Biochemical and Biophysical Research Communications 380 (2009) 153–159

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Hydrogen sulfide from adipose tissue is a novel insulin resistance regulator

Xuejuan Feng [a], Yu Chen [b,c], Jing Zhao [b], Chaoshu Tang [b,c], Zhisheng Jiang [a,*], Bin Geng [b,c,*]

[a] Institute of Cardiovascular Diseases, Nan Hua University, Hengyang 421001, China
[b] Department of Physiology and Pathophysiology, Peking University Health Science Center, Beijing, China
[c] Institute of Cardiovascular Research, First Hospital of Peking University, Xishiku Street 8, Xicheng District, Beijing 100034, China

ARTICLE INFO

Article history:
Received 8 January 2009
Available online 21 January 2009

Keywords:
Hydrogen sulfide
Cystathionine gamma lyase
Glucose uptake
Adipose
Insulin resistance

ABSTRACT

Recent data suggested that endogenous hydrogen sulfide (H_2S) contributes to the pathogenesis of diabetes. Here, we identified that cystathionine gamma lyase (CSE) was expressed in adipose tissue in rats and endogenously generated H_2S. The CSE/H_2S system exists in both rat adipocytes and pre-adipocytes. This system was up-regulated with aging, although a high level of glucose down-regulated the system in a concentration- and time-dependent manner. H_2S inhibited the basal and insulin-stimulated glucose uptake of mature adipocytes, whereas administration of CSE inhibitors enhanced the glucose uptake of adipocytes. The PI3K but not K_{ATP} channel pathway is involved in the inhibitory effect of H_2S on glucose uptake. Finally, in fructose-induced diabetes in rats, we confirmed the up-regulated CSE/H_2S system in adipose tissue, which was negatively correlated with glucose uptake in this tissue. Our findings suggest that H_2S might be a novel insulin resistance regulator.

Hydrogen sulfide (H_2S) is a poisonous gas with an offensive odor described as a rotten egg smell. H_2S is also endogenously generated by two pyridoxal-5′-phosphate-dependent enzymes, cystathionine beta synthase (CBS) and cystathionine gamma lyase (CSE), in mammals [1]. Endogenous H_2S is now recognized as a novel gastrotransmitter and takes part in the pathogenesis of cardiovascular diseases, nervous system diseases, gastric ulceration, and infection [2]. Recent data from beta cells showed that endogenous H_2S inhibits insulin secretion [3] and induces endoplasmic-reticulum stress response, which results in apoptosis of insulin-secreting beta cells [4]. In streptozotocin-induced diabetes in rats, endogenous H_2S production in the pancreas was significantly up-regulated [5]. These results imply that endogenous H_2S contributes to the pathogenesis of diabetes. L-Cysteine, synthesized from methionine, is a main precursor of H_2S. Adipose tissue is also an important organ of methionine metabolism to synthesize fatty acid [6]. Adipose tissue is also an insulin-sensitive organ that mediates glucose uptake and metabolism. Whether H_2S can be endogenously generated from adipose tissue and its role in glucose metabolism are unknown.

In the present study, we first identified the endogenous CSE/H_2S pathway in adipose tissue and adipocytes. Then we detected glucose uptake alteration induced by H_2S. Finally, we observed the change in the endogenous CSE/H_2S pathway in adipose tissue and its possible role in fructose-induced insulin resistance in a rat model.

Materials and methods

Materials. Seventy-six male Sprague–Dawley (SD) rats (200–250 g); 96 different age SD rats (from 1 month to 12 months old) were obtained from the Animal Department, Peking University Health Science Center. All animal care and experimental protocols complied with the Animal Management Rules of the Ministry of Science and Technology of the People's Republic of China (documentation no. 2, 1998). Collagenase type 1, L-cysteine, pyridoxal-5′-phosphate (PLP), insulin, DL-propargylglycine (PPG), beta-cyano-L-alanine (BCA), D(−)-fructose, and Medium 199 were purchased from Sigma (St. Louis, MO, USA). The primers for CSE (S-5′-CCGGATGGAGAAACACTTC-3′; A-5′-GCTGCCTTTAAAGCTGACC-3′), CBS (S-5′-GGGACTTCTTGGCTTTCA-3′; A-5′-TAGGTGCTTGTCGG GAAA-3′), and GAPDH (S-5′-GCAAGTTCAACGGCACAG-3′; A-5′-GCCAGTAGACTCCACGACAT-3′) were synthesized by SBS company (Beijing, China). CSE antibody (H00001491-M01) was purchased from Abnova Corp. (Taipei). Beta-actin antibody (SC-47778) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). [^{3}H]-2-Dexoy-D-glucose ([^{3}H]-2-DG) was purchased from PerkinElmer (Boston, MA, USA). The glucose assay kit (glucose oxidase method), TRIZol reagent and chemiluminescence kit were purchased from Applygen Technologies (Beijing). The H_2S-saturated solution (0.09 mol/L at room temperature) was freshly made by bubbling pure H_2S gas (99.99%, from Beijing XianHeYu). Other chemicals and reagents were of analytical grade.

