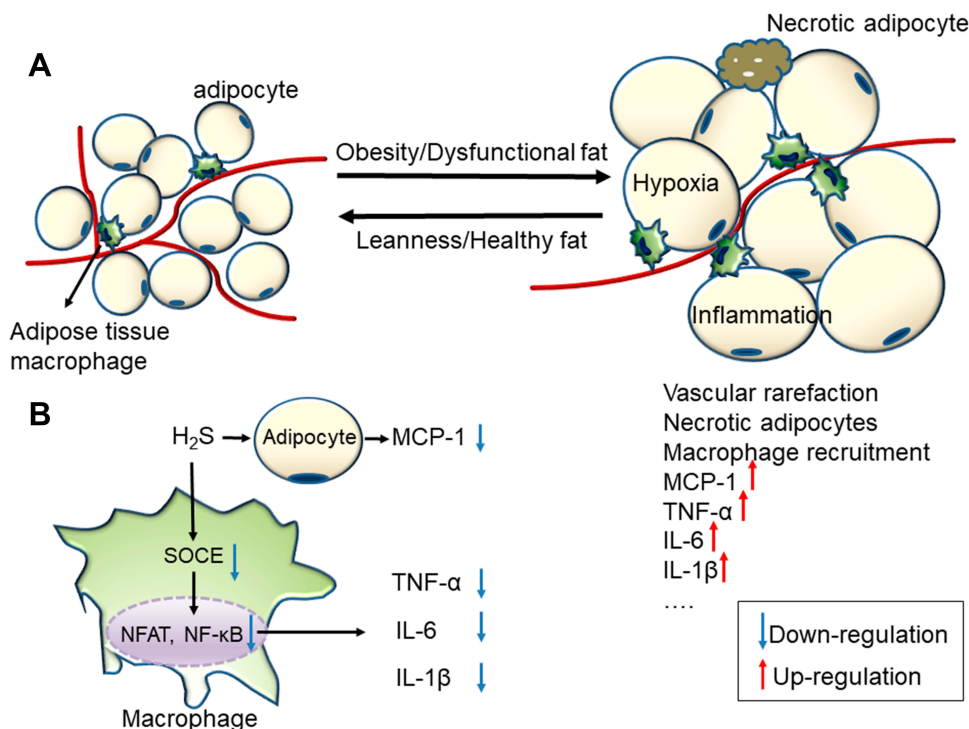
Diabetes-related obesity and general obesity are characterized by an increase in the AT mass, which can be defined as an increase in the BMI. In addition to the increase in AT mass (eg, increase in the size and number of adipocytes), histological and macroscopic changes and AT dysfunction, including hypersecretion of proinflammatory, proatherogenic and pro-diabetic adipocytokines, occurs in obesity.<sup>18–20</sup> Feng et al<sup>21</sup> first identified the endogenous CSE/H<sub>2</sub>S pathway in AT. They detected the rate of H<sub>2</sub>S production from visceral AT, including perirenal fat (2.93 ± 0.27 nmol/min/mg protein), epididymal fat (4.76 ± 0.92 nmol/min/mg protein) and brown fat tissue (4.65 ± 0.81 nmol/min/protein). RT-PCR results revealed that CBS and CSE mRNA were expressed in those fat tissues and illuminated the CSE-mediated primary pathway of H<sub>2</sub>S generation in AT. Meanwhile, they found H<sub>2</sub>S-generation rates in adipocytes and preadipocytes of 2.89 ± 1.34 and 2.17 ± 1.14 nmol/min/mg protein, respectively, and demonstrated that adipocytes also endogenously produce H<sub>2</sub>S through a CSE catalytic pathway. Furthermore, they found that H<sub>2</sub>S production and CSE protein expression in the epididymal fat pad and in perirenal fat increased in an age-dependent manner. The same year, Fang et al<sup>22</sup> first demonstrated that CSE protein expression and endogenous H<sub>2</sub>S production in rat PVAT (perivascular adipose tissue) were detectable and that the endogenous H<sub>2</sub>S generated by PVAT was predominantly CSE-catalysed.

In recent years, as increasing research has focused on AT-derived H<sub>2</sub>S and explored the pathophysiological roles of H<sub>2</sub>S in AT,<sup>23</sup> we have uncovered the effects of H<sub>2</sub>S on AT inflammation, apoptosis, adipokine secretion, glucose and lipid metabolism and vascular tension.

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## Hydrogen Sulfide and Inflammation of Adipose Tissue

A series of epidemiological studies have shown that circulating inflammatory markers are closely related to type 2 diabetes mellitus (T2DM) and are risk factors for the development of future T2DM.<sup>24</sup> Numerous preclinical and clinical studies now strongly support the view that obesity-induced inflammation plays an important role in the development of insulin resistance and T2DM.<sup>25,26</sup> Although obesity-induced inflammation is similar in many ways to inflammation observed in classical immunity, it is a low-grade form of inflammation that produces much lower levels of circulating cytokines.<sup>27</sup> It is also considered to be chronic inflammation, as it requires relatively long dietary therapy before it becomes clearly discernible in AT, which displays the most severe obesity-induced inflammation of all insulin-responsive tissues (as characterized by the increased cytokine/chemokine expression and immune cell infiltration).<sup>28</sup> Indeed, AT produces high levels of many inflammatory cytokines in obesity and is therefore considered to be the major inflammatory organ that mediates obesity-induced inflammation. Chronic inflammation and metabolic detrimental factors released by AT into the circulation are associated with some metabolic complications of obesity, such as T2DM and atherosclerosis.<sup>29</sup> AT inflammation is frequently observed in obesity and diabetes, is associated with the infiltration of macrophages, and may be caused by the death of adipocytes, the secretion of adipokines, such as TNF-α and IL-6, and adipocyte chemokines, such as monocyte chemo-attractant protein-1 (MCP-1) (Figure 2A).<sup>30,31</sup>



