



Hydrogen sulfide regulates insulin secretion and insulin resistance in diabetes mellitus, a new promising target for diabetes mellitus treatment? A review

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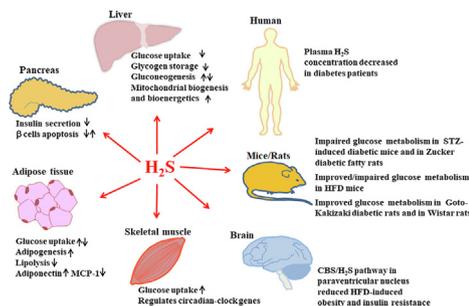
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GRAPHICAL ABSTRACT



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ABSTRACT

Background: Insulin resistance and impaired insulin secretion lead to disorders of glucose metabolism, which contributes to the development of diabetes. Hydrogen sulfide (H₂S), a novel gasotransmitter, is found to play important roles in regulation of glucose metabolism homeostasis.

Aim of Review: This study aimed to summarize and discuss current data about the function of H₂S in insulin secretion and insulin resistance regulation as well as the underlying mechanisms.

Key Scientific Concepts of Review: H₂S could be endogenously produced in islet β cells, liver, adipose, skeletal muscles, and the hypothalamus, and regulates local and systemic glucose metabolism. It is reported that H₂S suppresses insulin secretion, promotes or reduces the apoptosis of islet β cells. It plays important roles in the regulation of insulin sensitivity in insulin responsive tissues. H₂S inhibits glucose uptake and glycogen storage, and promotes or inhibits gluconeogenesis, mitochondrial biogenesis and mitochondrial bioenergetics in the liver. In adipose tissue, several investigators indicated that H₂S promoted glucose uptake in adipocytes, while other studies reported that H₂S inhibits this process. H₂S has also been shown to promote adipogenesis, inhibit lipolysis, and regulate adiponectin and MCP-1 secretion from adipocytes. In skeletal muscle, H₂S increases glucose uptake and improves insulin sensitivity. It is also observed that H₂S modulates circadian-clock genes in muscle. Hypothalamic CBS/H₂S pathway reduces obesity and

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improves insulin sensitivity via the brain-adipose interaction. Most studies indicated plasma H₂S levels decreased in diabetic patients. However, the mechanisms by which H₂S regulates systemic glucose metabolism remain unclear. Whether H₂S acts as a new promising target for diabetes mellitus treatment merits further studies.

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Introduction

Diabetes mellitus is increasing in prevalence and has emerged as a global health challenge [1,2]. Insulin resistance is an abnormal state with the impaired sensitivity of tissues, such as skeletal muscle, adipose and liver tissues, to the action of insulin. This condition is compensated by hyperinsulinemia resulting from pancreatic β -cell dysfunction and contributes to the development of diabetes [3–5]. The pathogenesis of insulin resistance and β -cells dysfunction remain important scientific issues. Studies showed that the novel gaseous signaling molecule, hydrogen sulfide (H₂S), could be produced in insulin-synthesizing pancreatic β cells and in target organs of insulin, such as liver, adipose and muscle [6–10]. Plasma H₂S concentration is decreased in diabetic subjects compared with the control ($45.1 \pm 15.5 \mu\text{mol/L}$ versus $54.0 \pm 26.4 \mu\text{mol/L}$ reported by Suzuki et al.; $10.5 [4.8, 22.0] \mu\text{mol/L}$ versus $38.9 [29.7, 45.1] \mu\text{mol/L}$ expressed in median plasma H₂S concentration [25th, 75th percentiles] reported by Whiteman et al.) [11–13]. Moreover, H₂S is found to regulate insulin sensitivity and insulin secretion [10]. 10–100 $\mu\text{mol/L}$ H₂S donor NaHS that may produce about 3–35 $\mu\text{mol/L}$ H₂S has important physiological effects on insulin-dependent metabolism, while 200–1000 μM NaHS that possibly releases about 70–350 $\mu\text{mol/L}$ H₂S leads to toxic effects [14–16]. Therefore, regulating H₂S level has emerged as a promising strategy in the treatment of diabetes mellitus. Herein, we have summarized and discussed current data about the function of H₂S in insulin secretion and insulin resistance regulation as well as the underlying mechanisms.

Endogenous production and elimination of H₂S

H₂S is colorless, characterized by a rotting egg odor, transparent and water-soluble. It can be generated from L-cysteine and/or homocysteine catalyzed by two enzymes, cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE) (Fig. 1). Production of H₂S from 3-mercaptopyruvate is catalyzed by 3-mercaptopyruvate sulfurtransferase (3-MST) [6]. Cysteine aminotransferase (CAT) could also catalyze H₂S generation in several tissues. The distribution of these enzymes is tissue specific. H₂S is mainly produced via CSE in the pancreas, adipose tissue, liver, cardiovascular system and respiratory system, while H₂S production in the central nervous system is predominantly catalyzed by CBS. In order to maintain the physiological levels of H₂S, the production and elimination of H₂S are precisely regulated and controlled. Oxidation, methylation, scavenging and expiration are responsible for H₂S elimination. For example, H₂S is oxidized in mitochondria to thiosulfate, then to sulfite or sulfate, and ultimately excreted in urine by the kidney. H₂S methylation occurs mainly in the cytosol. H₂S can also be scavenged by methemoglobin and other proteins and exhaled through the lung.

H₂S in islet β cells

Endogenous H₂S production in islet β -cells

An endogenous generation of H₂S via CBS and/or CSE was identified in pancreatic β -cells from several species (Table 1). A

different H₂S dominant synthase was identified in the pancreatic β cells depending on the species. A small amount of CBS was expressed by HIT-T15 Syrian hamster pancreatic β -cells with no CSE mRNA transcripts shown [17]. Both CBS and CSE were detected in mouse pancreases, mouse and rat pancreatic islets and MIN6 mouse islet β cells [18–21]. INS-1E rat insulinoma cells expressed CSE [22]. CSE inhibition by CSE siRNA or DL-propargylglycine (PPG) significantly depleted H₂S production from cultured INS-1E cells [22]. PPG also significantly reduced H₂S production in rat pancreatic islets [18]. These results suggested that CSE was the dominant enzyme catalyzing H₂S production in INS-1E rat insulinoma cell and in rat islet β cell. The distribution patterns of the two enzymes in the mouse pancreas are not identical. In immunohistochemistry results of the pancreas taken from male ICR mice, CBS existed in pancreatic exocrine and endocrine islet cell cytoplasm. CSE was primarily expressed in exocrine cell cytoplasm and showed faint levels in the islets [20]. No protein expression of 3-MST was detected by Western blot in mouse pancreases [21], but the contribution of 3-MST to H₂S production in pancreatic β -cells from other species has not been investigated. These studies indicated that H₂S dominant synthases in pancreatic β cells are species-specific.