* Corresponding authors. Address: Institute of Cardiovascular Research, First Hospital of Peking University, Beijing 100034, China (B. Geng). Fax: +86 010 82802183 (B. Geng), +86 0734 8282847 (Z. Jiang).
E-mail addresses: zsjiang2005@163.com (Z. Jiang), bingengch@yahoo.com (B. Geng).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.01.059

Adipocyte and pre-adipocyte isolation and culture [7]. Briefly, the epididymal fat pads of rats were removed and rinsed in Krebs–Ringer HEPES buffer (KRH). Following collagenase I digestion for 60 min, the isolated adipocytes were passed through a nylon mesh with a 250 μ-pore diameter and resuspended in KRH (pH 7.4). The cell suspension was washed twice by centrifugation at 200*g* for 30 s in KRH. The infranatant was collected into another tube and washed several times by centrifugation at 585*g* for 5 min. The isolated adipocytes and pre-adipocytes were resuspended in standard Medium 199. Cells were maintained in an incubator at 37 °C in 5% CO_2, and the next day, floating red blood cells were removed by aspiration, and the culture medium was replenished.

Assay of H_2S production. H_2S production was measured by the methylene blue method as previously described [8]. Briefly, adipose tissue homogenate or adipocyte lysis solution was prepared, then mixed with the reaction buffer containing (in mM) 100 potassium phosphate buffer (pH 7.4), 10 L-cysteine, and 2 pyridoxal 5′-phosphate. The reaction was performed at 37 °C for 90 min; 0.5 mL of 20% trichloroacetic acid was added to stop the reaction. Released H_2S was absorbed by zinc acetate, then dissolved in a hydrochloric acid solution of *N*,*N*-dimethyl-*p*-phenylenediamine, and methylene blue was formed within 10 min at room temperature in the presence of ferric chloride. The blue color of methylene blue was measured at 670 nm with use of a spectrophotometer (Bio-Rad M550). The H_2S concentration was calculated against the calibration curve of the standard S^{2-} solutions.

RT-PCR determination of CSE and CBS mRNA expression [9]. Total RNA from cells was extracted with use of TRIZol reagent. RT-PCR was performed in a total volume of 25 μL. After a denaturing at 95 °C for 5 min, PCR was run at 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 40 s for 30 cycles. The PCR products were separated on 1.2% agarose gel and stained with ethidium bromide. The optical density of the cDNA bands of CSE (400 bp), CBS (487 bp) and GAPDH (140 bp) was measured by use of the Gel Documentation System (Bio-Rad, Hercules, CA).

Western blot analysis [10]. Adipose tissues and adipocytes were lysed in laemmli sample buffer and denatured at 95 °C for 5 min. Protein samples were separated on 12% SDS–PAGE. The proteins were transferred to nitrocellulose membranes by electroblotting. Proteins were detected with use of monoclonal anti-CSE antibody (1:4000) and monoclonal anti-beta-actin antibody (1:8000). After incubation with horseradish peroxidase-conjugated secondary antibodies (1:10000), the membrane was washed, and color was developed by use of an enhanced chemiluminescence kit.

Determination of 2-deoxy-D-glucose uptake. Glucose transport into cells was determined by the [^{3}H]-labeled 2-deoxy-D-glucose (2-DG) method [11]. Adipocytes cultured in 24-well plates were washed with PBS and then incubated in KRH buffer containing 1% BSA for 15 min at 37 °C, 5% CO_2. 2-DG was added in a concentration of 50 μM containing 1 μCi/well of 2-deoxy-D-[^{3}H] glucose for 5 min at 37 °C, 5% CO_2. The uptake was stopped by the addition of 2 mL of ice-cold PBS containing 200 μM phloretin. The adipocyte glucose uptake was then quantified by determining radioactivity on liquid scientillation spectroscopy (Beckman, LS6500).

Adipose tissues were removed and placed in ice-cold PBS, cut into pieces approximately 1×1 mm^2 and transferred to 24-well plates containing 1 mL of KRH buffer per well to cover the tissue slices completely. Tissues were cultured in a shaking bath (60 beats/min) at 37 °C, in an atmosphere of 95% O_2 and 5% CO_2. The glucose uptake procedure in adipose tissues was the same as for adipocytes.

Preparation of animal insulin resistance model [12] *and experimental protocol.* Control rats ($n = 8$) were fed normal chow and water. Insulin-resistant animals ($n = 10$) were given water containing 10% fructose. After 12 weeks, the intraperitoneal glucose-tolerance test was performed after mice fasted for 12–14 h. After the basal blood glucose level was measured, conscious rats were injected intraperitoneally with 1.5 g/kg body weight of 50% (w/v) glucose solution in 0.9% (w/v) saline. Blood samples were then collected from the tail vein, and blood glucose level was measured by use of the Accu-Chek Active blood glucose monitoring system (Roche Diagnostic GmbH, Mannheim, Germany) at 0, 30, 60, and 120 min after the glucose injection. After continuous feeding for 2 days, rats were anesthetized intraperitoneally with pentobarbital sodium (30 mg/kg). Fasting blood glucose was measured by the glucose assay kit, and fasting blood insulin was measured by a radioimmunoassay kit (Beijing Atom HighTech Co.). Insulin sensitivity was calculated by use of the homeostasis model assessment (HOMA) index. With glucose in millimoles per liter and insulin in milliunits per liter, the HOMA index is calculated as (glucose × insulin)/22.5 [13]. The epididymal fat pads were removed quickly, and glucose uptake, H_2S production and CSE protein expression of adipose tissue were measured.

Statistical analysis. Results are expressed by means ± SD. Data were analyzed by means of unpaired Student's *t*-test or ANOVA followed by Student–Newman–Keuls test for comparison of groups. Correlation was analyzed by Pearson correlation. Differences at $P < 0.05$ were considered statistically significant.

