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Interplay between H6PDH and 11β-HSD1 implicated in the pathogenesis of type 2 diabetes mellitus

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Abstract

Extensive studies have been performed on the role of 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) in metabolic diseases. Our previous study reported glucose could directly regulate hexose-6-phosphate dehydrogenase (H6PDH) and 11β-HSD1. Recently, we further investigated the interplay of H6PDH and 11β-HSD1 and their roles in hepatic gluconeogenesis and insulin resistance to elucidate the importance of H6PDH and 11β-HSD1 in pathogenesis of type 2 diabetes mellitus (T2DM). T2DM rats model and H6PDH or 11β-HSD1 siRNA transfected in CBRH-7919 cells were used to explore the effect of H6PDH and 11β-HSD1 in T2DM. The results showed that the expression and activity of H6PDH and 11β-HSD1 in livers of diabetic rats were increased, with the expressions of PEPCK and G6Pase or liver corticosterone increased apparently. It also showed that H6PDH siRNA and 11β-HSD1 siRNA could inhibit the protein expression and enzyme activity by each other. With H6PDH siRNA, the enhancement of gluconeogenesis was blocked and insulin resistance stimulated by corticosterone was reduced. H6PDH and 11β-HSD1 might be the effective and prospective targets for T2DM and metabolic syndromes, based on the interplay between these two enzymes.

Graphical Abstract

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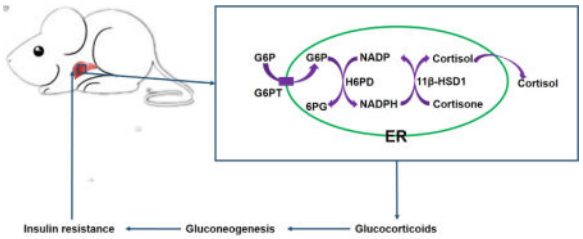

Keywords

H6PDH; 11β-HSD1; corticosterone; liver; type 2 diabetes mellitus

As essential endocrine hormones, glucocorticoids could regulate nearly all major physiological functions, from cell proliferation to differentiation, from glucose to lipid metabolism [1]. It has been reported that glucocorticoids are one of pivotal hormones involved in the pathogenesis of insulin resistance [2, 3]. Glucocorticoids promote gluconeogenesis and antagonize the hypoglycemic effect of insulin. It has also been documented that glucocorticoids stimulated its receptor GR (glucocorticoid receptor) to increase the expression of gluconeogenesis key enzymes, PEPCK or G6Pase in the liver of db/db mice [4, 5]. Several metabolic mechanisms are generalized in the mediation of glucocorticoid and its downstream signals. 11β-hydroxysteroid dehydrogenase 1 (11β-HSD1) is proved to be the key regulator of glucocorticoid on the pre-receptor level, which has been purified from hepatic microsomes of rats and cloned after it has been found several decades later [1, 6, 7].

11β-HSD1 is an enzyme in lumen of endoplasmic reticulum (ER) depending on NADPH, which has a high expression in liver, fat and skeleton muscle [8, 9]. It could be a dual enzyme. With the characteristic of reductase, it transferred inactivated glucocorticoid to activated glucocorticoid in local tissue. Activated 11β-HSD1 increased tissue glucocorticoid and induced insulin resistance, obesity and high blood glucose, which were all linked with T2DM [1]. On the contrary, inhibition of 11β-HSD1 suppressed generation of activated glucocorticoid and alleviated glucocorticoid reaction in liver and adipose tissue. Likewise, reductase activation of 11β-HSD1 was dependent on NADPH produced by H6PDH due to permeability of endoplasmic reticulum membrane to pyridine nucleotide [8].

H6PDH is located in ER lumen of hepatocytes and adipocytes as well, which was in the first reaction of pentose phosphate pathway catalysis. It has been reported previously that H6PDH knockout mice might have high fasting blood glucose because of no NADPH generation for hepatic 11β -HSD1 reductase impaired [10]. Nonetheless, there seems to be no specific discussion about interactions between 11β-HSD1 and H6PDH or their regulation of hepatic gluconeogenesis via insulin signaling pathway.