Hyperglycemia regulates endogenous H₂S in pancreatic β -cells

How high glucose regulates CSE/H₂S pathway is controversial. High glucose (20 mmol/L) was found to suppress H₂S production in INS-1E cells by downregulating CSE promoter activity and mRNA level, as glucose activates p38-MAPK/specificity protein 1 (SP1) pathway [22,23] (Fig. 2). Insulin secretion was promoted in high glucose-stimulated INS-1E cells [22], indicating that high glucose (20 mmol/L)-induced H₂S/CSE inhibition might lead to stimulating a long-term insulin release. On the contrary, high glucose (10 or 20 mmol/L) was observed to induce H₂S production in MIN6 cells by upregulating CSE expression. Similar phenomenon occurred in mouse islets [20]. The difference of the effect of glucose on H₂S/CSE pathway between mouse pancreatic β -cells and INS-1E cells may be explained in part by different glucose levels during the pre-incubation period before treatment, different H₂S responses based on animal species or cell types, and other experimental conditions. Therefore, further studies will be needed to explore the effect of hyperglycemia on endogenous H₂S in pancreatic β -cells and its mechanisms.

H₂S inhibits insulin secretion from pancreatic β -cells

Absolute or relative deficiency of insulin secretion caused by the loss or dysfunction of pancreatic β -cells contributes to the occurrence and development of diabetes mellitus. Several studies investigated the effects of H₂S on insulin secretion by pancreatic β -cells (Table 2).

Wu et al. [18] demonstrated that compared with control rats, impaired insulin secretion, reduced serum insulin level, elevated pancreatic CSE protein levels and H₂S production, hyperglycemia and insulin resistance were showed in Zucker diabetic fatty (ZDF) rats. Intraperitoneal injection of PPG to inhibit CSE activity and pancreatic H₂S production could restore normal pancreatic insulin

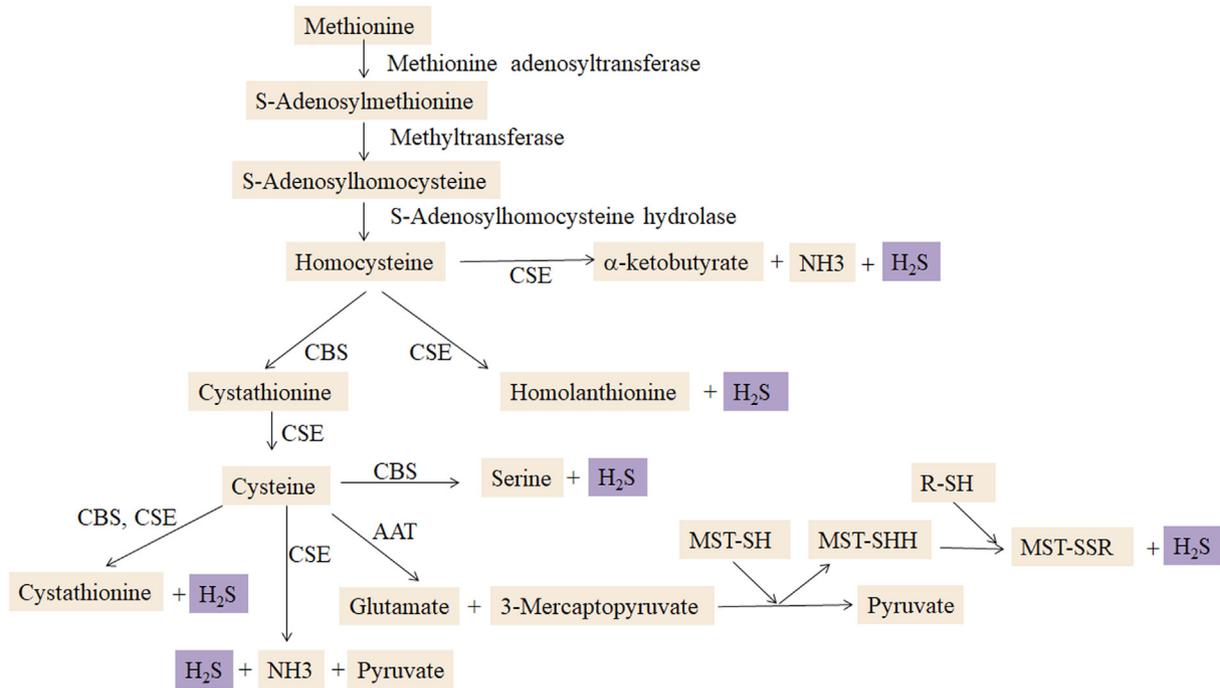


Fig. 1. Endogenous H₂S generation in mammals. H₂S, hydrogen sulfide; CSE, cystathionine γ -lyase; CBS, cystathionine β -synthase; 3-MST, 3-mercaptopyruvate sulfurtransferase; AAT, aspartate aminotransferase.

Table 1
Expression of H₂S-synthesizing enzymes in pancreases and islet β cells.

H ₂ S-synthesizing enzyme	Expression	Cells/ Model	Refs.
CBS	mRNA and protein were detected (low levels)	HIT-T15 cells	[17]
	mRNA was detected (low levels)	Rat pancreatic islets	[18]
	mRNA was detected	MIN6 cells	[19]
	mRNA and protein were detected	Mouse pancreatic islets	[19]
	mRNA and protein were detected (mRNA was moderately expressed)	Mouse pancreatic islets	[20]
CSE	protein was detected	Mouse pancreases	[20,21]
	No mRNA expression was detected	HIT-T15 cells	[17]
	mRNA and protein were detected (H ₂ S dominant synthase)	INS-1E cells	[22]
	mRNA and protein were detected (H ₂ S dominant synthase)	Rat pancreatic islets	[18]
	mRNA was detected	MIN6 cells	[19]
	mRNA was detected	Mouse pancreatic islets	[19]
3-MST	mRNA and protein were detected (low levels)	Mouse pancreatic islets	[20]
	protein was detected	Mouse pancreases	[20,21]
	No protein expression was detected	Mouse pancreases	[21]

secretion, upregulate the serum insulin, and improve high blood glucose as well as decrease hemoglobin A1c (HbA1c) in ZDF rats. These findings indicated that abnormally increased pancreatic

H₂S production might lead to the occurrence of impaired insulin secretion in diabetes. After the overexpression of CSE with adenovirus to increase H₂S production in INS-1E cells, insulin secretion at low glucose (5 mmol/L) did not change, whereas insulin secretion induced by high glucose (16 mmol/L) was significantly reduced [22]. Similar effects were observed in Syrian hamster pancreatic β -cells (HIT-T15) treated with exogenous (sodium hydrogen sulfide, NaHS) or endogenous (CBS overexpression) H₂S [17]. Additionally, NaHS inhibited high glucose-induced insulin release by mouse pancreatic islets and MIN6 cells. However, NaHS failed to regulate insulin release induced by 3 mmol/L glucose [19]. High glucose caused more insulin release by freshly isolated pancreatic islets of CSE-deficient mice than that by littermate wild-type mouse islets [24]. H₂S donor suppressed high glucose-induced insulin release in wild-type islets [24]. These data suggest that both endogenous H₂S generated from pancreatic β -cells and exogenous H₂S inhibit insulin secretion in the presence of high glucose, while the inhibition of pancreatic H₂S synthesis promotes insulin secretion, suggesting that abnormal H₂S synthesis is the cause and impaired insulin secretion is the effect.