Results and discussion

White adipose tissue is an active energy metabolism organ that uptakes glucose and amino acids, including methionine, to convert fatty acid [6]. H_2S is a intermediate production factor of methionine by trans-sulfuration metabolism [1]. Whether white adipose tissue could endogenously generate H_2S and its possible biological role and regulation is not known. Here, we detected the rate of H_2S production from visceral fat tissues, including epididymal fat (4.76 ± 0.92 nmol/min/mg protein), perirenal fat (2.93 ± 0.27 nmol/min/mg protein), and brown fat tissue (4.65 ± 0.81 nmol/min/protein) (Fig. 1A), and the production rate was approximately 40–50% that of liver tissues. H_2S accumulation was time dependent from 2 to 6 h (Fig. 1B). H_2S generation in mammals mainly depends on two enzymes, CBS and CSE, which need PLP as a coenzyme [1]. RT-PCR revealed both CBS and CSE mRNA in epididymal fat, perirenal fat and brown fat tissues, with liver and kidney used as a positive control (Fig. 1C and D). Pretreating adiopose tissues with the CSE inhibitor PPG (10 mmol/L) or BCA (2 mmol/L) for 20 min significantly decreased the endogenous H_2S production by 85% and 89%, respectively, as compared with controls (both $P < 0.01$, Fig. 1E), which suggests that the main pathway of H_2S generation in adipose tissues could be mediated by CSE. Furthermore, we measured the CSE protein expression in fat tissues and detected a 43-kD protein in visceral fat and brown fat (Fig. 1F).

H_2S-generating rates in adipocytes and pre-adipocytes were 2.89 ± 1.34 and 2.17 ± 1.14 nmol/min/mg protein, respectively (Fig. 1G). The CSE expression in adipocytes and pre-adipocytes is shown in Fig. 1H, which confirms that adipocytes endogenously produced H_2S through a CSE catalytic pathway.

Aging is associated with dysfunction of energy homeostasis of fat (increased lipolysis [14] or decreased insulin sensitivity [15]) and progressive redistribution of fat from subcutaneous to visceral regions. This redistribution of fat contributes to a feed-forward cycle of increased visceral fat mass, decreased insulin sensitivity and increased serum insulin, which increases the susceptibility to type 2 diabetes [16,17]. We selected different-aged rats and detected the alteration in the CSE/H_2S pathway from visceral fat tissues. With rat growth from 1 to 12 months, the