Therefore, the study hypothesized that H6PDH and 11β-HSD1 activated by corticosterone modulated each other and influenced PEPCK and downstream insulin signals, to promote glucocorticoid and insulin resistance. 11β-HSD1 and H6PDH would take the crucial roles in glucose metabolism regulation, according to this pathogenesis of T2DM.

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To elucidate the importance of 11β-HSD1 and H6PDH in T2DM, T2DM rat model [11] was established. The related biochemical parameters were measured and shown in Table 1. Compared with CON group, there was significant growth in FBG (22.57±2.85mmol/L versus 4.66±1.13mmol/L, $p<0.01$), TC (2.95±0.54mmol/L versus 1.62±0.11mmol/L, $p<0.01$) and TG (2.43±0.27mmol/L versus 0.44±0.08mmol/L, $p<0.01$), respectively. Conversely, blood insulin level was twice less in DM group than that in CON group. It suggested that pancreas islet β cells were impaired heavily and lacking in secretion of enough insulin. So the model of diabetes mellitus was at decompensation stage.

To examine the insulin sensitivity and glucose tolerance level of T2DM rats, glucose tolerance test [12] and insulin tolerance test [13] were carried on. After STZ injection for 4 weeks, the glucose tolerance level was shown (Fig. 1A). In CON group, blood glucose increased rapidly to the peak after glucose injection of 30min and went down gradually from 30min to 90min. In DM group, there was a similar trend of glucose level. Average glucose levels were much higher than that in CON group ($P<0.01$). For insulin tolerance (Fig. 1B), glucose level went down slower than that in CON group. The percentages of initial blood glucose in the time line of DM group were apparently higher than those in CON group ($P<0.01$). At 120min, in DM group blood glucose took up initial blood glucose of 80% approximately and in CON glucose was about 40%. So the rats were with hyperglycemia and insulin resistance. The suitable T2DM rat model was established successfully.

To measure the content of corticosterone in serum and liver of DM and CON group [14], it was shown by fluorescence analysis in Table 2. Compared with CON group, there seemed to be no differences in the amount of corticosterone in DM group serum. But hepatic corticosterone was increased significantly (261.27±20.81ng/100mg compared with 179.22±17.53ng/100mg, $p<0.01$). Thus the statistical difference of corticosterone level would be more obvious in tissue than in serum.

To analyze H6PDH and 11β-HSD1 expression [15] and activity [16], it was found that the expressions of H6PDH and 11β-HSD1 in DM group were higher than those in CON group (Fig. 1C and D). They had the same results for enzyme activity (Fig. 1E and F). As hepatic gluconeogenesis key enzymes, the expression of PEPCK and G6Pase were higher in DM group than in CON group (Fig. 1G and H).

For another, CBRH-7919 cell line was used in vitro for proving the importance of H6PDH and 11β-HSD1 or their interaction in T2DM. To establish the model and choose the optimal transfection ratio [17], the cells were cultured for 48h. It could be observed in the microscope that over 70% of cells were shown with green fluorescence with 100 times (Fig. 2A and C) and 200 times (Fig. 2B and D). Compared with normal cells, H6PDH expression was no difference after negative control interfering (Fig. 2E). Compared with negative control interfering, the optimal concentration was 3–4μg, which blocked the expression of H6PDH at most ($p<0.01$). Compared with negative control group, 3–4μg was also a good choice for 11β-HSD1 siRNA via CBRH-7919 transfection for 72h incubation (Fig. 2F), because 11β-HSD1 expression decreased apparently ($p<0.01$).