Such inhibition involves several mechanisms as follows (Fig. 2). First, H₂S decreases insulin release in islet β -cells via ATP-sensitive potassium (K_{ATP}) channels activation [17,18,22]. H₂S at 100 μ mol/L elevated K_{ATP} channel currents in islet β -cells from both ZDF and Zucker lean (ZL) rats, which were abolished by gliclazide, a K_{ATP} channel antagonist. Under resting conditions, K_{ATP} currents intensity was lower in ZDF islet β cells than in ZL β cells [18]. Patch-clamp whole-cell recording assay showed that treated INS-1E cells with high glucose (16 mmol/L) decreased K_{ATP} currents compared with cells by 5 mmol/L glucose treatment. H₂S (100 μ mol/L) application of INS-1E cells did not affect whole-cell K_{ATP} currents induced by 5 mmol/L glucose, but markedly upregulated K_{ATP} channel currents stimulated by 16 mmol/L glucose. Application of PPG or CSE siRNA to lower the cell H₂S content reduced K_{ATP} currents, which was abolished by H₂S donor supplementation. It was also found that H₂S facilitated K_{ATP} channel activity in INS-1E cells by upregulating single-K_{ATP}-channel open probability, but not the

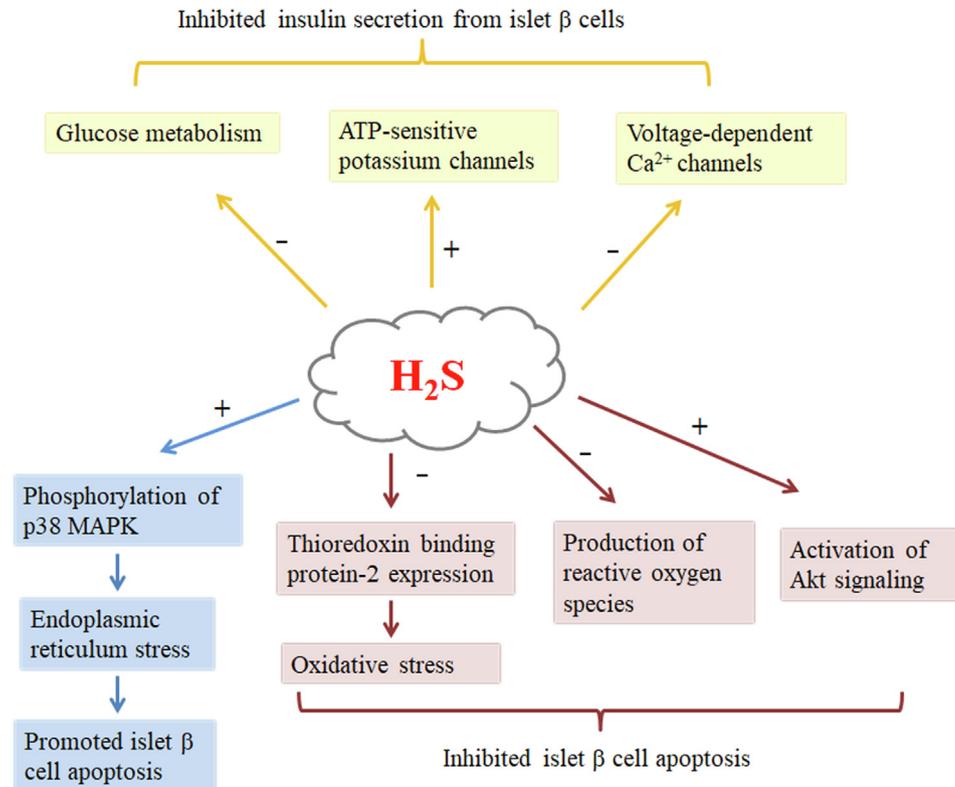


Fig. 2. H₂S inhibits insulin secretion and regulates apoptosis of islet β cells. H₂S inhibits insulin secretion from islet β cells through stimulating of K_{ATP} channels, inactivating VDCC and suppressing glucose metabolism. H₂S promotes islet β cell apoptosis through phosphorylation of p38 MAPK and subsequent enhances ER stress. H₂S inhibits islet β cell apoptosis through reducing thioredoxin binding protein-2 expression, inhibiting reactive oxygen species production and activating Akt signaling pathway. + indicates increase, – indicates inhibition.

Table 2
H₂S regulates insulin secretion and islet β cells apoptosis.

Action	Cells/Model	H ₂ S gas/donor application (concentration)	Effects	Refs.
Insulin secretion <i>In vitro</i> study	INS-1E cells	H ₂ S gas (100 μM)	Inhibited	[22]
	HIT-T15 cells	NaHS (100 μM)	Inhibited	[17]
	MIN6 cells	NaHS (10, 100 and 1000 μM)	Inhibited	[19]
	Isolated mouse islets	NaHS (100 and 300 μM)	Inhibited	[24]
	Isolated mouse islets	NaHS (100 and 1000 μM)	Inhibited	[19]
<i>In vivo</i> study	Zucker diabetic fatty rats	–	Inhibited	[18]
	Nonfasting wild-type mice	NaHS (39 μmol/kg)	Inhibited	[26]
Islet β cell apoptosis <i>In vitro</i> study	INS-1E cells	H ₂ S gas (100 μM)	Promoted	[25]
	Isolated mouse islets	NaHS (100 μM)	Inhibited	[20]
	Isolated mouse islets	NaHS (100 μM)	Inhibited	[28]
	MIN6 cells	NaHS (100 μM)	Inhibited	[28]
	<i>In vivo</i> study	Streptozotocin-induced diabetic mice	–	Promoted
High fat diet-induced obese mice		–	Inhibited	[21]

open time of single channels, single-channel conductance, linear current–voltage relation of single-channel currents, or ATP sensitivity of K_{ATP} channels. The results suggest that H₂S directly acts on K_{ATP} channel proteins [22]. Similarly, treatment of HIT-T15 cells with H₂S decreased intracellular K⁺ concentration, indicating that H₂S opens cell K⁺ channel to cause K⁺ outflow. Following K⁺ outflow, the HIT-T15 cell membrane was hyperpolarized by NaHS (100 μmol/L) treatment. This caused the voltage-gated Ca²⁺ channels to shut down, which in turn prevented Ca²⁺ from flowing into cells and lowered intracellular Ca²⁺ ion concentration. Glibenclamide, a K_{ATP} channel antagonist, reversed these effects and the inhibition of H₂S on insulin release [17]. These results demonstrate

that H₂S-activated K_{ATP} channels act as an important mechanism by which H₂S inhibits insulin secretion.

