Inhibition of Hepatic Glycogen Synthesis by Hyperhomocysteinemia Mediated by TRB3

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Recently, epidemiological and experimental studies have linked hyperhomocysteinemia (HHcy) to insulin resistance. However, whether HHcy impairs glucose homeostasis by affecting glycogenesis in the liver is not clear. In the present study, we investigated the effect of HHcy on hepatic glycogen synthesis. Hyperhomocysteinemia was induced in mice by drinking water containing two percent methionine. Mice with HHcy showed an increase in the phosphorylation of glycogen synthase and a significant decrease in hepatic glycogen content and the rate of glycogen synthesis. The expression of TRB3 (tribbles-related protein 3) was up-regulated in the liver of mice with HHcy, concomitantly with the dephosphorylation of glycogen synthase kinase-3β and Akt. The knockdown of TRB3 by short hairpin RNA suppressed the dephosphorylation of these two kinases. Homocysteine induced an increase in the levels of hepatic cAMP and cAMP response element-binding protein phosphorylation, which in turn up-regulated the expression of peroxisome proliferator-activated receptor (PPAR)-γ coactivator-1α and TRB3. The inhibition of PPAR-α by its inhibitor, MK886, or knockdown of PPAR-α by small interfering RNA significantly inhibited the expression of TRB3 induced by homocysteine. The current study demonstrates that HHcy impairs hepatic glycogen synthesis by inducing the expression of TRB3. These results provide a novel explanation for the development and progression of insulin resistance in HHcy. (Am J Pathol 2011, 178: 1489–1499; DOI: 10.1016/j.ajpath.2010.12.052)

The liver is a major organ for glucose metabolism; it maintains blood glucose levels in the fasting condition by production of glucose from intermediates, such as lactate, amino acids, and glycerol, through gluconeogene-

Supported by a grant from the National Natural Science Foundation of China (30560036) and a grant from Yunnan Department of Science and Technology (2009C045 to C.-G.Z.).

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Accepted for publication December 30, 2010.

The authors did not disclose any relevant financial relationships.

Supplemental material for this article can be found at http://ajp.amjpathol.org or at doi:10.1016/j.ajpath.2010.12.052.

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instance, the oral administration of homocysteine induces hyperinsulinemia and promotes the development of insulin resistance in rats. In contrast, prolonged folate treatment decreases homocysteine and insulin levels, thus improving insulin resistance in patients with metabolic syndrome. The molecular mechanism by which homocysteine promotes insulin resistance remains unclear. A recent study has demonstrated that homocysteine up-regulates the expression of resistin (a mediator of insulin resistance) and inhibits the phosphorylation of Akt in both primary rat adipocytes and adipose tissue of mice with HHcy. In vitro studies indicate that the oxidative stress produced by homocysteine thiolactone interrupts the insulin signaling pathway by inhibiting the phosphorylation of insulin receptor tyrosine kinase, phosphatidylinositol 3-kinase, and glycogen synthase kinase 3β (GSK3β) in HTC-HR hepatoma cells.

The accumulation of misfolded proteins within the endoplasmic reticulum triggers the activation of an unfolded protein response (UPR). The UPR leads to transcriptional up-regulation of several target genes, such as 78-kDa glucose-regulated protein and C/EBP homology protein/growth arrest and DNA damage-inducible protein 153 (CHOP). Accumulating evidence suggests that the UPR plays a crucial role in insulin resistance. Homocysteine has activated the UPR in a variety of cells, including hepatocytes. Recent studies have identified that TRB3, a mammalian homologue of Drosophila tribbles (tribbles-related protein 3), is a target gene of CHOP/ATF4. As a negative modulator of Akt, TRB3 plays an important role in insulin resistance. Homocysteine has activated the UPR in a variety of cells, including hepatocytes. Thus, it is possible that homocysteine induces insulin resistance by impairing glucose homeostasis through the activation of the UPR pathway. To test this hypothesis, we investigated the effect of HHcy on glycogenesis in the liver. Our results demonstrated that HHcy reduced glycogenesis in the liver by up-regulation of TRB3 expression. However, the induction of TRB3 expression was because of the cAMP–protein kinase A pathway, rather than the UPR pathway.