**Figure 2 (A)** Healthy and obese adipose tissue. Obese adipose tissue is characterized by inflammation and progressive infiltration by macrophages as obesity develops. **(B)** The anti-inflammation effect of H<sub>2</sub>S on obese adipose tissue.

The gaseous signalling molecule H<sub>2</sub>S has been identified as an important inflammatory mediator. At present, several research groups worldwide are focused on determining the role of H<sub>2</sub>S in inflammation. H<sub>2</sub>S has different effects on inflammation in different models.<sup>32–34</sup> The observation that an H<sub>2</sub>S donor significantly attenuates the production of cytokines triggered by the pro-inflammatory stimuli of primary macrophages<sup>35</sup> and macrophage cell lines<sup>36</sup> supports the anti-inflammatory action of H<sub>2</sub>S in macrophages. These findings are particularly intriguing for data that point to impaired H<sub>2</sub>S signalling in type 2 diabetes<sup>3,17,37–39</sup> and show a decrease in the plasma concentration of H<sub>2</sub>S in human diabetes and obesity.<sup>40</sup>

The overexpression of CSE and exogenous application of NaHS both increased H<sub>2</sub>S concentrations and significantly inhibited high glucose (HG)-induced MCP-1 secretions in mature 3T3-L1 adipocytes, indicating that H<sub>2</sub>S exhibited anti-inflammatory effects in mature adipocytes.<sup>41</sup> A further study<sup>42</sup> found that obesity increased the production of H<sub>2</sub>S while also increasing the consumption of H<sub>2</sub>S likely due to the promotion of H<sub>2</sub>S catabolism by radical species (the reactive oxygen and nitrogen species). In addition, H<sub>2</sub>S depletion was observed in chronic inflammation models [AT macrophages (ATMs) from obese mice]. In obesity, store-operated Ca<sup>2+</sup> entry (SOCE) increased concurrently with the consumption of H<sub>2</sub>S, and

a reduction in the concentration of cellular H<sub>2</sub>S promotes an increase in SOCE.<sup>43</sup> The activation of the SOCE pathway during pro-inflammatory stimulation provides the Ca<sup>2+</sup> signals required for the activation of nuclear factor of activated T cells (NFAT) and nuclear factor κB (NFκB), the transcription factors responsible for the expression of cytokine encoding genes.<sup>43,44</sup> During obesity, an increased abundance of M1-associated markers, reduced H<sub>2</sub>S bioavailability and increased SOCE were observed in ATMs. The inhibition of SOCE or the prevention of H<sub>2</sub>S loss from these cells also limited pro-inflammatory cytokine production. In parallel with the study by Pan,<sup>41</sup> this research implied that H<sub>2</sub>S plays an anti-inflammatory role in the ATMs from obese mice (Figure 2B).

Those reports suggested that therapeutic strategies designed to maintain H<sub>2</sub>S bioavailability may be effective in a number of inflammatory pathological contexts. However, further studies are required to specifically examine the effects of H<sub>2</sub>S on AT inflammation in diabetes, obesity and other pathological conditions.

## Hydrogen Sulfide and Insulin Resistance of Adipose Tissue

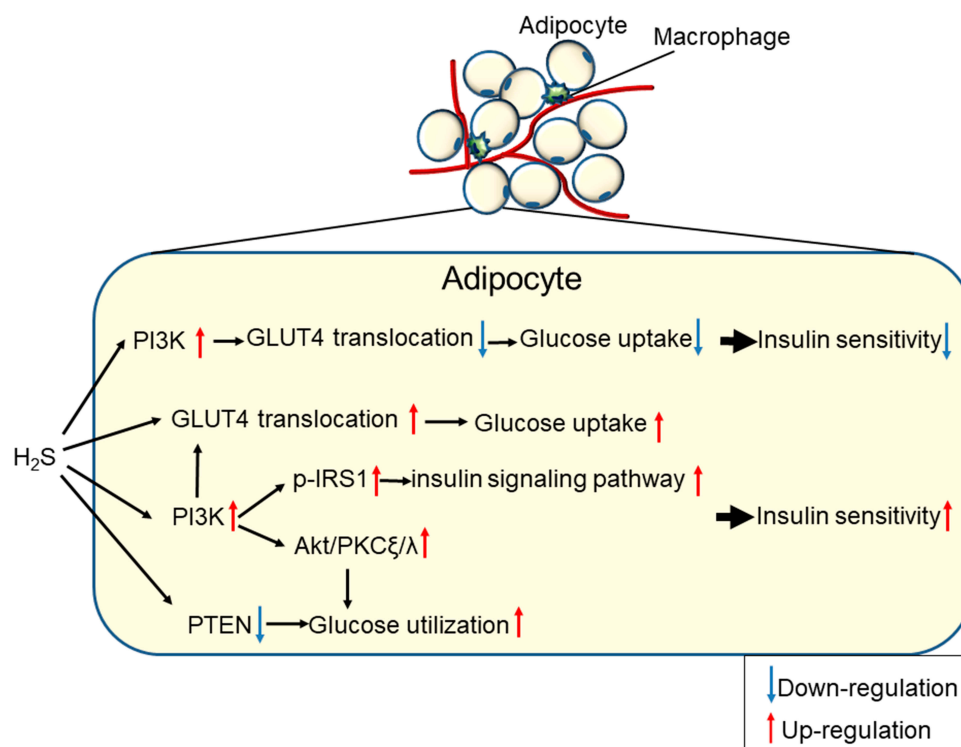
T2DM consists of two independent but related alterations: insulin resistance and dysfunction of the β-cells in the