Several studies reported that diazoxide, an activator of K_{ATP} channels, could not completely abolish the inhibitory effect of NaHS on insulin release, suggesting that the underlying mechanism involves a K_{ATP} channel-independent pathway [19]. Kaneko *et al.* found that H₂S inhibited glucose-stimulated [Ca²⁺]_i oscillation in mouse pancreatic β-cells [19]. The H₂S donors NaHS and ACS67 decreased L-type voltage-dependent Ca²⁺ channel (VDCC) currents in the wild-type islet β-cells, whereas an L-type VDCC antagonist nifedipine inhibited it [24]. Moreover, the inhibitory effect of NaHS on L-type VDCCs was reversible. NaHS administration delayed the

channel recovery from depolarization-caused inactivation but failed to alter the channel current–voltage relationship curve. Application of CSE inhibitor PPG to mouse islet β -cells promoted the basal activity of L-type VDCCs. Moreover, CSE-deficient islet β cells showed higher density of L-type VDCC current than that in wild-type β -cells. Nifedipine co-application enhanced the inhibitory role of NaHS in insulin secretion. L-type VDCC activator Bay K-8644 increased insulin secretion stimulated by high glucose, which was counteracted by NaHS or nifedipine addition [24]. These results demonstrate that H₂S reduces insulin secretion at least partially by inhibiting L-type VDCCs in islet β -cells. Therefore, NaHS suppresses insulin release through K_{ATP}-channel-dependent mechanisms and -independent mechanisms. K_{ATP} channels activated by H₂S resulted in islet β cell hyperpolarization and decreased L-type VDCC activity, suggesting K_{ATP}-channel-dependent mechanisms. Data demonstrating that H₂S directly suppressed L-type VDCC suggest K_{ATP}-channel-independent mechanisms involved in H₂S action [24] (Fig. 2).

H₂S regulates apoptosis of islet β -cells

Impaired islet β -cell survival arising from an imbalance between cell proliferation and death constitutes one of the major pathogenic factors for diabetes. The pattern of islet β -cell death in the development of diabetes is mainly apoptosis. H₂S regulation of islet β -cell apoptosis is controversial (Table 2). Endogenously produced H₂S by CSE overexpression or exogenously produced H₂S (100 μ mol/L, within physiologically relevant concentration [15]) incubation for 12 h induced INS-1E cell apoptosis, which may not be associated with the changed redox status [25]. The study further indicated that H₂S increased the phosphorylation of p38-MAPK, and then enhanced endoplasmic reticulum (ER) stress, demonstrated by increased expression of Bip, CHOP and SREBP-1c, thus inducing apoptosis of β -cell. (Fig. 2). The phenomenon of H₂S-induced INS-1E cell apoptosis suggests a suppressive effect of H₂S on insulin secretion by decreasing β -cell mass. Type 1 diabetes was formed in wild-type mice 24 days after the first intraperitoneal administration of streptozotocin (STZ), while CSE knockout delayed it. CSE inhibitor PPG treatment protected against hypoinsulinemia and hyperglycemia in mice subjected to STZ. Moreover, STZ markedly promoted CBS mRNA expression, H₂S generation and islet β cell apoptosis in pancreatic tissue of wild-type mice, while CSE knockout partly abolished STZ-induced islet β -cell apoptosis [26,27]. Treatment of INS-1E cells with STZ increased H₂S generation and decreased the viability of the cells, while PPG co-treatment partially reversed the decreased cell viability. Mechanistically, STZ induced K_{ATP} channel currents in wild-type islet β cells, which was blocked by PPG treatment or CSE knockout. Glucose intolerance, hyperglycemia as well as low insulin secretion were observed in the wild-type mice with NaHS administration [26]. Collectively, the results demonstrated that the H₂S produced by CSE accelerated diabetes in STZ mice through inducing β -cell apoptosis and activating K_{ATP} channels. Inhibition of the CSE/H₂S pathway is thought to be a novel strategy for protection against diabetes mellitus.

In contrast, several studies reported a protective role of H₂S in regulating pancreatic β -cell apoptosis. NaHS (100 μ mol/L) inhibited high glucose (20 mmol/L)-stimulated cell apoptosis and elevated the glutathione concentration in mouse islet β cell line MIN6 [20,28], indicating that H₂S might protect pancreatic β -cells from glucotoxicity. The inhibitory effect of H₂S on pancreatic β -cell apoptosis was also confirmed in cultured islets [28]. Interestingly, H₂S suppressed fatty acid-, cytokines- or H₂O₂-induced β -cell apoptosis, while H₂S did not prevent mouse islets and MIN6 cells from apoptosis stimulated by thapsigargin and tunicamycin, both of which led to ER stress. It indicates that the anti-oxidative

capacity and the activation of Akt signaling but not ER stress may be involved in H₂S-mediated prevention from β -cell apoptosis (Fig. 2). In addition, Thioredoxin binding protein-2 (TBP-2) plays an important role in β -cell apoptosis and the onset and development of diabetes. TBP-2 expression was increased in the pancreas from high fat diet (HFD)-fed CSE-deficient mice which showed elevated β -cell apoptosis, decreased pancreatic insulin contents, and glucose intolerance [21]. The administration of the H₂S donor NaHS abolished 20 mmol/L glucose exposure-enhanced TBP-2 expression in CSE knockout pancreatic β cells. These data suggested that the CSE/H₂S pathway protected against the occurrence of HFD-induced diabetes mellitus by inhibiting TBP-2-mediated β -cell apoptosis (Fig. 2). However, CSE knockout delayed the onset of STZ-induced type 1 diabetes, decreased hypoinsulinemia and hyperglycemia, and reduced islet β -cell apoptosis [26]. These findings put forward the possibility that H₂S induces apoptosis of extremely impaired β -cells and inhibits apoptosis of not extremely impaired β -cells. While, the effects of the endogenous H₂S on β -cell functions may depend on the age or stage/type of diabetes.

Hydrogen sulfide regulates insulin sensitivity

Insulin resistance acts as a fundamental pathology of type 2 diabetes [5]. Insulin resistance means that the responsiveness of target tissues to insulin is reduced, leading to reduced glucose uptake to skeletal muscle and adipose and elevated hepatic glucose output. Previous studies have shown that H₂S is important for regulating insulin sensitivity in the liver, adipose and skeletal muscle.

H₂S and liver glucose metabolism

Hepatic insulin resistance refers to the failure of insulin to inhibit glycogenolysis and gluconeogenesis in the liver to maintain normal plasma glucose levels [5,29]. The enzymes responsible for endogenous H₂S, CSE, CBS, and 3-MST are found in the liver and contribute to liver H₂S production. The effect of diabetes mellitus and its related conditions on H₂S production system in the liver is controversial. Compared with non-diabetic rats, H₂S production and CSE and CBS mRNA levels in the liver were increased in STZ diabetic rats, while insulin treatment reversed these effects [27]. Alternatively, H₂S formation and CSE activity and protein expression in the liver were suppressed in STZ-induced type 1 diabetic rats [30]. Livers taken from insulin sensitizer metformin-treated SJL mice (100 mg/kg b.w. per day) exhibited an increased H₂S concentration [31]. Further study will be needed to explore the regulatory mechanism of diabetes mellitus and its related conditions on the production of H₂S in the liver.

H₂S regulates glucose uptake, glycogen storage and gluconeogenesis

H₂S function in liver glucose metabolism has been studied extensively (Table 3). One view is that H₂S suppresses glucose uptake and glycogen storage but promotes gluconeogenesis. NaHS (10, 30, and 100 μ mol/L) inhibited glucose uptake and impaired glycogen storage in HepG2 cells by inhibiting glucokinase activity [32]. CSE overexpression decreased glycogen content in HepG2 cells, whereas CSE knockout increased glycogen content in liver tissues. Primary hepatocytes from CSE knockout mice showed increased glucose consumption but reduced glucose production via gluconeogenesis and glycogenolysis pathways. H₂S promoted the rate of gluconeogenesis and increased glycogenolysis in HepG2 cells, while CSE-deficient hepatocytes showed a decreased rate of gluconeogenesis and glucose release. The research of Untereiner et al. also confirmed that H₂S increased the hepatic glucose production and gluconeogenesis [33].