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To examine the interplay of H6PDH and 11β-HSD1 in RNA level [18], protein level [15] and protein activity [19] with H6PDH and 11β-HSD1 siRNA, RT-PCR, WB and activity assay were conducted. There were no differences in RNA, expression and activity of H6PDH and 11β-HSD1 from negative control group. Compared with negative control, RNA levels were decreased significantly ($p<0.01$) for H6PDH and 11β-HSD1 (Fig. 3A and B). It was found that transfected with H6PDH siRNA, the expression of H6PDH could decrease significantly and 11β-HSD1 expression was suppressed (Fig. 3C). However, when cells were incubated with 11β-HSD1 siRNA, 11β-HSD1 expression could be decreased with suppression of H6PDH expression (Fig. 3D). Additionally, in H6PDH siRNA transfected group, H6PDH activity could be affected apparently with 11β-HSD1 activity decreased by one fourth (Fig. 3E). In 11β-HSD1 siRNA transfected group, 11β-HSD1 and H6PDH activity were both decreased (Fig. 3F).

To examine whether the influence of interplay between H6PDH and 11β-HSD1 could affect hepatocyte gluconeogenesis, protein level changes were recorded by western blot [15]. 10^{-6}M corticosterone stimulation [20] could upregulate the expression of H6PDH (Fig. 4A) and 11β-HSD1 (Fig. 4B). This reaction was blocked by insulin. After H6PDH expression decreased by H6PDH siRNA interfering, corticosterone still upregulated H6PDH and 11β-HSD1 expression. The suppression of insulin to H6PDH was weakened, but the suppression of insulin to 11β-HSD1 was still existed. 10^{-6}M corticosterone also upregulated gluconeogenesis key enzyme PEPCK in RNA (Fig. 4C) and protein level (Fig. 4D). The expression of PEPCK towards insulin was not significantly suppressed.

To further examine the influence of hepatocyte insulin signaling pathway by H6PDH, protein expressions were measured by western blot [15]. Without stimulation of corticosterone, the expressions of IR-β, IRS1, Akt, p-Akt were upregulated after insulin stimulation. When CBRH-7919 cells were incubated in corticosterone, the expressions of IR-β, IRS1, Akt, p-Akt were not upregulated after the same insulin stimulation (Fig. 4E). In addition, when H6PDH expression decreased by H6PDH siRNA transfection, insulin resistance induced by corticosterone was alleviated, and the expressions of IR-β and p-Akt were increased obviously after insulin stimulation (Fig. 4F). Thus blocking of H6PDH attenuated insulin resistance induced by corticosterone.

Previously, a great number of evidence proved that glucocorticoid took a crucial role in the development of obesity or T2DM. Hepatic gluconeogenesis was activated by glucocorticoid, which inhibited glucose reduction via insulin. These were thought to be the main causes leading to high blood glucose level of T2DM patients [21, 22]. It was also reported that glucocorticoid level in insulin target tissues might be regulated by 11β-HSD1, such as liver, skeleton muscle, visceral fat, etc. [4, 23]. 11β-HSD1 might have glucocorticoid combine with cellular GR to show the tissue specific activation of glucocorticoid. Additionally, according to the structure characteristics, limited and selective permeability of ER membrane to pyridine nucleotide, reductase activity of 11β-HSD1 located in lumen of ER depended on providing NADPH from H6PDH [8, 24, 25]. 11β-HSD1 was regulated by H6PDH in ER of liver and adipose tissue catalyzing G6P and $NADP^+$ to generate NADPH [26]. It has been announced that reductase activation of hepatic 11β-HSD1 in H6PDH knockout mice might be impaired due to no generation of NADPH [10]. In our studies, rats with stable blood