Material and Methods

Induction of HHcy

Twelve-week-old male BALB/c mice were obtained from Yunnan Baiyao Pharmacological Co, Kunming, China. Animals were kept under a constant 12-hour light-dark cycle and were allowed to eat and drink ad libitum. Generally, mild HHcy can be induced by increasing total methionine. The animals were fed one of the following diets: control diet (LM-485 chow; Harlan Teklad, Madison, WI) or a high-methionine diet (LM-485 chow with drinking water supplemented with two percent L-methionine). Mice were sacrificed after three months on the diets. Homocysteine levels in the plasma of mice were determined by using an enzyme-linked immunosorbent assay kit (Axis-Shell, Dundee, UK). The investigation conforms to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health. The protocol of the experiments was approved by the Animal Care and Use Committee of Yunnan University, Yunnan, China.

Tissue Glycogen Content

Animals had ad libitum access to both food and water. Hepatic glycogen concentrations were measured after perchloric acid digestion, as previously described. Briefly, frozen powder of liver (100 mg to 150 mg) was extracted with 1.5 mL of 6% (w/v) HClO4 and centrifuged for 15 minutes at 2000 × g; 0.5 mL of the supernatant was neutralized with 10% (w/v) KOH. The glycogen in 0.2 mL of supernatant was hydrolyzed by α-amylglucosidase (50 U/mL; Sigma, St Louis, MO) in sodium acetate buffer (50 mmol/L; pH, 4.8) overnight at room temperature. The concentration of glycogen in the liver was estimated as the measurement of glucose released from glycogen by a glucose assay kit (Sigma). Hepatic glycogen levels were calculated by determining the difference between the glucose level with and without amyloglucosidase incubation. Glycogen concentration was expressed as glucose units (in milligrams) per protein (in grams). The liver was stained with periodic acid-Schiff for glycogen staining.

Measurement of Glycogen Synthesis Rates

Mice were injected intraperitoneally with tritiated water (2 mCi/mouse; Atom High-Tech Co, Beijing, China) 2 hours before they were sacrificed. Samples of liver were immediately collected and frozen in liquid nitrogen. The hepatic glycogen synthesis rate in vivo was determined by extracting total glycogen, which includes unlabeled and 3H-labeled glycogen, with perchloric acid, as previously described.

Cell Culture and Homocysteine Treatment

Mouse hepatocytes were isolated as previously described. Hepatocytes were plated in serum-free William’s medium E (Gibco, Gaithersburg, MD), including insulin (20 mU/mL; Sigma) and epidermal growth factor (10 ng/mL; Sigma). After attachment, the cells were cultured with Dulbecco’s modified Eagle’s medium (DMEM; Gibco) without serum overnight.

The human hepatocarcinoma cell line HepG2 was obtained from the Kunming Cell Center (Chinese Academy of Sciences, Kunming, China). The cells were grown in DMEM (Gibco) with 10% fetal bovine serum and maintained at 37°C with five percent CO2. When the cells were 60% to 80% confluent, the culture medium was changed to DMEM lacking fetal bovine serum and maintained for 24 hours. Experiments were initiated with fresh DMEM with five percent fetal bovine serum.
Cells were lysed on ice for 30 minutes in lysis buffer (containing 0.15 mol/L NaCl, 30 mmol/L Tris, 1 mmol/L phenylmethanesulfonyl fluoride, one percent Triton X-100, 1 mmol/L EDTA, 10 μg/ml leupeptin, 2 μg/ml pepstatin, 2 μg/ml aprotinin, and 2 mmol/L Na₂VO₄). Cell lysates (25 μg) of total protein were loaded per well and separated on a 10% SDS polyacrylamide gel. Proteins were then transferred to polyvinylidene difluoride membrane. Primary antibodies were as follows: anti-cAMP response element-binding protein (CREB), anti-phospho (Ser9) CREB, anti-Akt, anti-phospho (Ser473) Akt, anti-actin antibodies (1:1000 dilution; Sigma), anti-peroxisome proliferator–activated receptor (PPAR)–γ coactivator-1α (PGC-1α) anti-PPAR-α, anti-phospho (Ser52) eIF2α, anti-eIF2α, anti-phospho (Ser21) GSK3β, and anti-GSK3β antibodies (1:1000 dilution; Santa Cruz Biotech, Santa Cruz, CA). The secondary antibody was a peroxidase-coupled anti-rabbit or mouse IgG (1:5000 dilution; Amersham Biosciences, Piscataway, NJ). The membrane was exposed to ECL Hyperfilm (Amersham Biosciences), and the film was developed.