Table 3
H₂S regulates glucose uptake, glycogen storage, gluconeogenesis and mitochondrial function in liver.

Action	Cells/Model	H ₂ S gas/donor application (concentration)	Effects	Refs.
Glucose uptake <i>In vitro</i> study	HepG2 cells/primary mouse hepatocytes	NaHS (10, 30 and 100 μM)/–	Inhibited	[32]
Glycogen storage <i>In vitro</i> study <i>In vivo</i> study	HepG2 cells	NaHS (10, 30 and 100 μM)	Inhibited	[32]
	Liver from mice under nonfasting or 6-h fasting condition	–	Inhibited	[32]
Gluconeogenesis <i>In vitro</i> study <i>In vivo</i> study	HepG2 cells/primary mouse hepatocytes	NaHS (10, 30 and 100 μM)/–	Promoted	[32]
	Primary mouse hepatocytes	NaHS (30 μM)	Promoted	[33]
	HepG2 cells/primary mouse hepatocytes	NaHS (50 μM)/–	Promoted	[34]
	HepG2 cells/primary mouse hepatocytes	NaHS (100 μM)/–	Inhibited	[35]
	Pyruvate tolerance test on overnight-fasted mice Pyruvate tolerance test on high fat diet-fed mice	NaHS (39 and 63 μM/kg) NaHS (50 μM/kg/day)	Promoted Inhibited	[33] [35]
Mitochondrial function Biogenesis Bioenergetics	Primary mouse hepatocytes	NaHS (30 μM)	Promoted	[38]
	HepG2 cells	NaHS (0.01 and 0.1 μM)	Promoted	[39]

Mechanistically, the fact that H₂S attenuated AMP-activated protein kinase (AMPK) activation was involved in H₂S-induced inhibition of glucose uptake by HepG2 cells. Further study indicated that H₂S promoted glucose production via upregulated phosphoenolpyruvate carboxykinase (PEPCK) activity but decreased glucokinase (GK) activity [32]. In addition, H₂S-induced upregulation of peroxisome proliferator-activated receptor-γ coactivator (PGC)-1α and phosphoenolpyruvate carboxykinase expression through the glucocorticoid receptor (GR) pathway, upregulation of PGC-1α expression through the cAMP/PKA pathway, and the activation and upregulation of PGC-1α S-sulfhydration may explain, at least in part, CSE-generated H₂S regulation of hepatic glucose production [33]. And H₂S upregulates FBPase and G6Pase expression might contribute mechanistically as well (by sulfhydration). Furthermore, H₂S sulfhydrated pyruvate carboxylase at cysteine 265, and increased its activity, thus promoting gluconeogenesis in hepatocytes [34]. These results demonstrated that H₂S in the liver reduced glucose uptake and promoted glucose output, contributing to hyperglycemia and insulin resistance. Downregulation of hepatic H₂S synthesis or CSE activity is a potential approach to preventing the development of insulin resistance.

In contrast, another view is that H₂S inhibits hepatic gluconeogenesis. Guo *et al.* [35] reported that CSE deficiency promoted hepatic gluconeogenesis. Knockdown of CSE in HepG2 cells increased glucose production under insulin stimulation, while NaHS (100 μM) attenuated CSE knockdown-induced glucose production. Furthermore, CSE knockout inhibited insulin and AMPK signaling pathways and increased nuclear accumulation of Forkhead box protein O1 (FoxO1), thus promoting hepatic gluconeogenesis. But NaHS treatment reversed the effects. CSE knockout mice exhibited exacerbated HFD-induced obesity and insulin resistance, while administration of NaHS to wild-type mice alleviated HFD-caused metabolism disorders. These data suggest that H₂S inhibits hepatic gluconeogenesis through a FoxO1-dependent mechanism, thus attenuating HFD-induced insulin resistance.

H₂S regulates hepatic mitochondrial biogenesis and mitochondrial bioenergetics

The mitochondria are crucial in regulating energy homeostasis, β-oxidation of fatty acids, and reactive oxygen species (ROS) production. Mitochondrial dysregulation occurs in insulin resistance [36,37]. The amount of mitochondria was decreased in CSE-deficient hepatocytes compared with control [38]. NaHS treatment restored the mitochondrial content in CSE-knockout hepatocytes.

The expressions of NRF-1, NRF-2, Tfam and peroxisome proliferator-activated receptor-γ coactivator-related protein (PPRC) were each downregulated in CSE-knockout hepatocytes. NaHS incubation increased PPRC levels but reduced PGC-1β expression in mouse hepatocytes. NaHS induced PPRC sulfhydration, while CSE knockout reduced it. However, the sulfhydration of PGC-1β was not detected. PGC-1α silencing reduced H₂S donor-induced hepatic mitochondrial biogenesis. The same phenomenon was observed in PPRC-silence hepatocytes. The effect of H₂S donor-stimulated mitochondrial biogenesis was completely blocked by knockdown of both PGC-1α and PPRC genes in wild-type and CSE-knockout hepatocytes. These data suggested that the endogenous H₂S-stimulated hepatic mitochondrial biogenesis through PGC-1α and PPRC pathway [38]. NaHS sulfhydrated ATP synthase alpha subunit at cysteine 244 and 294, and activated this enzyme, thereby stimulating mitochondrial bioenergetics in HepG2 cells and in the liver [39] (Table 3).

Hydrogen sulfide and adipose tissue function

Adipose tissue serves as the main energy storage organ. Adipocytes uptake and store much triglycerides under the energy sufficiency conditions, but consume them during fasting or energy insufficiency conditions. During fasting or stress conditions, triglycerides undergo lipolysis by lipases including adipocyte triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and monoglyceride lipase (MGL). The products of triglyceride hydrolysis, glycerol and free fatty acids (FFA), are released into the blood and taken up by other tissues. For example, FFA is transported to skeletal muscles and heart to provide energy for these organs. They serve as energy substrates while glycerol is taken up by the liver. Adipose tissue is also thought to be an active endocrine organ. Cytokines and FFA released from adipocytes regulate systemic metabolic process. Several adipokines are produced and released from adipocytes, such as leptin, adiponectin and resistin. Maintaining normal adipose tissue function helps to maintain systemic metabolic homeostasis. Adipose tissue dysfunction is an important cause of metabolic diseases, such as diabetes, obesity and atherosclerosis [40,41]. How H₂S modulates adipose tissue function is discussed below (Table 4).

Hydrogen sulfide exists in adipose tissues

H₂S was first reported in 2009 to be produced in rat adipose tissues, such as epididymal adipose, perirenal adipose, and brown

Table 4H₂S regulates glucose uptake, adipogenesis and lipolysis in adipocytes.