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glucose showed some typical syndromes of clinical T2DM, such as high blood glucose, high blood lipid, insulin resistance and insufficient insulin secretion. It was also illustrated that the expression and activity of H6PDH and 11β-HSD1 in T2DM rats were significantly enhanced, and hepatic corticosterone amount was higher than that in CON group. In our cellular experiments, it illuminated further that H6PDH and 11β-HSD1 might have a bidirectional regulation mechanism. Furthermore, the crucial factors of development in insulin resistance competition for mankind is partial production of hydrocortisone and 11β-HSD1 expression increasing via seizing more GR [27]. The ratio of hydrocortisone/cortisone was also higher in patients with hypothalamic obesity mainly based on the consequence of increased 11β-HSD1 activity. Likewise, it has been reported that in db/db mice, excess corticosterone might result in high blood glucose, insulin resistance and obesity. When corticosterone level increased in circulatory system, hepatic H6PDH would be activated and level went up for more NADPH production and 11β-HSD1 reductase activity [28–30]. Inhibition of hepatic 11β-HSD1 activity could improve insulin sensitivity and decrease the expression of PEPCK [31, 32]. Our results also proved that PEPCK and G6Pase expression in T2DM group apparently higher than CON group. Therefore, it speculated that H6PDH activated by corticosterone upregulated 11β-HSD1 and 11β-HSD1 could strengthened gluconeogenesis in T2DM via stimulating GR and activating gluconeogenesis key enzymes. 11β-HSD1 might also suppress H6PDH.

Subsequently, to study the mechanism how H6PDH and 11β-HSD1 regulate the gluconeogenesis and insulin resistance, we built up the cellular models. It was reported when preadipocytes 3T3-L1 was stimulated with 10^{-6}M dexamethasone over 24h, insulin resistance might occurred [33]. Similarly, insulin resistance happened when CBRH-7919 was incubated in 10^{-6}M corticosterone about 48h in our studies. After transfected with H6PDH siRNA for 24h, CBRH-7919 was incubated in 10^{-6}M corticosterone for 48h. With insulin stimulation, it was found that corticosterone up-regulated the expression of H6PDH, improved NADPH production with 11β-HSD1 activity increasing and alleviated the inhibition of PEPCK by insulin. It was also found that cellular protein expressions stimulated by insulin were up-regulated, including IR-β, IRS-1, Akt and p-Akt. But their expressions with corticosterone and insulin stimulation weren't upregulated. Thus, it elucidated that corticosterone might induce insulin resistance in rat hepatocytes. Moreover, H6PDH siRNA transfection inhibited PEPCK expression, weakened insulin resistance induced by corticosterone, and upregulated IR-β and p-Akt expression under insulin resistance condition. It suggested that inhibition of H6PDH not only blocked gluconeogenesis induced by corticosterone stimulation, but also impaired corticosterone-induced insulin resistance, which was the possible mechanism for H6PDH in gluconeogenesis and insulin sensitivity.

In summary, all the results above illustrated that H6PDH and 11β-HSD1 might be the potential targets of T2DM and metabolism disorders. The interaction between H6PDH and 11β-HSD1 might be a prospective option for developing a novel treatment of T2DM. These studies might provide the theoretical support for clinical T2DM treatment.

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Acknowledgments

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11. 30 male Wistar rats (Experimental animal center holding of Jilin University) were purchased to develop the diabetic rat model, according to the method of high-fat diet with multiple low-doses of streptozotocin (STZ) injection [34, 35]. Firstly, rats were divided randomly into control group (CON) with standard chow (5% calories from fat) and diabetic group (DM) with multiple STZ injection (DM) with high-fat diet feeding (22% calories from fat) (The artificial diet center of the experimental animal holding facility). After standard or high-fat diets feeding for 4 weeks, DM group and CON group were injected intraperitoneally with STZ (30mg/kg) and equal volume of vehicle citrate buffer (pH4.4), respectively. STZ was dissolved in 0.1M sodium citrate buffer at pH4.4. Then, they were injected again in the next week with the same dose of STZ or vehicle citrate buffer. The diagnostic criteria of diabetic rat model was that fasting blood glucose (FBG) should be over 7.8mmol/L. So FBG was measured after STZ injection for another 4 weeks. If model was established successfully, the rats were fasting for 12–16h. Blood samples were collected from tail vein and centrifuged for 15min at 3500g. FBG, total cholesterol (TC) and triglyceride (TG) were measured with kits (Beijing BHKT Clinical reagent Co., Ltd). Fasting insulin was measured by radioimmunoassay kit (Shanghai Institute of Biological Products). They were sacrificed under anesthesia with liver collected in −80°C for analysis.
12. After a 12–16h fasting period, FBG was measured as 0min samples. The rats for IPGTT were conducted with an intraperitoneal injection of 40% glucose (2g/kg of body weight). The other samples were collected at 30min, 60min and 120min from tail vein.