**Glucose Tolerance Test**

For glucose tolerance tests, mice fasted overnight. After baseline blood collection, mice were injected intraperitoneally with glucose (2 mg glucose/g body weight). Blood samples were taken from the tail of animals at 20, 40, 60, 90, and 120 minutes after glucose load. The blood glucose concentration was measured (OneTouch Ultra Glucometer; Lifescan, Milpitas, CA). Plasma insulin levels were determined by a radiomunoassay kit (Linco Research, St Charles, MO).

**Plasmid Construction for the Short Hairpin RNA**

To induce stable RNA interference-mediated knockdown of the TRB3 gene, pSilencer2.1-U6 plasmids were constructed to express short hairpin RNAs. Phosphorlated and annealed 64-mer double-stranded DNA oligonucleotides, incorporating BamHI and HindIII restriction sites, were ligated into the respective sites in the pSilencer2.1-U6 vector. The RNA interference sequence was 5′-CGAGCTCGAAGTGGGCCCC-3′ for TRB3.

**xbp1 mRNA Splicing Assay**

X-box binding protein-1 (xbp1) mRNA splicing was detected as previously described. In brief, PCR primers, 5′-ACACGGCTTGGAATGACAC-3′ and 5′-CCATGGGAAAGATGTCTGGG-3′, encompassing the spliced sequences in xbp1 mRNA were used for PCR amplification with polymerase (TianScript M-MLV; Tiangen Biotech, Beijing, China). The PCR products were separated by electrophoresis on a 2.5% agarose gel and visualized by ethidium bromide staining.

**RNA Interference**

The PPAR-α–targeted small interfering RNA (siRNA) (sense, 5′-pGAUCGAGCUGCAAGAUAUCDAdT-3′; antisense, 5′-pGGAUCUUGAGACUCUCUACUAUdT-3′) and the control siRNA targeting the secreted alkaline phosphatase reporter gene (GenBank accession No. U89937) (sense, 5′-pAGGGCAACUUCAGACCCAUdTD-3′; antisense, 5′-pAUGGGUCUGAGAUUGCUCUUdTD-3′) were obtained from Takara. The siRNAs were trans-
duced according to the methods previously described. Briefly, hepatocytes at a density of $5 \times 10^5$ cells were transfected with siRNA at a final concentration of 200 nmol/L using TransIT-siQuest transfection reagent (Mirus Bio Corporation, Madison, WI), according to the manufacturer’s protocol. Hepatocytes were harvested 24 hours after transfection, and total RNA or protein was extracted.

### Statistical Analysis

Data from experiments were expressed as the mean ± SD. The statistical difference between the groups was analyzed using one-way analysis of variance, followed by a Student-Newman-Keuls test. $P < 0.05$ was considered statistically significant.

### Results

#### Hepatic Glycogen Synthesis Is Reduced in HHcy

In this study, HHcy in mice was induced by drinking water containing two percent methionine. After 3 months on the diet, mice demonstrated an approximately nine-fold increase in plasma levels of homocysteine compared with control mice fed a normal diet ($26.5 \pm 2.7$ versus $2.9 \pm 0.4$ μmol/L). After ad libitum–fed animals were sacrificed, the effect of HHcy on hepatic glycogen content was analyzed. The levels of hepatic glycogen were markedly reduced, with HHcy mice displaying approximately 48% less glycogen than control mice (Figure 1, A and B). The reduced glycogen content in the liver is probably because of an alteration of enzymes involved in glycogenesis. To test this possibility, the phosphorylation levels of glycogen synthase were determined in the liver. The phosphorylation levels of glycogen synthase were significantly increased in the liver from HHcy mice (Figure 1C), indicating decreased glycogen synthase activity. In the fed state, liver preferentially stores glucose as glycogen. To further study the effect of HHcy on glycogen metabolism, we assessed the rate of glycogenesis in the liver using a tritiated water method. As shown in Figure 1D, glycogenesis in the liver was approximately twofold lower in HHcy mice than in control mice.

#### HHcy Produces Glucose Intolerance

The reduction in glycogenic capacity of HHcy mice prompted us to evaluate possible changes in glucose homeostasis. First, we determined blood glucose and plasma insulin levels after a 16-hour fast. Compared with control mice, HHcy mice exhibited significantly increased fasting plasma insulin levels (Figure 2A). In contrast, fasting blood glucose levels in HHcy mice remained unchanged (Figure 2B). Next, we performed a glucose tolerance test in mice, evaluating the ability of the body to adjust glucose levels after an immediate glucose injection.
tion. When injected i.p. with glucose (2 g/kg), the glucose levels of normal mice peaked at 20 minutes and returned to the basal levels at 60 minutes (Figure 2B). In contrast, HHcy mice showed a delayed response, indicating glucose intolerance.