Action	Cells/Model	H ₂ S gas/donor application (concentration)	Effects	Refs.
Glucose uptake				
3T3-L1 adipocytes		H ₂ S gas (10, 25 and 50 μM)	Promoted	[45]
3T3-L1 adipocytes		Na ₂ S (10 and 100 μM)	Promoted	[44]
3T3-L1 adipocytes		NaHS (25, 50 and 100 μM)	Promoted	[45]
3T3-L1 adipocytes		NaHS (100 μM)	Promoted	[43]
3T3-L1 adipocytes		NaHS (100, 500 and 1000 μM)	Inhibited	[48]
Adipogenesis				
3T3-L1 adipocytes		H ₂ S gas (100 μM)	Promoted	[43]
3T3-L1 adipocytes		NaHS (30 μM)	Promoted	[50]
3T3-L1 adipocytes		NaHS (50 μM)	Promoted	[49]
3T3-L1 adipocytes		GY4137 (50 μM)	Promoted	[49]
3T3-L1 adipocytes		GY4137 (100 μM)	Promoted	[43]
Lipolysis				
3T3-L1 adipocytes		NaHS (50 μM)	Inhibited	[49]
3T3-L1 adipocytes		GY4137 (50 μM)	Inhibited	[49]
Primary rat adipocytes		GY4137 (1000 μM)	Inhibited	[57]
Adipokines or inflammatory factors secretion				
3T3-L1 adipocytes		NaHS (50 μM)	Attenuated high glucose-induced decrease in adiponectin and the increase in MCP-1 secretion	[58]
High fat diet-induced diabetic mice		SG-1002 (20 mg/kg/day)	Increased the plasma adiponectin level	[59]

adipose tissues [42]. RT-PCR analysis showed that epididymal adipose, perirenal adipose and brown adipose tissues all expressed the mRNA of CSE and CBS, the two H₂S-producing enzymes. CSE protein expression was also detected in these adipose tissues. After the adipose tissues were pretreated with the CSE inhibitor PPG, endogenous H₂S generation was decreased to less than 20%, indicating that CSE was a key enzyme to catalyze this gasotransmitter generation from adipose tissues. In addition, CSE/H₂S pathway existed both in pre-adipocytes and adipocytes [42,43]. The study also demonstrated that in rat visceral adipose, CSE/H₂S level increased with age [42].

H₂S regulates insulin sensitivity in adipose tissue

Several studies reported that H₂S directly regulated insulin sensitivity in adipocytes. H₂S improved high glucose-induced insulin resistance [44]. Manna *et al.* demonstrated that H₂S and its precursor L-cysteine upregulated insulin signaling pathways. They found that both H₂S and L-cysteine increased phosphatidylinositol 3,4,5-trisphosphate (PIP3), downstream phosphorylated Akt level as well as glucose uptake by cultured 3T3-L1 cells in the presence of 25 mmol/L glucose. H₂S mediated the effect of L-cysteine on PIP3 and glucose uptake. Furthermore, H₂S activated PI3K but inhibited PTEN. L-cysteine, H₂S or PIP3 upregulated Akt and PKCζ/λ phosphorylation, glucose transporter type 4 (GLUT4) activity, and glucose utilization in high glucose-stimulated cells. These results suggested that PIP3 participated in the effect of L-cysteine or of H₂S on glucose utilization. PKCζ phosphorylation was more effective than AKT2 for glucose uptake in L-cysteine-, H₂S-, and PIP3-incubated adipocytes incubated with high glucose. These results suggested that H₂S or L-cysteine improved glucose metabolism by upregulating the cellular PIP3 level, which was mediated by suppressing PTEN/NF-κB pathway as well as activating PI3K/PI3K/Akt/PKCζ/λ insulin signaling in adipocytes [44].

Xue *et al.* [45] indicated that both NaHS and H₂S gas solution treatment significantly increased glucose uptake in adipocytes. Application of NaHS to cultured adipocytes incubated with 5.5 mM glucose or 25 mM glucose increased phosphorylated insulin receptor (p-IR), p-PI3K and p-Akt levels. Insulin receptor (IR) knockdown abolished the effect of NaHS, which indicated that IR mediated the promotion of H₂S on glucose utilization. Further-

more, H₂S donor activated IR in purified IR protein. Chronic treatment of NaHS (30 μmol/kg/day) was found to improve insulin resistance in Goto-Kakizaki diabetic rats. This study suggested that NaHS/H₂S improved insulin resistance through activating the insulin receptor signaling pathway [45].

Adipogenesis participates in obesity and insulin resistance regulation [46]. CSE expression and H₂S generation were upregulated during the differentiation of 3T3L1 preadipocytes to mature adipocytes. Treatment with H₂S or upregulated CSE increased triglyceride deposition in adipocyte differentiation, while inhibiting CSE activity with PPG reduced intracellular triglyceride deposition. H₂S donor GY4137 inhibited PDE activity and promoted adipocyte differentiation. CSE/H₂S pathway upregulated PPARγ level in the process of adipocyte differentiation. H₂S sulfhydrated cysteine-139 in PPARγ, and then induced activation of PPARγ, which enhanced glucose uptake and lipid storage. Mutation of cysteine 139 abolished sulfhydration of PPARγ and upregulated PPARγ activity. *In vivo* experiments further confirmed that H₂S improved insulin resistance and promoted lipid accumulation by sulfhydrating PPARγ in high-fat-diet-induced obese mice. These data suggested that under physiological conditions or at the early stage of obesity, the CSE/H₂S pathway converted glucose into triglyceride storage in adipocytes by sulfhydrating PPARγ, upregulating its activity and maintaining systemic glucose metabolism homeostasis [43].

Manna *et al.* revealed that 1,25-dihydroxyvitamin D₃ [1,25-(OH)₂D₃] upregulated GLUT4 protein expression, GLUT4 translocation and glucose uptake inhibited by high glucose in 3T3L1 adipocytes. The 1,25-(OH)₂D₃-caused GLUT4 translocation and glucose uptake were dependent on 1,25-(OH)₂D₃-upregulated CSE activity and H₂S formation. Inhibiting CSE activation using PPG or knocking down CSE using siRNAs blocked the positive function of 1,25-(OH)₂D₃ upon GLUT4 activation and glucose uptake. These results indicated that CSE/H₂S pathway in adipocytes might mediate the improvement of 1,25-(OH)₂D₃ on glucose utilization impaired by high glucose in 3T3L1 adipocytes [47].

In contrast, Huang *et al.* reported that H₂S mediated tumor necrosis factor-α (TNF-α)-stimulated insulin resistance. The study showed that treatment with TNF-α increased H₂S concentration of cultures medium from 3T3-L1 adipocytes, and decreased glucose consumption and uptake. TNF-α also increased CSE protein expres-

sion and activity. CSE inhibitors PPG and β -cyano-L-alanine (BCA) attenuated TNF- α -induced H₂S generation in the cells, and improved TNF- α -caused impaired glucose consumption and uptake. Treatment with H₂S (100, 500 and 1000 mM) inhibited glucose consumption and uptake in adipocytes. The results indicated the H₂S mediated TNF- α -induced insulin resistance in adipocytes [48].

Above all, these studies suggested that H₂S directly regulated insulin sensitivity in adipocytes. How H₂S regulates insulin sensitivity in adipocytes needs to be further studied.