pancreas. AT is an insulin-sensitive organ that mediates glucose uptake and metabolism<sup>45</sup> and is an important source of methionine metabolism for the synthesis of fatty acids.<sup>46,47</sup> Increasing evidence has shown that excess AT accumulation alters AT secretion profiles,<sup>48</sup> and only a few adipokines have been shown to regulate insulin sensitivity, including TNF- $\alpha$ , adiponectin, interleukin-6, resistin and leptin (Figure 3A). TNF- $\alpha$  provides a molecular basis for insulin resistance and contributes by inhibiting the expression of genes that are essential for insulin signalling and adipocyte differentiation, including adiponectin.<sup>49</sup> Single-nucleotide polymorphisms (SNPs) in the promoter region of the adiponectin gene may be associated with the development of insulin resistance, obesity, and T2DM.<sup>50,51</sup>

Researchers have found H<sub>2</sub>S-reduced insulin sensitivity in adipocytes, and CSE inhibitors might reverse this effect. A high level of glucose (20 mmol/L) inhibited the H<sub>2</sub>S production of adipocytes in a time- and concentration-dependent manner. Under basal and insulin stimulating conditions, H<sub>2</sub>S inhibited glucose transport into adipocytes in a concentration-dependent manner. The CSE inhibitors PAG (propargylglycine) and BCA ( $\beta$ -cyano-L-alanine) increased basal and insulin-stimulated adipocyte glucose uptake.<sup>21</sup> Further research<sup>52</sup> indicated that the upregulation of the endogenous CSE/H<sub>2</sub>S system contributes to TNF- $\alpha$  induced insulin

resistance in 3T3-L1 adipocytes, and exogenous H<sub>2</sub>S causes a decrease in insulin-stimulated glucose consumption and uptake, which can be blocked by pretreatment with LY294002, a PI3K inhibitor, but not with glibenclamide, a non-selective K<sup>+</sup><sub>ATP</sub> channel inhibitor. Pinacidil, a non-selective K<sup>+</sup><sub>ATP</sub> channel opener, also has no effect on the glucose uptake of adipocytes. Therefore, H<sub>2</sub>S might inhibit GLUT4 (glucose transporter 4) translocation through the PI3K (phosphatidylinositol 3-kinase) pathway, rather than through the K<sup>+</sup><sub>ATP</sub> channel pathway, to inhibit glucose uptake by adipocytes.<sup>21</sup>

However, the effect of H<sub>2</sub>S on the insulin resistance of adipocytes is controversial. Prasenjit et al<sup>53</sup> argued that H<sub>2</sub>S plays an important role in vitamin D-induced GLUT4 translocation to the cell surface for insulin-stimulated glucose uptake and utilization in 3T3-L1 adipocytes. Interestingly, they also indicated that LC and H<sub>2</sub>S caused an increase in PIP3 (phosphatidylinositol-3,4,5 trisphosphate, a positive regulator of glucose metabolism), Akt (serine/threonine protein kinase) phosphorylation, and glucose utilization in HG (25 mM) treated 3T3-L1 adipocytes.<sup>54</sup> In addition, H<sub>2</sub>S mediated the above effect of LC. H<sub>2</sub>S or PIP3 increased GLUT4 activation, glucose utilization and the phosphorylation of IRS1 (insulin receptor substrate 1), Akt and PKC $\xi/\lambda$  (protein kinase C  $\xi/\lambda$ ) in



**Figure 3** The bilateral regulation of H<sub>2</sub>S on glucose metabolism and insulin sensitivity in adipocytes.

HG-treated cells. They provide evidence for a molecular mechanism by which H<sub>2</sub>S can upregulate the insulin-signalling pathways by increasing PIP3 and glucose utilization by inhibiting PTEN (Phosphatase and Tensin Homolog) protein and activating the PI3K/AKT/PKC $\xi$ / $\lambda$  pathway in 3T3-L1 adipocytes.<sup>55</sup>