**Homocysteine Inhibits the Phosphorylation of Akt and GSK3β**

The GSK3β is a critical component in the regulation of glycogen synthase activity by inducing its phosphorylation.29 In insulin-responsive tissue, insulin may inhibit the activity of GSK3β via Akt-mediated phosphorylation of an Ser residue (Ser21).29 To determine the effect of HHcy on the phosphorylation of GSK3β in the liver of mice, animals that fasted were injected with insulin and liver tissue was collected at 10 minutes after intravenous injection. As shown in Figure 3A, the phosphorylation levels of GSK3β (Ser21) in the liver of HHcy mice were lower than those in the liver of control mice. Because GSK3β is a major downstream target of Akt (Ser473), which results in its activation. On activation, Akt phosphorylates and inactivates GSK3β.30 As shown in Figure 3B, the phosphorylation levels of Akt (Ser473) were significantly lower in the liver of HHcy mice.

**HHcy Induces Up-Regulation of Hepatic TRB3 Expression**

Previous studies31,32 have shown that HHcy induces the UPR in liver, which is responsible for abnormal lipid metabolism. To clarify whether the UPR is involved in HHcy-mediated impairment in glycogen metabolism, we examined the markers of UPR activation in the liver of mice (fed ad libitum). However, neither xbp1 mRNA splicing nor increased elf2α phosphorylation was observed in the liver of mice with HHcy (see Supplemental Figure S1 at [http://ajp.amjpathol.org](http://ajp.amjpathol.org)). Meanwhile, the mRNA levels of ATF4 and the two UPR target genes (78-kDa glucose-regulated protein and CHOP) in HHcy mice were similar to those in normal mice (Figure 4A). In contrast, the mRNA and protein levels of TRB3 were significantly up-regulated in the liver of HHcy mice compared with those in the liver of normal mice (Figure 4, A and B). Although TRB3 is thought to be a target gene of CHOP and ATF4,18 these results suggest that the up-regulation of hepatic TRB3 expression is not because of the UPR in HHcy mice. Furthermore, in vitro studies also demonstrated that homocysteine treatment induced an increase in the mRNA and protein levels of TRB3 in HepG2 cells (Figure 4, C and D).

The TRB3 inhibits insulin signaling by preventing the phosphorylation of Akt.20 Thus, the induction of TRB3 is a potential mechanism by which homocysteine could block Akt activation. To further confirm these results, the effect of homocysteine on insulin-induced Akt phosphorylation was determined in HepG2 cells. Serum-starved HepG2 cells were preincubated with 100 μmol/L homocysteine for 6 hours before stimulation for 10 minutes with 100 nmol/L insulin. As shown in Figure 4, E and F, insulin-mediated phosphorylation of Akt (Ser473) and GSK3β (Ser21) was prevented by preincubation with homocysteine. To determine whether the induction of TRB3 was responsible for the inhibition of Akt and GSK3β phosphorylation by homocysteine, we disrupted endogenous TRB3 expression in HepG2 by RNA interference (see Supplemental Figure S2 at [http://ajp.amjpathol.org](http://ajp.amjpathol.org)). As shown in Figure 4, E and F, knockdown of TRB3 by short hairpin RNA blunted the inhibitory effect of homocysteine on the phosphorylation of Akt and GSK3β induced by insulin.

**Induction of TRB3 Expression in HHcy Mice Is Mediated by the PGC-1–PPAR-α Pathway**

A previous study33 has demonstrated that TRB3 is induced by PGC-1α through PPAR-α in liver and hepatocytes. The elevated expression of TRB3 has also been observed in skeletal muscle of PGC-1α-transgenic mice.34 Thus, we determined the effect of HHcy...
on the expression of PGC-1α and PPAR-α. As shown in Figure 5, A and B, the mRNA and protein levels of PGC-1α were significantly up-regulated in the liver of HHcy mice. Furthermore, homocysteine treatment also induced the mRNA and protein levels of PPAR-α in the liver of HHcy mice. (Figure 5, A and B). To test the role of PPAR-α in the induction of TRB3 expression, primary cultured hepatocytes were pretreated with an inhibitor of PPAR-α, MK886. As shown in Figure 6, A and B, MK886 (50 μmol/L) significantly inhibited the expression of TRB3 induced by homocysteine (Figure 6, C and D).