H₂S promotes adipogenesis in adipocytes

Adipogenesis is the process of conversion of preadipocytes into adipocytes, contributing to increasing numbers of adipocytes in adipose tissue and adipose tissue expansion [46]. Tsai *et al.* reported that H₂S promoted adipogenesis in 3T3L1 adipocytes [49]. They found that CSE, CBS and 3-MST levels were increased in the process of 3T3L1 cell differentiation. Treating 3T3L1 adipocytes during differentiation with GYY4137 or NaHS elevated the expression of adipogenesis-associated genes and increased lipid accumulation in adipocytes. Treating the cells with the CSE inhibitor, PPG, CSE small interference RNAs, the CBS inhibitor, aminooxyacetic acid, or CBS small interference RNAs decreased the expression of adipogenesis-related genes. Aminooxyacetic acid or PPG inhibited lipid droplet formation in mature adipocytes. These results indicated that H₂S promoted adipogenesis likely by increasing fatty acid binding protein 4 expression [49]. In addition, Cai *et al.* recently reported the effects of the CSE/H₂S pathway on lipid storage in adipocytes. They found that treatment with an H₂S donor or CSE overexpression sulfhydrated PPAR γ at cysteine 139 to induce PPAR γ activation, thereby promoting adipogenesis in adipocytes [43]. In accordance with these results, Yang *et al.* [50] indicated that CSE/H₂S levels increase in the process of adipocyte differentiation. H₂S sulfhydrated PPAR γ to induce its activation and promote adipogenesis. Studies in genetic mouse models also confirmed positive regulation of H₂S on adipogenesis. Lower body weight and less adipose tissue were observed in CSE knockout mice and CBS knockout mice [51].

H₂S inhibits lipolysis in adipocytes

Lipolysis causes triglyceride hydrolysis to release glycerol and FFA. Lipolysis increases in obesity and excessive FFA are released into the bloodstream, contributing to local oxidative stress, inflammation and ectopic fat deposition in target tissues, which leads to insulin resistance and diabetes [52–54]. Lipolysis is regulated by multiple endogenous factors, including catecholamines, insulin, and cytokines [55,56]. Studies have investigated whether H₂S regulates lipolysis. Geng and colleagues studied the effect of H₂S on lipolysis in adipocytes. The CSE inhibitor PPG elevated lipolysis, while H₂S precursor L-cysteine and H₂S donor GYY4137 inhibited lipolysis in adipocytes. PPG upregulated phosphorylation of PKA substrate and HSL as well as perilipin 1 expression, while H₂S donor GYY4137 reduced these protein expressions. Results from *in vivo* studies indicated that PPG upregulated lipolysis and GYY4137 suppressed adipose lipolysis in HFD-fed mice. However, both PPG and GYY4137 improved insulin sensitivity in HFD mice. These findings suggested the CSE/H₂S pathway inhibited lipolysis through PKA-perilipin/HSL signaling pathway and improved insulin sensitivity [57].

Lipolysis inhibition by H₂S was confirmed by Tsai *et al.* They reported that both GYY4137 and NaHS inhibited CL-316, 243 (β -adrenoceptor agonist)-stimulated lipolysis in mature 3T3-L1 adipocytes, while the CBS inhibitor AOAA or CSE inhibitor PPG pro-

moted it, suggesting that endogenous and exogenous H₂S inhibit lipolysis [49].

Role of H₂S in regulation of adipokines and inflammatory factors in adipose

Adipokines secreted from adipose tissue, like leptin and adiponectin, regulate systemic glucose and lipid metabolic homeostasis. Pan and colleagues found high glucose (25 mmol/L) reduced CSE expression in differentiated 3T3-L1 adipocytes. CSE overexpression or supplementation with NaHS abolished the elevated monocyte chemoattractant protein 1 (MCP-1) and reduced adiponectin secretion from mature 3T3-L1 adipocytes induced by high glucose. These findings suggested that inhibiting the CSE/H₂S pathway at least partly mediated the regulation of high glucose in adipocyte inflammation [58]. Barr found that in HFD-induced diabetic cardiomyopathy mice, HFD feeding induced lower levels of circulating H₂S and adiponectin. Treatment with an orally active H₂S donor, SG-1002, increased the plasma H₂S level and restored adiponectin levels. These results indicated that H₂S might increase adiponectin secretion from adipose tissue in HFD-induced diabetic mice [59]. Further studies in humans indicated the relationship between plasma H₂S and adiponectin levels. In 36 healthy volunteers, plasma H₂S levels positively correlated with the adiponectin concentration [60].

H₂S and skeletal muscle

Skeletal muscle serves as one of the chief insulin responsive organs and regulates systemic glucose metabolism. CBS and CSE are abundant in human skeletal muscles similar to their expressions in human kidney and liver [61–63]. CBS, CSE and 3-MST were detected in rat skeletal muscle, but their expressions were much lower than those in rat kidney and liver tissue [64]. The levels of three H₂S synthases as well as H₂S production capacity were also detectable in mouse skeletal muscle [65]. Skeletal muscles in both ovine and bovine expressed very little 3-MST. Therefore, the expression of the H₂S synthases in skeletal muscle varies between species.

H₂S might promote glucose uptake in the muscle. Administration of NaHS to L6 myotubes promoted glucose uptake and upregulated phosphorylated insulin receptor (IR), PI3K and AKT levels, which were blocked by the IR inhibitor [45]. Either PI3K inhibitor or IR knockdown attenuated NaHS-induced upregulation of glucose uptake. Moreover, NaHS could directly activate IR. Chronic NaHS administration improved insulin resistance accompanied by elevated phosphorylated PI3K and Akt levels in the muscles from Goto-Kakizaki (GK, a spontaneous type 2 diabetic model) diabetic rats. The same phenomenon was found in Wistar rats [45]. These results indicated that H₂S might promote glucose uptake in skeletal muscles through sensitizing the IR-PI3K-Akt signaling pathway, thus ameliorating insulin resistance. Moreover, the promotion of glucose uptake by H₂S was further confirmed in C₂C₁₂ mouse myotubes [66]. NaHS administration (10 or 20 μ M; 6 h) increased glutathione (GSH) biosynthesis, glucose uptake and utilization, but decreased ROS generation. In contrast, inhibition of endogenous H₂S in C₂C₁₂ myotubes by CSE knockdown reduced GSH biosynthesis, GLUT4 level and glucose uptake, but increased ROS generation [66]. Persistent hyperglycemia is one of the main causes of oxidative stress. NaHS treatment improved diaphragm contractility and ultrastructural damage in diabetic rat, which might be related to the inhibition of oxidative damage and cell apoptosis in diaphragmatic muscle [67]. These results indicated that H₂S might be crucial for maintaining glucose homeostasis in the muscle.

H₂S regulates circadian clock in the skeletal muscle. Disruption of core clock genes (CCG) contributes to the progression of metabolic disorders [68]. H₂S synthesis enzymes (CBS, CSE and 3-MST), antioxidant genes, CCG and clock-controlled genes were all decreased in muscle of HFD-induced mice, but markers of oxidative stress and expression of oxidative stress related genes were increased [65]. In cultured mouse myoblast cell line C₂C₁₂, high glucose or palmitate treatment decreased CSE/H₂S level and CCG expression, but increased oxidative stress. TNF and MCP-1 treatment displayed similar effects. Inhibiting CSE and glutamate cysteine ligase catalytic subunit (GCLC) with siRNA or pharmacological inhibitors decreased H₂S production, Bmal1 and Clock mRNA expression, and increased oxidative stress. Application of H₂S donor NaHS or GSH precursor improved expression of CCG [65]. These results suggest that H₂S may be an important endogenous regulator of the circadian clock and metabolic disorder.