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13. For ITT, the rats with an 8h-fasting period were conducted with an intraperitoneal injection of insulin (0.75IU/kg, Novolin R; Eli Lilly). The samples were also collected at 0min, 30min, 60min and 120min for glucose oxidase kit measurement (Beijing BHKT Clinical reagent Co., Ltd).
14. 100μg/ml corticosterone standard buffer should be prepared first. 10mg corticosterone was dissolved in 100ml total ethanol for storage buffer. 0.4μg/ml standard buffer was prepared with 40μl storage buffer and 10ml 0.04mol/L NaOH. The sulfuric acid and ethanol for coloring was prepared with 7 volumes of sulfuric acid and 3 volumes of pure ethanol. For standard curve establishing, standard buffer was diluted with 0.04mol/L NaOH for 0.02μg/ml, 0.04μg/ml, 0.06μg/ml, 0.08μg/ml, 0.10μg/ml, 0.12μg/ml, 0.16μg/ml and 0.20μg/ml. Fluorescence intensity was measured by standard curve. Next, 0.5ml serum or liver tissue was mixed with 0.5ml or 1ml 0.04mol/L NaOH buffer. The mixture was added with 5ml dichloromethane, shaking vigorously for 2min. After separation of distinct phases, water phase was dropped. Dichloromethane phase was collected and added with 2.5ml sulfuric acid including ethanol, shaking for 2min vigorously. After standing for 30min, the sulfuric acid and ethanol part was meas ured by fluorescence spectrophotometer. The results of fluorescence intensity were used for calculating the content of corticosterone.
15. Protein samples were extracted from liver and stimulated hepatocytes with examining of concentrations via Bradford assay (Bio-Rad protein assay kit). For western blot (WB) measurement, primary antibody was prepared and diluted with TBS, including H6PDH (1:1000), 11β-HSD1 (1:1000), PEPCK (1:1500), G6 Pase (1:1000), IRS-1 (1:1000), IR-β (1:1500), Akt (1:1500), p-Akt (1:1000) and β actin (1:5000) (Santa Cruz). The secondary antibody was goat-anti-rabbit antibody (1:2000, Santa Cruz). After incubation, membranes were pictured with ECL (Thermo Fisher Scientific Inc., USA) and X-ray film, analyzing by Quantity one.
16. H6PDH activity in vivo was determined by level of NADPH production [26, 36]. 20mg microsomes collected via liver homogenization and differential centrifugation, 0.5–5mM glucosamine-6-phosphate (G6P) and 1–5mM nicotinamide-adenine dinucleotide phosphate ($NADP^+$) were incubated in 100mM glycine buffer for 30min. NADPH production was measured as absorbance at 340nm by the spectrophotometer (Synergy 2 SL, BioTek) every 5min. Based on the results, the standard curve of NADPH would be calculated. Then, nanomoles of NADPH production per mg protein in one minute could represent H6PDH activity. 11β-HSD1 activity in vivo was determined by the content of corticosterone with ELISA kit (R&D, USA) [4, 37]. 20mg microsomes, 2μM 11-dehydrocorticosterone and 200μM NADPH were incubated in 50mM PBS for 1h at 37°C. According to the instructions of kit, 11β-HSD1 activity was presented by moles of corticosterone per mg protein in one minute.
17. Rat hepatocyte CBRH-7919 (TCR2) (Shanghai institute of Biochemistry and Cell Biology, Chinese Academy of Sciences) were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) with 10% fetal bovine serum (FBS). For plasmid transfection, CBRH-7919 were seeded in 6-well plate for 24h. After 70%–90% confluence, they were incubated in Opti-MEM for 30min. 1–4μg plasmids and 6μl FuGENE HD were mixed with 100μl Opti-MEM for 15min before culturing the hepatocytes. After culturing for 4–6h, medium were changed with DMEM. The negative control was the cells transfected by FuGENE HD with no homology sequence of target gene. H6PDH siRNA, 11β-HSD1 siRNA and negative control siRNA has been designed with GFP plasmid in pRNATin-H1.2 (Ambion company).
18. Cellular total RNA were collected from hepatocytes by TRIzol reagent (Invitrogen), according to the manufacturer's instructions. 2μg total RNA were used for cDNA synthesis with SuperScript reverse transcriptase and for reverse transcription production amplifying. The specific primers for rat contains *h6pdh* (forward: 5′-ATCATTACCTGGGCAAGC-3′, reverse: 5′-GCCATACTCCTCGTAGAAACT-3′), *11β-hsd1* (forward: 5′-GAAGAAGCATGGAGGTCAAC-3′, reverse: 5′-GCAATCAGAGGTTGGGTCAT-3′), *pepck* (forward: 5′-AGCCTCGACAGCCTGCCC CAGG-3′, reverse: 5′-CCAGTTGTTGACCAAAGGCTTTT-3′) and *gapdh* (forward: 5′-CCATGG AGAAGGCTGGG-3′, reverse: 5′-CAAAGTTGTCATGGATGACC-3′) [34]. The amplification conditions of h6pdh, 11β-hsd1, pepck, and gapdh were optimized in preliminary studies to result in amplification within the linear range. Specifically, the amplification conditions were as follows. h6pdh was for 94°C 30s, 57°C 30s and 72°C 15s with 30 cycles. 11β-hsd1 was for 94°C 30s, 60°C 30s and 72°C 15s with 32 cycles. pepck was for 94°C 30s, 59°C 30s and 72°C 35s with 30 cycles.