**HHcy Activates the cAMP-CREB Pathway**

The expression of PGC-1α is strongly induced by cAMP in primary cultures of hepatocytes. In this study, we found that both the levels of hepatic cAMP (Figure 7A) and the phosphorylation of CREB were markedly higher in HHcy mice than control mice (Figure 7B). Homocysteine treatment also induced the phosphorylation of CREB in hepatocytes (Figure 7C). These results are consistent with observations from Woo et al. As expected, pretreatment with H89 (5 μmol/L) (a specific inhibitor of protein kinase A) or adenylyl cyclase toxin (100 μmol/L) (a specific inhibitor of adenylyl cyclase) markedly suppressed the effect of the phosphorylation of CREB in hepatocytes (Figure...
To determine whether the cAMP-CREB pathway is responsible for the induction of PGC-1 and TRB3 expression by homocysteine, hepatocytes were preincubated with H89 or adenylyl cyclase toxin. As shown in Figure 7, D–F, H89 and adenylyl cyclase toxin attenuated the expression of PGC-1 and TRB3 induced by homocysteine. These data suggest that the elevation of hepatic cAMP levels is involved in the induction of PGC-1 and TRB3 expression in mice with HHcy.

Figure 5. The expression of PGC-1α is up-regulated in mice with HHcy. A: Total RNA was extracted from the liver of mice. The mRNA levels were detected by real-time PCR. All results are standardized to the levels of β-actin and are the mean ± SD (n = 6 in each group). *P < 0.05 versus control (CTR). B: The levels of proteins were measured using Western blotting analysis. Representative Western blots are shown. *P < 0.05 versus control (CTR).

Figure 6. Induction of TRB3 expression by homocysteine is PPAR-α dependent. Primary cultured hepatocytes were preincubated with MK886 (50 μmol/L) for 30 minutes after treatment with homocysteine (100 μmol/L) (Hcy) for 6 hours. Total RNA was extracted and subjected to real-time PCR. All results are standardized to the levels of β-actin and are the mean ± SD of five experiments. *P < 0.05 versus CTR (without Hcy). D: The levels of proteins were measured using Western blotting analysis. Representative Western blots are shown. *P < 0.05 versus CTR (without Hcy) (n = 5).
Discussion

Although in vivo and in vitro studies suggest that homocysteine causes insulin resistance, the mechanisms of homocysteine-induced insulin resistance are incompletely understood. In the present study, we found that the fasting basal levels of serum insulin were elevated twofold in HHcy mice, suggesting compensatory hypersecretion due to peripheral insulin resistance.GL Meanwhile, glucose tolerance test results demonstrated that glucose tolerance was impaired in HHcy mice, indicating lowered insulin sensitivity. The development of progressive glucose intolerance in HHcy mice may result from a reduction of glycogen in liver. To test this hypothesis, we studied the impact of homocysteine on glycogen metabolism. The hepatic glycogen content and the rate of glycogen synthesis were significantly lower in HHcy mice and were reduced by approximately 40% to 50% relative to normal mice. Moreover, HHcy mice exhibited an increase in the phosphorylation of glycogen synthase in the liver from HHcy mice, reflecting a decrease in glycogen synthase activity. In liver, the main consequence of insulin resistance is unrestrained hepatic glucose production; underlying mechanisms include decreased glycogen synthesis and failure to suppress gluconeogenesis. The elevations of homocysteine cause activation of the UPR in the liver of mice. Because the activation of UPR has been involved in insulin resistance, we first tested whether the effect of homocysteine on glycogen metabolism is the result of activation of the UPR. However, xbp1 mRNA splicing, the phosphorylation of eIF2α, and the expression of 78-kDa glucose-regulated protein and CHOP did not significantly change in the liver of HHcy mice, which had an eightfold elevation in plasma levels of homocysteine. Recently, Gupta et al reported that even cystathionine beta-synthase-deficient mice with a mean serum homocysteine concentration of 169 μmol/L do not exhibit the activation of the UPR. These results suggest that the UPR, per se,
is unlikely to play a role in the inhibitory effect of HHcy on glycogen synthesis. Interestingly, a significant increase in the expression of TRB3 was observed in the liver of HHcy mice or HepG2 cells after homocysteine treatment. Although TRB3 has been the target of CHOP and ATF4, a direct causal role for CHOP and ATF4 mediating the up-regulation of TRB3 expression in mice with HHcy is excluded.