H₂S and hypothalamus

CBS and 3-MST are the major endogenous enzymes in the brain that produce H₂S [69–73]. CBS/H₂S pathway in the hypothalamic paraventricular nucleus (PVN) was found to improve obesity and insulin sensitivity via the brain-adipose interaction [74]. CBS was detected in thyrotropin-releasing hormone (TRH) and CRH producing neurons. Hypothalamus CBS protein levels was downregulated in HFD-induced obese rats and in db/db obese mice. Overexpression of PVN CBS decreased food intake and improved obesity and insulin sensitivity in high-fat-diet rats. Moreover, CBS overexpression in PVN upregulated pre-TRH level and downregulated adrenocortico-tropic-hormone (ACTH) and corticosterone content in the blood. The activation of mTORC1 signaling pathway is involved in the upregulation of CBS/H₂S in pre-TRH level. Further results [74] indicated that leptin activated FOXO3a to increase CBS expression and H₂S production in the hypothalamus, regulating neuroendocrine hormone (TRH and ACTH) to impact systemic glucose metabolism and energy homeostasis. However, the roles of H₂S in regulating glucose metabolism control by the hypothalamus and its mechanism need to be further studied.

Roles of H₂S in glucose metabolism *in vivo*

Endogenous H₂S plays vital roles in regulating pancreatic β-cells, liver, adipose, skeletal muscle and hypothalamus function to modulate glucose metabolism. How H₂S regulates glucose metabolism *in vivo* has attracted great attention. Guandong and colleagues found that CSE knockout mice showed a delayed onset of STZ-induced diabetes and the reduced apoptotic β-cell death. STZ upregulated pancreatic H₂S generation, and the treatment of PPG improved glucose metabolism disorder in STZ wild type mice. But NaHS injection impaired glucose metabolism [26]. The inhibition of H₂S production with PPG upregulated serum insulin levels, and decreased blood glucose in Zucker diabetic fatty (ZDF) rats, indicating that inhibiting H₂S production improved hyperglycemia [18]. In contrast, other studies reported that middle-aged CSE-deficient mice showed increased blood glucose levels and impaired glucose intolerance [21]. Xue *et al.* showed that chronic NaHS administration improved glucose metabolism disorder in Goto-Kakizaki diabetic rats. Application of NaHS (60 μmol/kg·day, 10 weeks) improved insulin sensitivity in Wistar rats [45]. Guo *et al.* indicated that CSE knockout promoted HFD-induced mouse obesity and insulin resistance and increased hepatic gluconeogenesis. A low dose of NaHS treatment (25 μmol/kg/d, intraperitoneally for 12 weeks) improved HFD-induced obesity and insulin resistance but a high dose of NaHS treatment (50 μmol/

kg/d, intraperitoneally for 12 weeks) promoted HFD-induced obesity and insulin resistance [35]. Treatment with H₂S gas buffer, or the H₂S donor GYY4137 improved insulin resistance in HFD-induced obese mice [43]. Geng *et al.* reported that both upregulation and downregulation of H₂S level could improve insulin resistance in HFD mice [57]. Therefore, the roles of H₂S in glucose metabolism *in vivo* are not consistent, and need to be further investigated.

H₂S in humans

Most studies indicated plasma H₂S levels decreased in diabetic patients. Compared with lean subjects, overweight and type 2 diabetes patients had decreased blood H₂S concentrations. The median plasma H₂S levels (25th, 75th percentiles) in lean, overweight and type 2 diabetes subjects were 38.9 (29.7, 45.1) μmol/L, 22.0 (18.6, 26.7) μmol/L and 10.5 (4.8, 22.0) μmol/L, respectively [12]. There was a negative correlation between blood H₂S level and fasting glucose. The negative correlations between blood H₂S level and insulin sensitivity were also observed. Adiposity emerged as an independent predictor of blood H₂S. Similarly, Jain *et al.* and other groups reported that plasma H₂S levels were reduced in type 2 diabetes patients compared to control (45.1 ± 15.5 μmol/L versus 54.0 ± 26.4 μmol/L reported by Suzuki *et al.*) [11,13,75]. H₂S synthesis enzyme expression and H₂S production were also decreased in diabetic conditions. CSE protein expression and activity in peripheral blood mononuclear cells (PBMCs) from type 1 diabetes patients were decreased significantly compared with normal subjects. A significantly negative correlation was seen between CSE protein expression and HbA1c levels in the type 1 diabetes patients [30]. Xiaonan *et al.* reported that the leukocyte-derived H₂S production rate was much lower in both obese and overweight hypertensive than in normal weight hypertensive patients. H₂S production from leukocyte was negatively correlated with the levels of homeostasis model assessment of insulin resistance in overweight and obese hypertensive patients. CSE expression in leukocytes of patients with high insulin levels was decreased compared with patients with high insulin levels. The production rate of leukocyte-derived H₂S positively correlated with the plasma IL-10 levels [75]. High glucose inhibited CSE/H₂S levels in peripheral blood monocytes [76]. These results indicated that the plasma H₂S concentration decreased in diabetic patients. Whether this decrease contributes to the development of diabetes in humans needs to be further investigated.

Conclusions and future perspectives

In summary, H₂S could be endogenously produced from islet β cells, the liver, adipose, skeletal muscles and the hypothalamus, and regulates local and systemic glucose metabolism. It inhibits insulin secretion from islet β cells and promotes or suppresses β-cell apoptosis. It regulates insulin sensitivity in insulin responsive tissues. In the liver, H₂S inhibits glucose uptake and glycogen storage, and promotes or inhibits gluconeogenesis, mitochondrial biogenesis and mitochondrial bioenergetics. Several research groups reported that H₂S promoted glucose uptake in adipocytes while others reported H₂S inhibited this process. It is also reported that H₂S promotes adipogenesis, inhibits lipolysis, and regulates adipokine secretion from adipocytes. H₂S increases glucose uptake, modulates CCG in myotubes and muscle from HFD mice, inhibits oxidative damage and cell apoptosis in diaphragmatic muscle. CBS/H₂S pathway in the paraventricular nucleus improved obesity and insulin sensitivity via the brain-adipose interaction. Plasma H₂S levels are downregulated in diabetes patients compared with controls.

Although numerous studies have been performed to explore how H₂S regulates insulin resistance, β -cell function, and glucose metabolism, the results are sometimes controversial and many important issues need to be further studied. This discrepancy might be due to several mechanisms. For example, varying H₂S concentrations may cause different effects. Low concentrations of H₂S may play a protective role while high concentrations of H₂S may cause toxic effects. Differences under experimental conditions, species-specificity, and specificity of H₂S-synthesizing enzyme inhibitors and donors might also contribute to this discrepancy. The roles of tissue-specific H₂S in systemic glucose metabolism have been far from clear. The molecular mechanisms through which H₂S regulates β cell function and insulin resistance require further study. How H₂S regulates glucose metabolism in human remains largely unknown and needs further investigation.

Compliance with ethics requirements

This article does not contain any studies with human or animal subjects.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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