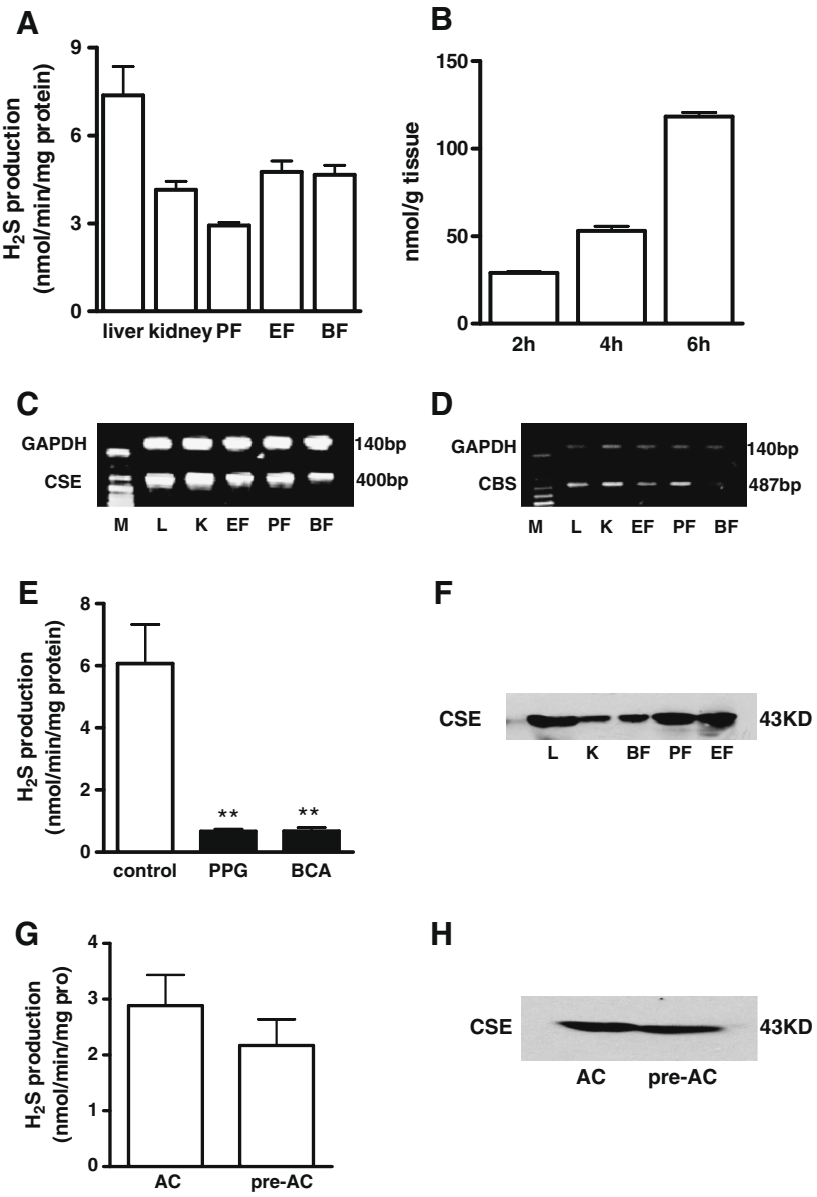

Fig. 1. The cystathionine gamma lyase (CSE)/hydrogen sulfide (H_2S) system in adipose tissues. (A) Preparation of tissue homogenate (liver, kidney, perirenal fat [PF], epididymal fat [EF] and brown fat [BF]), addition of L-cysteine plus pyridoxal 5′-phosphate, and measurement of H_2S production. (B) Supplementation of L-cysteine and pyridoxial 5′-phosphate to epididymal fat homogenate, incubation for 2, 4 or 6 h, and measurement of generated H_2S. Measurement of CSE (C) and cystathionine beta synthase (CBS) activity in tissue. (D) RT-PCR results of gene expression. (E) Epidermal fat was pretreated with CSE inhibitors DL-propargylglycine (PPG) or beta-cyano-L-alanine (BCA) for 20 min, then H_2S production was measured. (F) CSE protein expression detected by western blot analysis. Isolated adipocytes and pre-adipocytes (pre-AC) underwent collagenase I assay, then H_2S production (G) or CSE protein expression (H) was detected. All data are means plus SD. $^{**}P < 0.01$ versus control.

body mass and visceral fat mass (weight of epididymal fat plus perirenal fat) gradually increased; after 10 months, the speed of increase in body weight became slower; however, fat weight progressively increased (Figs. S1 and 2A), for an increased ratio of visceral fat weight to body weight (Fig. S2). With increased growth by age, the H_2S production of the epididymal fat pad increased moderately, from months 1 to 4, was maintained from months 4 to 8, and then was greatly elevated from months 9 to 12 (Fig. 2B). Furthermore, the CSE protein expression increased continually in an age-dependent manner, from months 1 to 12 (Fig. 2C). The alteration in H_2S production and CSE protein expression in perirenal fat was the same as for epididymal fat (Figs. S3 and S4). These data suggest that with age, the endogenous CSE/H_2S system was up-regulated in adipose tissues. Indeed, with increasing age, adipose eNOS and iNOS expression was found to increase [18], and over-produced nitric oxide can up-regulate H_2S generation [19].

Adipose tissue is an important organ of glucose metabolism, and elevated blood glucose might impair adipocyte functions [20]. We examined the H_2S production rate in the original cultured adipocytes from epididymal fat pads with different concentrations of glucose (5–20 mM) incubated for 30 min (Fig. 2D). The H_2S generation rates were significantly reduced with high glucose incubation, but 20 mM mannitol did not affect the H_2S generation (Fig. 2D). High glucose (20 mM) inhibited H_2S production of adipocytes in a time-dependent manner (Fig. 2E). These results suggest that aging and blood glucose could regulate the endogenous CSE/H_2S pathway in adipose. Elevated glucose induces reactive oxygen