Moreover, Rong et al<sup>56</sup> found that NaHS treatment may ameliorate insulin resistance by sensitizing insulin receptor (IR)-mediated signals. The phosphorylation of IR, PI3K and Akt increased in adipocytes treated with NaHS. However, the ability of NaHS to activate PI3K was blocked by the IR inhibitor HNMPA (C<sub>11</sub>H<sub>11</sub>O<sub>4</sub>P). In addition, NaHS directly activated the IRs in a cell-free system, indicating that IRs may act as a direct target molecule for NaHS and that PI3K may act as a downstream element of IR in the actions of NaHS. This hypothesis was further supported by the notion that increased NaHS-induced glucose uptake was blocked by siRNA-mediated IR knockdown or by pretreatment with the IR inhibitor HNMPA or the PI3K inhibitor LY294002. NaHS may react with some amino acid residues of IRs, leading to certain chemical modifications of the residues and to subsequent conformational changes in the IRs. Changes in IR conformation induced by NaHS may promote the trans-autophosphorylation of IRs and, thus, enhance IR activation. The stimulating effects of NaHS on the IRs may have potential clinical relevance. Fasting blood glucose levels were decreased and glucose tolerance was increased in GK rats chronically treated with NaHS at a dose of 30  $\mu$ mol/kg $\cdot$ day. The activation of the PI3K/Akt pathway was also observed in some tissues in GK rats treated with NaHS. Increased insulin sensitivity and reduced plasma fasting glucose levels were also observed in Wistar rats treated with NaHS.<sup>56</sup> These data further confirm that the insulin sensitizing effects of exogenous H<sub>2</sub>S treatment are also present in subjects without diabetes. Another study<sup>57</sup> found that H<sub>2</sub>S ameliorated insulin resistance by upregulating IRS1 protein, and the CSE/H<sub>2</sub>S system might regulate the gene transcription of glucose metabolic enzymes or transporter proteins through a nuclear receptor.

The bilateral regulation of the CSE/H<sub>2</sub>S system in glucose metabolism and insulin sensitivity suggested that CSE/H<sub>2</sub>S might be an energy balancer<sup>58</sup> (Figure 3B). Under physiological conditions, the CSE/H<sub>2</sub>S system tended to reduce energy consumption, resulting in a slight decrease in glucose utilization, whereas under stress or inflammation, the CSE/H<sub>2</sub>S system antagonized

injury and increased glucose utilization, resulting in increasing insulin sensitivity. Clarifying the regulation of CSE/H<sub>2</sub>S during energy metabolism may help elucidate the complex interactions among glucose, fat and sulfur-containing amino acids in physiology and diseases.

## Hydrogen Sulfide and Adipocyte Lipid Metabolism

During AT expansion, adipocytes become either hyperplastic, when their number increases through adipogenesis, or hypertrophic, when their size increases through lipogenesis.<sup>59</sup> The increased number of adipocytes is primarily determined by the process of adipocyte differentiation, termed adipogenesis, which refers to the conversion of undifferentiated preadipocytes to mature adipocytes. In contrast, lipolysis is one of the most important processes for reducing adipose mass in which triglycerides stored in adipocytes are broken down into glycerol and fatty acids.<sup>60</sup> The dysregulation of AT metabolism (including lipogenesis and lipolysis) is related to obesity, insulin resistance and diabetes in human and experimental animal models.<sup>19,61</sup>

The role of H<sub>2</sub>S in the regulation of AT metabolism is complex. Geng et al<sup>57</sup> found that the H<sub>2</sub>S precursor LC or the donor-GYY4137 (morpholin-4-ium 4 methoxyphenyl (morpholino) phosphinodithioate) suppressed lipolysis while PAG induced basal and isoproterenol-stimulated lipolysis in rat epididymal adipocytes. PAG also increased lipolysis in normal chow and high-fat diet (HFD) fed mice by increasing serum glycerol levels without affecting food consumption and then blunting fat deposition and weight gain. GYY4137 reduced lipolysis in HFD mice without increasing fat mass and body weight. Moreover, they found that the PKA-HSL/perilipin 1 pathway may be involved in the lipolytic regulation of H<sub>2</sub>S.

Endogenous H<sub>2</sub>S was increased after 3T3-L1 differentiation.<sup>62</sup> The expression levels of the H<sub>2</sub>S-synthesizing enzymes CSE, CBS and 3-MST were increased in a time-dependent manner during 3T3-L1 differentiation. GYY4137 and NaHS increased the expression of genes involved in adipogenesis, which include fatty acid binding protein 4 (FABP4/aP2), a key regulator of adipogenesis, and significantly increased the size and number of lipid droplets in mature adipocytes, which also compromised the ability of CL316,243 (a  $\beta$ 3-agonist) to promote lipolysis in these cells. In contrast, aminoxyacetic acid (AOAA) and PAG had the opposite effect. Moreover, the inhibition of H<sub>2</sub>S production by CBS/CSE inhibitors seriously prevented