The phosphatidylinositol 3-kinase–Akt pathway plays a central role in regulating glucose transport, gluconeogenesis, and glycogen synthesis. In vitro studies have demonstrated that homocysteine thiolactone disrupts insulin signaling by inhibiting insulin-stimulated phosphatidylinositol 3-kinase activity and phosphorylation of GSK3β and glycogen synthesis in HTC hepatoma cells. We observed that the phosphorylation of Akt in the liver was significantly lower in HHcy mice than in control mice. The GSK3β is a major downstream target of Akt, which can phosphorylate GSK3β to make it inactive. In this study, our results also demonstrated that the phosphorylation levels of GSK3β were dramatically decreased in the liver of HHcy mice. The GSK3β can phosphorylate and thereby inactivate glycogen synthase, resulting in reduced glycogenesis. Thus, dephosphorylation of GSK3β by homocysteine may explain the inhibitory effect on glycogen synthesis. The TRB3 has interacted directly with Akt and suppressed the phosphorylation of this kinase in liver. In this study, knockdown of TRB3 by short hairpin RNA suppressed the inhibitory effect of homocysteine on the phosphorylation of Akt and GSK3β in HepG2. Thus, TRB3 is probably a critical component in the glycogen metabolism disorders mediated by homocysteine. Interestingly, reduced activity of Akt by inducing the expression of TRB3 is also observed in hepatic tissues in rats fed long-term with ethanol. It is well established that long-term ethanol consumption leads to a marked decrease in hepatic glycogen content, which is related to a depressed rate of synthesis. Elevated phosphorylation levels of CREB and protein kinase A activities in the liver of HHcy mice have been previously reported. Our results demonstrated that the levels of intracellular cAMP and CREB phosphorylation were increased in the liver of HHcy mice. Both H89 and adenylyl cyclase toxin significantly suppressed homocysteine-induced CREB phosphorylation in hepatocytes. Both in vivo and in vitro studies indicate that PPAR-α promotes TRB3 expression in liver and hepatocytes in a PGC-1α–dependent manner. Because CREB is the main positive regulator of PGC-1α expression in liver, the up-regulation of TRB3 expression induced by homocysteine is probably mediated by the PGC-1α–PPAR-α pathway. To test this hypothesis, we determined the effect of homocysteine on the expression of PGC-1α. In the liver of HHcy mice, the mRNA and protein levels of PGC-1α, rather than PPAR-α, were markedly elevated. Homocysteine treatment (25 to 100 μmol/L) also resulted in an increase in the expression of PGC-1α in hepatocytes but did not alter the expression of PPAR-α (data not shown). However, both the PPAR-α inhibitor MK886 and knockdown of PPAR-α by siRNA significantly suppressed the homocysteine-mediated up-regulation of TRB3 in hepatocytes. The PGC-1α–PPAR-α pathway is located downstream of CREB. Our results demonstrated that the inhibition of CREB phosphorylation by H89 (5 μmol/L) or adenylyl cyclase toxin attenuated the expression of PGC-1α and TRB3. Taken together, these data suggest that the cAMP–CREB–PGC-1α–PPAR-α pathway probably plays a critical role in homocysteine-induced expression of TRB3 in liver.

Based on the data presented herein and on previously published data, we propose the following scheme by which HHcy inhibits glycogen synthesis in liver (Figure 8). Homocysteine elicits the levels of intracellular cAMP and CREB phosphorylation, which, in turn, induces the expression of PGC-1α, leading to increased phosphorylation levels of glycogen synthase and reduced hepatic glycogen synthesis. Our study expands our view of the role of HHcy in insulin resistance.

Acknowledgments
We thank Dr. Jian-Guo Wu, Ph.D. (Wuhan University, Wuhan, China), for providing the pSilencer2.1-U6 plasmids; and Dr. Yu-Chen Xie, M.D. (The Second People’s Hospital of Yunnan, Kunming, China), for her technical help in glycogen staining.

References

![Figure 8. The proposed mechanism of homocysteine-induced inhibition of hepatic glycogen synthesis. Homocysteine induces an increase in the intracellular levels of cAMP and the phosphorylation (P) of CREB in hepatocytes. Activation of this pathway promotes the expression of PGC-1α, which, in turn, up-regulates the expression of TRB3. The TRB3 inhibits the phosphorylation of Akt and GSK3β. The GSK3β induces the phosphorylation of glycogen synthase (GS), leading to reduced glycogen synthesis.](image-url)