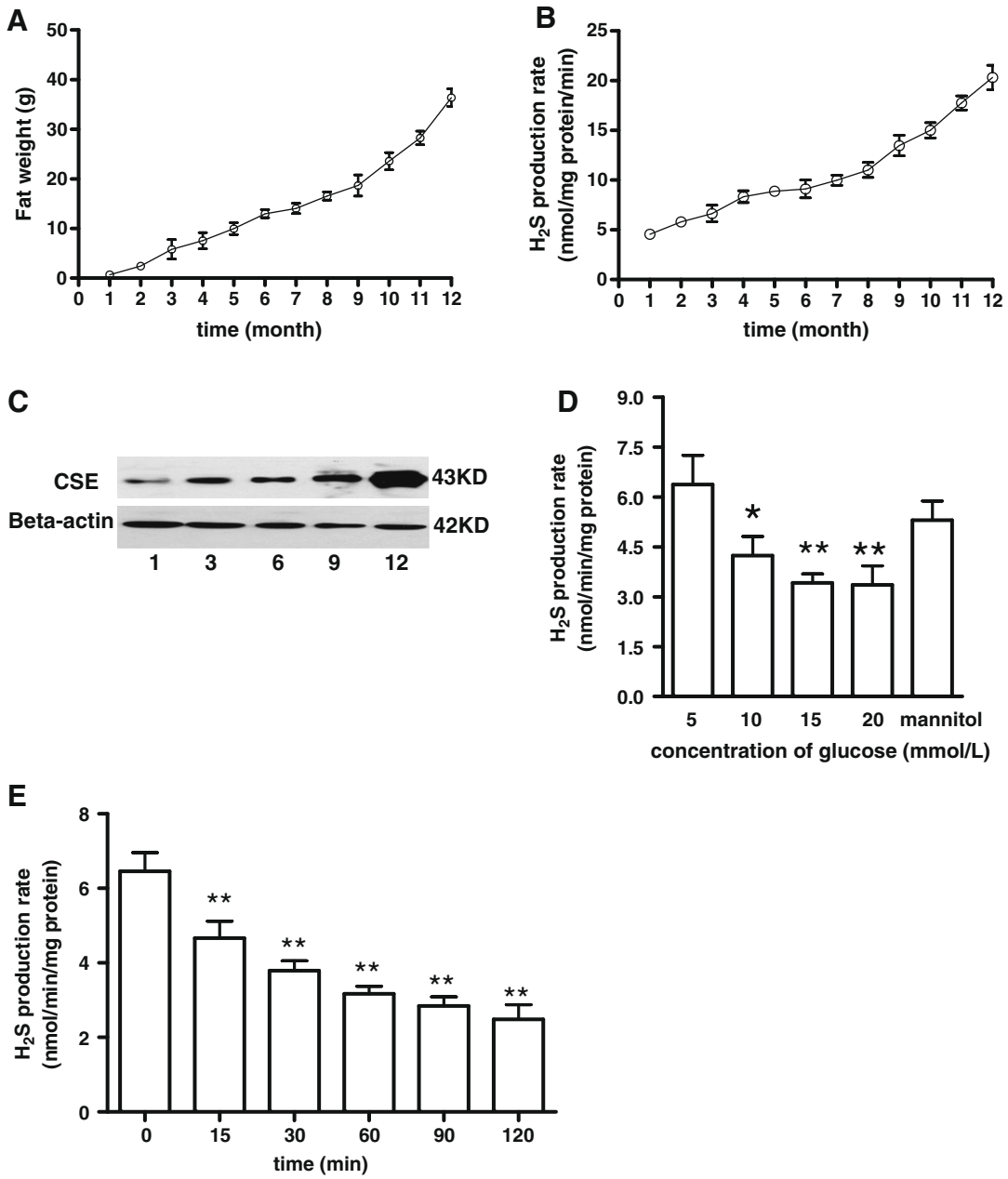

Fig. 2. The effects of aging on the CSE/H_2S system in adipose tissues. 96 rats from age 1 to 12 months were recruited (8 rats for each month age); visceral fat (epididymal fat plus perirenal fat) weight (A) was measured. In isolated epididymal fat, H_2S production (B) and CSE protein (C) were detected in rats of different ages. (D) Different concentrations of glucose (5, 10, 15 and 20 mM) were pre-incubated in epididymal fat for 30 min, and H_2S production was assayed by the methylene blue method. Data are means plus SD. $^{*}P < 0.05$, $^{**}P < 0.01$ versus 5-mM glucose incubation. (E) Epididymal fat was incubated with 20 mM glucose for different times, and the alteration in H_2S generation was compared. $^{**}P < 0.01$ versus 0-min incubation.

species (ROS) generation in adipocytes [21], so ROS may reduce endogenous H_2S production [22].

Adipose tissue is an important insulin-sensitive organ to regulate blood glucose [23]. We observed the effects of H_2S on glucose uptake in adipocytes with or without insulin stimulation. After incubation with H_2S (from 10 to 1000 μm) for 30 min, the capacity of glucose transport into adipocytes was significantly inhibited in a concentration-dependent manner (Fig. 3A). The glucose uptake in adipocytes was sensitive to insulin. Insulin, 10 nM, increased [^{3}H]-2-DG uptake to approximately 2.67-fold that of the basal level (Fig. 3B); H_2S also concentration-dependently inhibited the insulin stimulatory action in adipocytes (Fig. 3B). Administration of L-cysteine plus PLP increased endogenous H_2S production with reduced adipocyte glucose uptake by 39% as compared with controls and also lowered glucose uptake stimulated by insulin (all $P < 0.01$, Fig. 3C). In contrast, the CSE inhibitor PPG or BCA increased adipocyte basal glucose uptake by 48% and 17%, respectively, and increased insulin-stimulated glucose uptake by 37.6% and 20.2%, respectively (all $P < 0.01$, Fig. 3C). These data elucidated that endogenous H_2S-reduced insulin sensitivity of adipocytes, and CSE inhibitors might reverse this effect.

The K_{ATP} channel is a important signal target to regulate insulin secretion in pancreas beta cells [24]. In the present