the induction of adipogenic marker genes,<sup>63</sup> PPAR $\gamma$ 2 (peroxisome proliferator-activated receptor gamma 2) and its target gene perilipin1, Fabp4 and stearoyl-CoA desaturase-1 (Scd1). In addition, Cai et al<sup>64</sup> confirmed the upregulation of an endogenous CSE/H<sub>2</sub>S system during adipocyte differentiation. Increased H<sub>2</sub>S promoted adipogenesis and inhibited lipolysis, resulting in the storage of triglycerides in lipid droplets. More interestingly, using H<sub>2</sub>S instead of 3-isobutyl-1-methylxanthine (IBMX) in the differentiation cocktail also upregulated adipogenesis markers and slightly increased the intracellular triglyceride levels. Based on the IBMX mechanism of action, H<sub>2</sub>S might directly promote PPAR $\gamma$  expression in part by inhibiting PDE (phosphodiesterase) activity. Therefore, H<sub>2</sub>S sulfhydrates PPAR $\gamma$ , enhancing its activity to promote adipogenesis, increase insulin sensitivity and inhibit basal lipolysis, causing a decrease in the circulating free fatty acid level and thereby ameliorating target injury in obesity.<sup>65</sup>

In summary, H<sub>2</sub>S increases the expression of adipogenesis-related proteins, increases lipid synthesis, promotes the differentiation of preadipocytes into mature adipocytes and reduces the lipolysis of adipocytes (Figure 4). H<sub>2</sub>S synthase inhibitors or siRNAs have the opposite effect.

## Hydrogen Sulfide Is a Vascular Tone Modifier

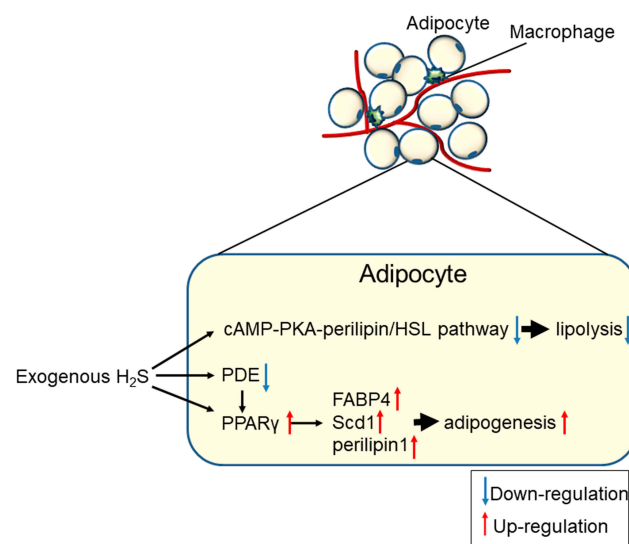
The contractile force of aortas induced by norepinephrine<sup>66</sup> and other vasoconstrictors was mitigated by PVAT in a paracrine manner.<sup>67,68</sup> The anti-contractile effect of

PVAT was also observed for both large and small vessels in mice, pigs and humans.<sup>69,70</sup> Bioassay experiments showed that the adipocyte derived relaxing factor (ADRF), produced by PVAT, activates the K<sup>+</sup> channels in vascular smooth muscle cells, exerting its anti-contractile effect on systemic peripheral arteries.<sup>68</sup>

Fang et al<sup>22</sup> first demonstrated that the CSE protein was expressed in both the PVAT and aorta and in the adipocytes of PVAT and vascular smooth muscle cells (VSMCs) of aorta media. The rate of endogenous H<sub>2</sub>S generation was similar to that of aortic tissues and was primarily catalysed by CSE. The H<sub>2</sub>S donor NaHS produces dose-dependent vasorelaxation effects, which are blocked by XE991 (the KCNQ inhibitor). Therefore, H<sub>2</sub>S may be a candidate or modulator of ADRF, which activates the K<sub>v</sub> channels encoded by KCNQ genes to achieve the paracrine control of vascular tone by PVAT. It is unclear how the PVAT derived H<sub>2</sub>S activates KCNQ channels, where it activates the channel directly or produces sulfhydryl radicals [HS(\*)/S(\*-)], which, coupled to the formation of superoxide radical anions,<sup>71</sup> are powerful KCNQ channel activators.<sup>72</sup> Further research confirmed that H<sub>2</sub>S generated by PVAT was a releasable vascular relaxation factor, acting in a paracrine manner by opening the K<sup>+</sup><sub>ATP</sub> channel in a NO-, endothelium- and Ca<sup>2+</sup> channel-independent manner. Inhibitors of CSE, such as BCA (5 mmol/l) and PAG (10 mmol/l), inhibit the anti-contractile effects of PVAT.<sup>73</sup>

Moreover, the anti-contractile effect of PVAT on rat aortic rings has been demonstrated to be blunted not only by XE991 or 4-AP (4-aminopyridine, the K<sub>v</sub> channel inhibitor) but also by glibenclamide. In addition, the dilation of serotonin-precontracted aortic rings without PVAT induced by NaHS was also abolished by glibenclamide, 4-AP or XE991.<sup>74</sup> It is unclear whether this phenomenon results from the nonspecific effects of these inhibitors on different types of K<sup>+</sup> channels or from the fact that both K<sup>+</sup><sub>ATP</sub> and KCNQ channels are necessary for vasodilation induced by PVAT-derived H<sub>2</sub>S. Interestingly, the expression levels of CSE in PVAT surrounding mouse aortas is much lower than that in rats, and PVAT also has an anti-contractile effect on mouse aortic rings; this effect is not mediated by H<sub>2</sub>S, but it is still inhibited by XE991. These data indicate that H<sub>2</sub>S is the ADRF in rat but not mouse aorta, but the H<sub>2</sub>S-independent anti-contractile effects of PVAT on mouse aorta are also mediated by KCNQ channels.<sup>74</sup>