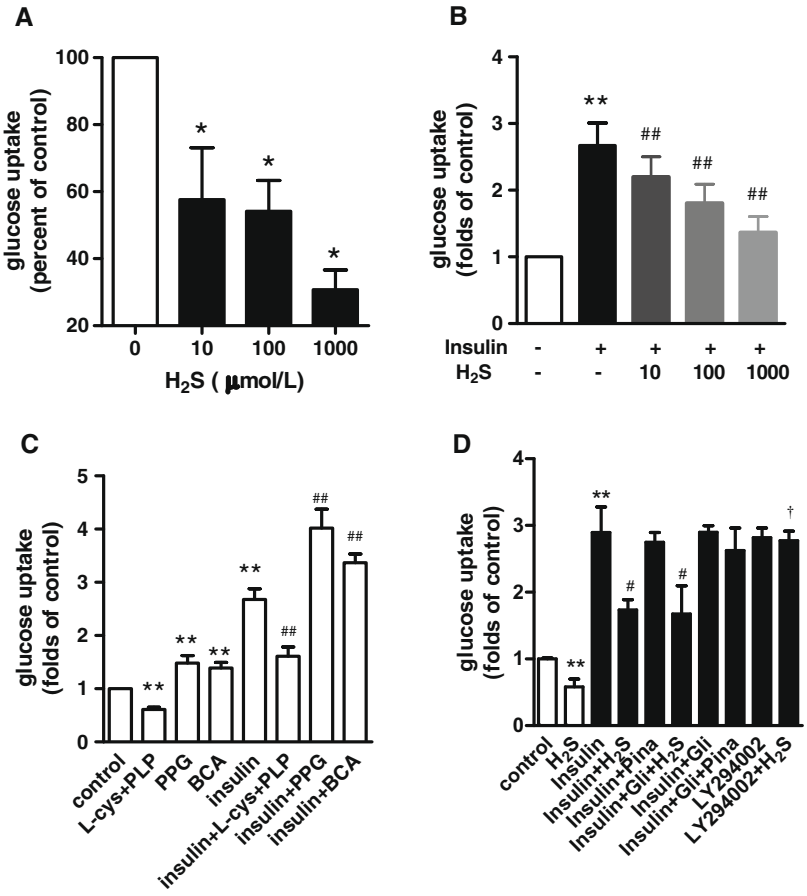

Fig. 3. The effect of H_2S on glucose uptake of adipocytes. We prepared mature adipocytes from epididymal fat, treated them with different concentrations of H_2S (10, 100, 1000 μM), then glucose uptake was measured by 2-deoxy-D-[^{3}H] glucose uptake without (A) or with (B) insulin (10 nM). (C) On incubation with L-cysteine plus PLP in the presence or absence of the CSE inhibitor PPG or BCA, the glucose uptake in adipocytes was detected with or without insulin stimulation. (D) On reincubation with galibenclamide (K_{ATP} channel inhibitor), pinacidil (Pina; non-selective K_{ATP} channel opener) or PI3K inhibitor LY294002, the changes in glucose uptake inhibited by H_2S were measured. All data are means plus SD. $^{**}P < 0.01$ versus control; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ versus single insulin stimulation. $^{\dagger}P < 0.05$ versus insulin plus H_2S treatment.

study, we added the non-selective K_{ATP} channel inhibitor glibenclamide or PI3 K inhibitor LY294002 for 20 min before the addition of H_2S. H_2S, 100 μM, significantly reduced the insulin-stimulated glucose uptake by 41.3% ($P < 0.01$), which was blocked by pre-treatment with LY294002 (10 μM) but not glibenclamide (10 μM) ($P > 0.05$) (Fig. 3D). The non-selective K_{ATP} channel opener pinacidil, 10 μM, had no effect on glucose uptake by adipocytes. H_2S-regulated glucose uptake of adipocytes may be mainly through a PI3K but not K_{ATP} channel pathway. LY294002 causes the accumulation of GLUT4-containing vesicles just beneath the cell surface, and these vesicles could be mobilized by insulin, which results in increased glucose uptake by adipocytes [25]. These data imply that H_2S might inhibit GLUT4 translocation through a PI3K pathway, which causes an inhibition of glucose uptake by adipocytes.

According to our *in vitro* data, H_2S derived from adipocytes might regulate insulin sensitivity. To determine the *in vivo* role of adipose-generated H_2S in insulin resistance, we prepared insulin-resistant rats by fructose feeding for 12 weeks. In rats with insulin resistance, intraperitoneal glucose-tolerance test curves showed high blood glucose level at each time point (Fig. 4A, $P < 0.01$) and increased fasting plasma insulin concentration approximately 1-fold that of controls (Fig. 4B, $P < 0.01$), which resulted in an increased HOMA index approximately 2.9-fold that of controls (Fig. 4C, $P < 0.01$). To investigate the insulin resistance of adipose tissues, [^{3}H]-2-DG uptake was measured in incubated epididymal fat tissues from control or insulin-resistant rats. At 20 min after administration of insulin (10 nM), adipose tissue glucose uptake activity in insulin resistant rats was only 33% that of controls (Fig. 4D, $P < 0.01$). Furthermore, we detected CSE protein expression significantly up-regulated in insulin-resistant rats as compared with controls. The adipose-released H_2S level in insulin-resistant rats also increased to approximately 1.9-fold that of control rats (Fig. 4F, $P < 0.01$). Of interest, we analyzed the correlation between endogenous H_2S production and adipose glucose uptake, finding a significant negative correlation ($R^2 = 0.67$, Fig. 4G, $P < 0.01$). These *in vivo* data confirmed that H_2S generated by adipose tissues might regulate insulin sensitivity and contribute to the pathogenesis of diabetes mellitus.

Taken together, our findings suggest that adipose could synthesize and secrete the gasotransmitter H_2S, which acts as an autocrine factor and inhibits basal or insulin-stimulated glucose metabolism in adipose tissues and affects the pathogenesis of insulin resistance. H_2S is an endogenous K_{ATP} channel opener according to previous data. The K_{ATP} channel blocker glibenclamide increases the insulin secretion of beta cells [26] but does not change the insulin sensitivity in adipose [27], which limits the use of these drugs to treat diabetes. The CSE/H_2S pathway in adipose tissues might be a new therapeutic target for type 2 diabetes mellitus and insulin resistance.

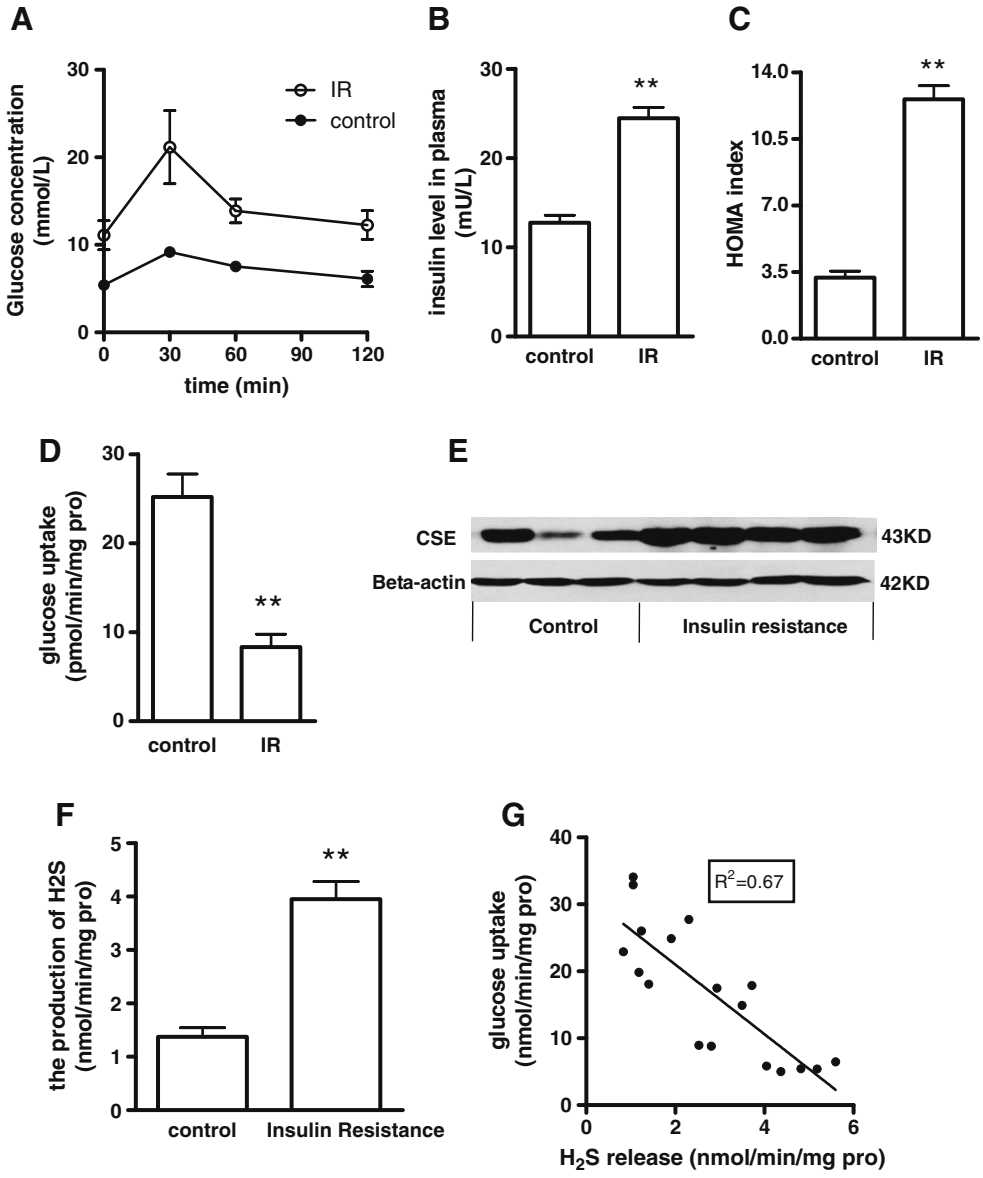

Fig. 4. The pathophysiological role of the adipose CSE/H_2S system in diabetic rats. We fed fructose to rats with diabetes and insulin resistance for 12 weeks. (A) The intraperitoneal glucose-tolerance test was carried out after 12- to 14-h fasting. (B) The insulin level of plasma was measured by radioimmunoassay, and insulin resistance (IR) was calculated by the homeostasis model assessment (HOMA) index. With glucose in millimeters per liter and insulin in milliunits per liter, the HOMA index is calculated as (glucose × insulin)/22.5 (C). In isolated epididymal fat, the glucose uptake (D), CSE protein expression (E) and endogenous H_2S production (F) were assayed. (G) Pearson correlation analysis of H_2S production and glucose uptake. All data are means plus SD. $^{**}P < 0.01$ versus control.

Acknowledgments

This work was supported by The Major State Basic Research Development Program of the People's Republic of China (no. 2006CB503807) and the National Natural Science Foundation of China (NSFC: 30570766). We also thank Professor *Guoheng Xu* for technical support in adipocyte isolation and culture.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.01.059.

References

[1] P. Kamoun, Endogenous production of hydrogen sulfide in mammals, Amino Acids 26 (2004) 243–254.
[2] C. Tang, X. Li, J. Du, Hydrogen sulfide as a new endogenous gaseous transmitter in the cardiovascular system, Curr. Vasc. Pharmacol. 4 (2006) 17–22.
[3] W. Yang, G. Yang, X. Jia, L. Wu, R. Wang, Activation of KATP channels by H2S in rat insulin-secreting cells and the underlying mechanisms, J. Physiol. 569 (2005) 519–531.
[4] G. Yang, W. Yang, L. Wu, R. Wang, H2S, endoplasmic reticulum stress, and apoptosis of insulin-secreting beta cells, J. Biol. Chem. 282 (2007) 16567–16576.
[5] M. Yusuf, B.T. Kwong Huat, A. Hsu, M. Whiteman, M. Bhatia, P.K. Moore, Streptozotocin-induced diabetes in the rat is associated with enhanced tissue hydrogen sulfide biosynthesis, Biochem. Biophys. Res. Commun. 333 (2005) 1146–1152.
[6] D.D. Feller, E. Feist, Conversion of methionine and threonine to fatty acids by adipose tissue, Can. J. Biochem. Physiol. 41 (1963) 269–273.
[7] H. Jiang, J. He, S. Pu, C. Tang, G. Xu, Heat shock protein 70 is translocated to lipid droplets in rat adipocytes upon heat stimulation, Biochim. Biophys. Acta 1771 (2007) 66–74.
[8] J.K. Fogo, M. Popowsky, Spectrophotometric determination of hydrogen sulfide, Anal. Chem. 21 (1949) 732–734.
[9] B. Geng, J. Yang, Y. Qi, J. Zhao, Y. Pang, J. Du, C. Tang, H2S generated by heart in rat and its effects on cardiac function, Biochem. Biophys. Res. Commun. 313 (2004) 362–368.