The contractions of arteries induced by 5-HT (5-hydroxytryptamine) in diabetic and healthy rats with intact



**Figure 4** Generalised overview of the effects of H<sub>2</sub>S on adipocyte lipolysis and adipogenesis.

PVAT were significantly different from those in arteries without PVAT. At the highest studied concentrations of 5-HT ( $10^{-5}$  mol/L), the arteries of diabetic rats with PVAT had significantly stronger contractions than those without PVAT. The different regulatory roles of PVAT in diabetic and control rats could be explained by the decreased levels of  $H_2S$  caused by diabetes.<sup>75</sup>

Almost all of the blood vessels are surrounded by a different amount of PVAT. PVAT may have a paracrine function in the regulation of arterial tone, vascular reactivity, and more.<sup>76</sup> This paracrine adipose-vascular coupling is achieved by the production and function of various ADRFs, which might be a volatile, gaseous mediator, namely,  $H_2S$ .<sup>75</sup> There is evidence that the opening of myocyte  $K^+$  channels plays a critical role in the paracrine regulation of arterial tone by  $H_2S$ . KCNQ (Kv7) channels could represent, at least in part, the subtypes of the voltage-dependent  $K^+$  (Kv) channels involved.<sup>77</sup> Additional ion channels, such as  $Ca^{2+}$ -activated  $K^+$  ( $K^+-Ca^{2+}$ ) channels, and cellular mechanisms appear to be involved in the vasoactive effects.<sup>78</sup> Therefore, PVAT- $H_2S$  S-smooth muscle crosstalk in the artery wall (Figure 5) may constitute a therapeutic approach against the harmful effects of diabetes and obesity in different vascular beds.

## Hydrogen Sulfide and Adipokines

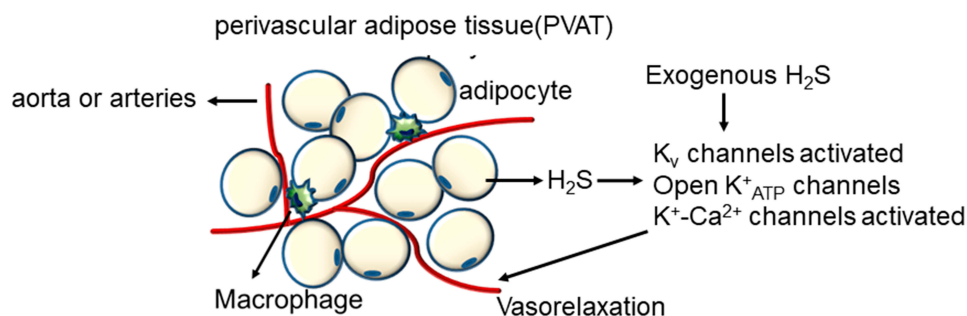
Adipocytes and AT produce a wide range of hormones and cytokines involved in glucose metabolism (eg, adiponectin, resistin), lipid metabolism (eg, cholesteryl ester transfer protein, CETP; stearoyl-CoA desaturase-1, adipose triglyceride lipase, hormone-sensitive lipase), inflammation [eg, interleukin (IL)-1 $\beta$ , IL-6, IL-8, IL-10, tumour growth factor (TGF)- $\beta$ , TNF- $\alpha$ , osteopontin (OPN)], the acute-phase and immune response (eg, serum amyloid A, adipsin, PAI-1), blood pressure (eg, angiotensinogen, angiotensin II) and feeding behaviour (eg, leptin), thus affecting the metabolism and function of many organs

and tissues, including muscle, liver, vascular and brain tissues<sup>79–84</sup> (Table 1). Approximately 20% of all genes in subcutaneous adipose tissue (SAT) and approximately 30% of genes in visceral adipose tissue (VAT) encode adipokine secretion.<sup>85</sup> AT also expresses many of the receptors for most of these factors.<sup>86</sup>

Adiponectin is an anti-diabetic and anti-atherogenic adipokine. Although the plasma adiponectin levels are reduced in obesity, other plasma adipocytokine levels increase with increases in AT and adipocyte volume.<sup>87,88</sup>  $H_2S$  is a recently identified endogenous gasotransmitter. Pan et al<sup>41</sup> indicated that the overexpression of CSE and exogenously supplied NaHS, which both increase the concentration of  $H_2S$  could significantly increase the adiponectin secretion that had been depressed by HG (25 mM) in mature 3T3-L1 adipocytes, whereas reduced  $H_2S$  production may contribute to deregulated adipokine secretion under HG conditions.  $H_2S$  may exert anti-diabetic effects by increasing adiponectin levels.