[10] B. Geng, Y. Cui, J. Zhao, F. Yu, Y. Zhu, G. Xu, Z. Zhang, C. Tang, J. Du, Hydrogen sulfide downregulates the aortic L-arginine/nitric oxide pathway in rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (2007) R1608–R1618.
[11] I.S. Wood, B. Wang, S. Lorente-Cebrian, P. Trayhurn, Hypoxia increases expression of selective facilitative glucose transporters (GLUT) and 2-deoxy-D-glucose uptake in human adipocytes, Biochem. Biophys. Res. Commun. 361 (2007) 468–473.
[12] K. Kamata, K. Yamashita, Insulin resistance and impaired endothelium-dependent renal vasodilatation in fructose-fed hypertensive rats, Res. Commun. Mol. Pathol. Pharmacol. 103 (1999) 195–210.
[13] M.O. Goodarzi, X. Guo, K.D. Taylor, M.J. Quinones, M.F. Saad, H. Yang, W.A. Hsueh, J.I. Rotter, Lipoprotein lipase is a gene for insulin resistance in Mexican Americans, Diabetes 53 (2004) 214–220.
[14] P. Engfeldt, P. Arner, Lipolysis in human adipocytes, effects of cell size, age and of regional differences, Horm. Metab. Res. Suppl. 19 (1988) 26–29.
[15] M. Cnop, P.J. Havel, K.M. Utzschneider, D.B. Carr, M.K. Sinha, E.J. Boyko, B.M. Retzlaff, R.H. Knopp, J.D. Brunzell, S.E. Kahn, Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex, Diabetologia 46 (2003) 459–469.
[16] M. Frisard, E. Ravussin, Energy metabolism and oxidative stress: impact on the metabolic syndrome and the aging process, Endocrine 29 (2006) 27–32.
[17] R. Curtis, B.J. Geesaman, P.S. DiStefano, Ageing and metabolism: drug discovery opportunities, Nat. Rev. Drug. Discov. 4 (2005) 569–580.
[18] I. Knerr, C. Schirl, T. Horbach, A. Stuppy, R. Carbon, W. Rascher, J. Dotsch, Maturation of the expression of adrenomedullin, endothelin-1 and nitric oxide synthases in adipose tissues from childhood to adulthood, Int. J. Obes. (Lond.) 29 (2005) 275–280.
[19] W. Zhao, J.F. Ndisang, R. Wang, Modulation of endogenous production of H_2S in rat tissues, Can. J. Physiol. Pharmacol. 81 (2003) 848–853.
[20] W.W. Hay Jr., Recent observations on the regulation of fetal metabolism by glucose, J. Physiol. 572 (2006) 17–24.
[21] Y. Lin, A.H. Berg, P. Iyengar, T.K. Lam, A. Giacca, T.P. Combs, M.W. Rajala, X. Du, B. Rollman, W. Li, M. Hawkins, N. Barzilai, C.J. Rhodes, I.G. Fantus, M. Brownlee, P.E. Scherer, The hyperglycemia-induced inflammatory response in adipocytes: the role of reactive oxygen species, J. Biol. Chem. 280 (2005) 4617–4626.
[22] L. Chang, B. Geng, F. Yu, J. Zhao, H. Jiang, J. Du, C. Tang, Hydrogen sulfide inhibits myocardial injury induced by homocysteine in rats, Amino Acids 34 (2008) 573–585.
[23] M.E. Trujillo, P.E. Scherer, Adipose tissue-derived factors: impact on health and disease, Endocr. Rev. 27 (2006) 762–778.
[24] Y.M. Leung, E.P. Kwan, B. Ng, Y. Kang, H.Y. Gaisano, SNAREing voltage-gated K+ and ATP-sensitive K+ channels: tuning beta-cell excitability with syntaxin-1A and other exocytotic proteins, Endocr. Rev. 28 (2007) 653–663.
[25] A. Bose, S. Robida, P.S. Furcinitti, A. Chawla, K. Fogarty, S. Corvera, M.P. Czech, Unconventional myosin Myo1c promotes membrane fusion in a regulated exocytic pathway, Mol. Cell. Biol. 24 (2004) 5447–5458.
[26] T. Miki, K. Minami, L. Zhang, M. Morita, T. Gonoi, T. Shiuchi, Y. Minokoshi, J.M. Renaud, S. Seino, ATP-sensitive potassium channels participate in glucose uptake in skeletal muscle and adipose tissue, Am. J. Physiol. Endocrinol. Metab. 283 (2002) E1178–E1184.
[27] F.M. Tayo, Failure of glibenclamide to stimulate glucose uptake by adipose tissue in vitro [proceedings], West Afr. J. Pharmacol. Drug Res. 3 (1976) 79P–80P.