## Hydrogen Sulfide and Adipocytes Apoptosis

In several disease states, including autoimmune lipodystrophy,<sup>89</sup> tumour cachexia,<sup>90</sup> and HIV patients under highly active anti-retroviral therapy,<sup>91</sup> the apoptosis of adipocytes is accompanied by a loss of AT. In obese individuals, AT is poorly oxygenated,<sup>92</sup> which may lead to localized hypoxia, promote free fatty acid (FFA) release and inhibit glucose uptake in adipocytes by inhibiting the insulin signalling pathway.<sup>93</sup> Hypoxia is associated with ER (endoplasmic reticulum) stress in 3T3-L1 adipocytes, as demonstrated by elevated levels of GRP78 (glucose-regulated protein, 78 kDa) and CHOP (C/EBP homologous protein).<sup>94</sup> Excessive ER stress induces apoptosis. Adipocyte apoptosis is still poorly understood, as adipocytes are known to be resistant to apoptosis. Studies have reported that weight loss in obese models could be attributed to apoptosis in adipocytes.<sup>95–97</sup>



**Figure 5** The mechanisms of the vasorelaxation effect of adipocytes-derived and exogenous  $H_2S$ .

**Table 1** Multiple Products Called Adipokines Involving Different Biological Processes Expressed and Secreted by White Adipose Tissue May Act at Both the Systemic (Endocrine) and Local (Autocrine and/OR Paracrine) Levels

Effects On	Adipokine	Full Name
Inflammation	IL-1R $\alpha$	Interleukin-1 Receptor $\alpha$
	IL-1 $\beta$	Interleukin-1 $\beta$
	IL-8	Interleukin-8
	IL-10	Interleukin-10
	CRP	C-reactive protein
	MCP-1	Monocyte chemotactic protein 1
	OPN	Osteopontin
	Visfatin	
Glucose metabolism	FFA	Free fatty acids
	Resistin	
Lipid metabolism	ApoE	Apolipoprotein E
	LPL	Lipoprotein lipase
	Glycerol	
	CD36	Leukocyte differentiation antigen CD36
	HSL/ATGL	Hormone-sensitive lipase/Adipose triglyceride lipase
Vasoactive factors	Angiotensinogen	
	Adiponectin	
	PAI-1	Plasminogen activator inhibitor 1
	VEGF	Vascular endothelial growth factor
	Tissue factors	
	NO	Nitric oxide
Growth factors	TGF- $\beta$	Transforming growth factor $\beta$
	IGF-1	Insulin-like growth factor 1
	HGF	Hepatocyte growth factor
	NGF	Nerve growth factor
	LIF	Leukaemia-inhibitory factor
	Fibronectin	
Immune response	Adipsin	
	ASP	Acylation stimulating protein
	Factors B and C3	
	CSFs	Colony-stimulating factor
	IL-17D	Interleukin-17D
	SAA3	Serum amyloid A3
Cytokines	Leptin	
	TNF- $\alpha$	Tumour necrosis factor $\alpha$
	Chemerin	

(Continued)

**Table 1** (Continued).

Effects On	Adipokine	Full Name
Other proteins	CAV-1	Caveolin 1
	AQP-7	Aquaporin 7
	RBP-4	Retinol binding protein 4
	LCN-2	Lipocalin-2

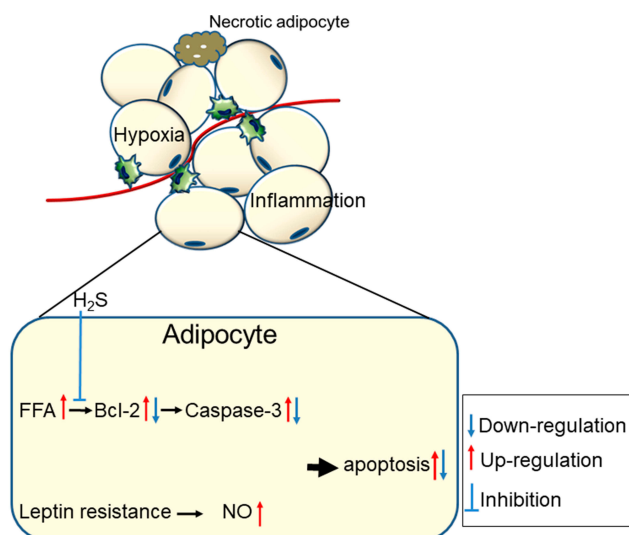
Adipokines such as leptin and TNF- $\alpha$  mediate apoptosis through caspase-dependent pathways and regulate body fat mass.<sup>98,99</sup> In addition, a variety of natural compounds, including xanthohumol, isoxanthohumol and ajoene, have been reported to induce apoptosis in adipocytes.<sup>100,101</sup> Interestingly, these compounds have been independently studied for their anti-obesity effects,<sup>102,103</sup> which means that the anti-obesity effects of some natural compounds may be achieved by inducing the brown fat-like phenotype and apoptosis of white adipocytes. Controlling apoptosis in mature white AT appears to be an ideal strategy for combating obesity and related metabolic syndromes.<sup>104</sup>

H<sub>2</sub>S has become an effective cytoprotective mediator in various models of tissue and cellular injuries.<sup>105,106</sup> After the ischaemic period, mature adipocytes were treated with NaHS (0, 0.1, 1, 10, 100, and 1000  $\mu$ mol/L as an H<sub>2</sub>S donor), and the late apoptosis level of mature adipocytes decreased in every dose group.<sup>107</sup> In addition, the gene expression level of caspase-3 decreased while that of Bcl-2 increased. These results imply that H<sub>2</sub>S has a protective effect on mature adipocytes under ischaemic conditions that is mediated by elevating anti-apoptotic gene expression (Figure 6).

The current knowledge regarding the effects of H<sub>2</sub>S on adipocyte apoptosis is inadequate, and it is necessary to explore it further and elucidate the roles of hypoxia, ER stress and pro-apoptotic and anti-apoptotic molecules in this process. H<sub>2</sub>S may become a new anti-obesity strategy.

## The H<sub>2</sub>S/CSE System as a Target for Pharmacotherapy

The anti-inflammatory and insulin-sensitizing properties, the protective effects on mature adipocytes, and the beneficial effects on vascular tone of H<sub>2</sub>S suggest that increasing H<sub>2</sub>S signaling could be a potential new therapeutic strategy for the treatment of diabetes. The application of H<sub>2</sub>S donors and the augmentation of endogenous H<sub>2</sub>S has



**Figure 6** The mechanisms of adipocytes apoptosis in obese adipose tissue and the anti-apoptosis effect of H<sub>2</sub>S.

attracted the most attention as possible therapeutic approaches for diabetes, even if reducing H<sub>2</sub>S could be protective in some instances. However, the available CBS and CSE inhibitors have limited membrane permeability and are not very specific. In addition, there is no 3-MST inhibitor available. CBS, CSE or 3-MST knockout mice exhibit many abnormalities, which precludes considering such an approach in humans. Therefore, in the subsequent section, we will focus on only the approaches aimed at increase H<sub>2</sub>S signaling.

In most studies on H<sub>2</sub>S, inorganic sulfide salts such as sodium hydrosulfide (NaHS) and sodium sulfide (Na<sub>2</sub>S) were used, as they increase the H<sub>2</sub>S concentration rapidly and in the short term.<sup>108</sup> NaHS may activate K<sup>+</sup><sub>ATP</sub> channels, protein kinase B/Akt or Nrf2-induced signalling<sup>109</sup> to exert its protective effects. To overcome the limitations of sulfide salts, several organic, slow-releasing H<sub>2</sub>S compounds have been synthesized, with GYY4137 being the most popular; however, they may possess H<sub>2</sub>S-independent activities mediated by the parent compound.<sup>110</sup> LC is widely used in experimental studies to augment endogenous H<sub>2</sub>S production. However, LC is not a good H<sub>2</sub>S precursor because it is metabolized in many other pathways. In recent years, a new group of H<sub>2</sub>S donors has been developed. This group consists of a traditional NSAID that has been structurally modified to release H<sub>2</sub>S (S-NSAID). S-NSAIDs possess anti-inflammatory effects and are potentially protective for digestive and circulatory systems.<sup>108</sup> Garlic, originally grown in central Asia, has many medicinal properties based on its antibacterial, antifungal, and antiviral activities and usually acts through the

cardiovascular system. Garlic contains approximately 2,000 active compounds, including various sulfur-containing chemicals that may release H<sub>2</sub>S. It reduces blood pressure, suppresses blood platelet aggregation and has anti-atherosclerotic properties.<sup>111</sup> Moreover, garlic lowers cholesterol. Nevertheless, the development of H<sub>2</sub>S donors is critical for understanding the biological functions of H<sub>2</sub>S. There will be more interesting work arising in this field.

## Conclusions and Future Perspectives

The altered expression of H<sub>2</sub>S-synthesizing enzymes, as well as endogenous H<sub>2</sub>S levels, were observed in diabetic and obese animals. H<sub>2</sub>S is synthesized in AT and participates in the regulation of adipose tissue metabolism and function (Table 2). Although increasing research been performed in this field, some effects of H<sub>2</sub>S on AT, such as its role in the regulation of insulin sensitivity, are controversial, and its roles in adipokine secretion and adipocyte apoptosis have been incompletely demonstrated. Moreover, the mechanisms through which H<sub>2</sub>S acts on AT metabolism, such as glycolipid metabolism, have not been fully clarified; therefore, much work remains to be performed. Because of these controversies, the notion of using H<sub>2</sub>S donors or enhancing H<sub>2</sub>S signaling to alter AT dysfunction in common metabolic pathologies should be treated with caution.

**Table 2** Effects of H<sub>2</sub>S on Adipose Tissue/Adipocyte Pathophysiological Functions

Adipose Tissue/Adipocyte Pathophysiological Function	Effects of H <sub>2</sub> S	References
Inflammation	Anti-inflammation	41,42,112
Insulin resistance	Ameliorate insulin resistance	53,54,56,57,64
	Exacerbate insulin resistance	21,52
Lipogenesis/lipolysis	Promote lipogenesis	62–64
	Reduce lipolysis	57,64
Vascular tone	Anti-contractile	22,71,73–75
	Pro-contractile	75
Adipokines	Abnormal secretion	41
Apoptosis	Anti-apoptosis	107

## Data Sharing Statement

All data included in this study are available upon request by contact with the corresponding author.

## Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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## Disclosure

The authors report no conflicts of interest in this work.

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