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gapdh was for 94°C 30s, 58°C 30s and 72°C 15s with 25 cycles. Then, PCR products were visualized on agarose gels with ethidium bromide staining. Finally, gels were taken pictures under UV light. Relative gene expression was analyzed by densitometry with analysis software.

19. H6PDH activity in vitro was also determined by NADPH production [26, 38]. 20mg cellular protein, 2mM G6P and 0.5mM $NADP^+$ were incubated in 100mM glycine buffer for 30min. After absorbance results collection every 5min, H6PDH activity was present by nanomoles of NADPH per mg protein in one minute. 11β-HSD1 activity in vitro was also represented by measurement of corticosterone with ELISA [4, 39]. 200nM 11-dehydrocorticosterone was added to cell medium and cultured at 37°C for 1h. Supernatant was centrifuged at 1000g for 10min as ELISA kit samples. Moles of corticosterone per mg protein in one minute represented 11β-HSD1 activity.
20. After transfection of CBRH-7919 with H6PDH siRNA and negative control plasmid for 24h, 10^{-6}M corticosterone was added for another 48h incubation. Before protein collection, 10^{-7}M insulin was added for measurements of insulin signaling proteins in hepatocytes.

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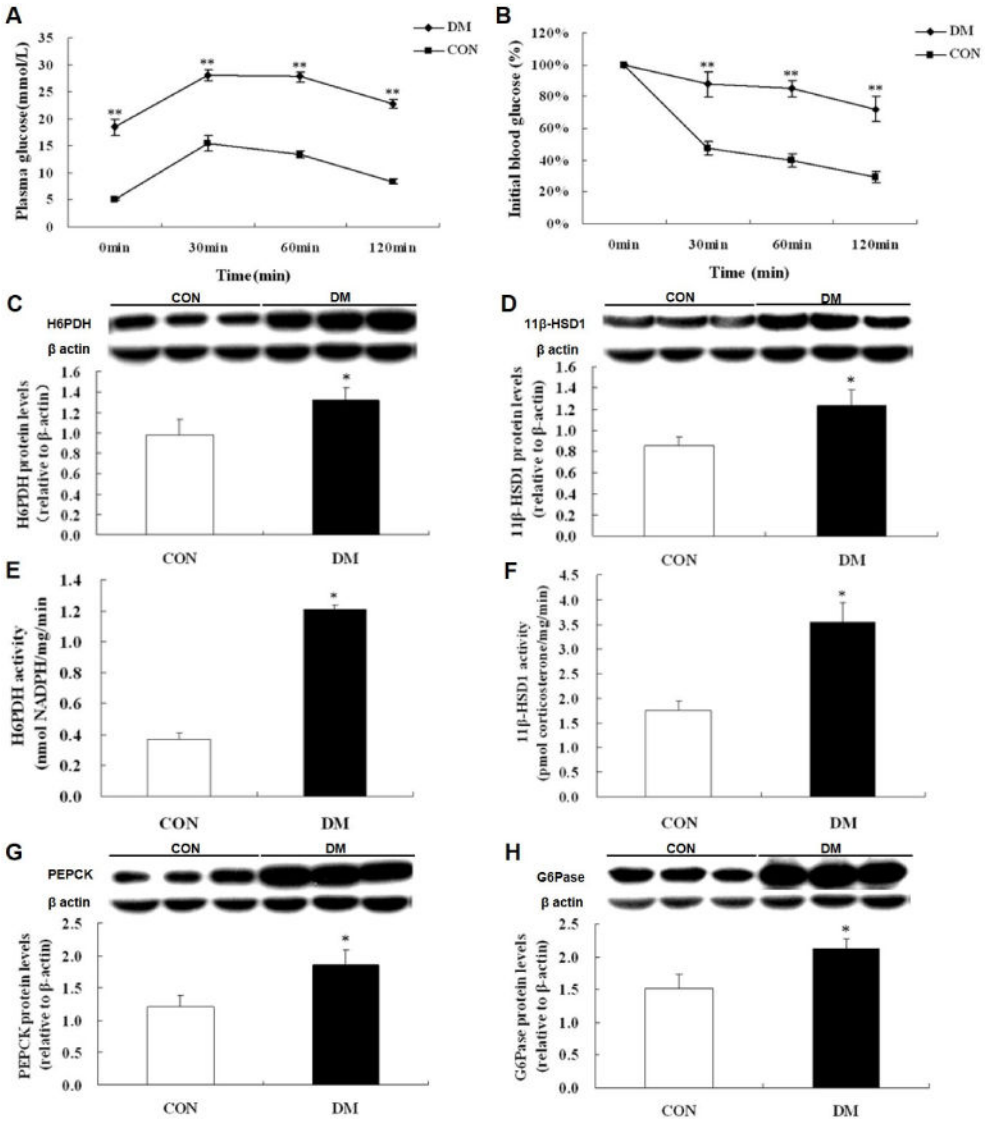

Fig. 1. Insulin resistance measurement, the expression and activity of H6PDH or 11β-HSD1 and key enzymes of gluconeogenesis in T2DM rats

A–B. IPGTT and ITT was performed compared between CON group and DM group after STZ injection for 4w. C–D. The protein expression of H6PDH and 11β-HSD1 in CON and DM group of liver. E–F. The activity of H6PDH and 11β-HSD1 in CON and DM group of liver. G–H. The protein expression of PEPCK and G6Pase in CON and DM group of liver.n≥5, **p<0.01, *p<0.05 compared with CON group.

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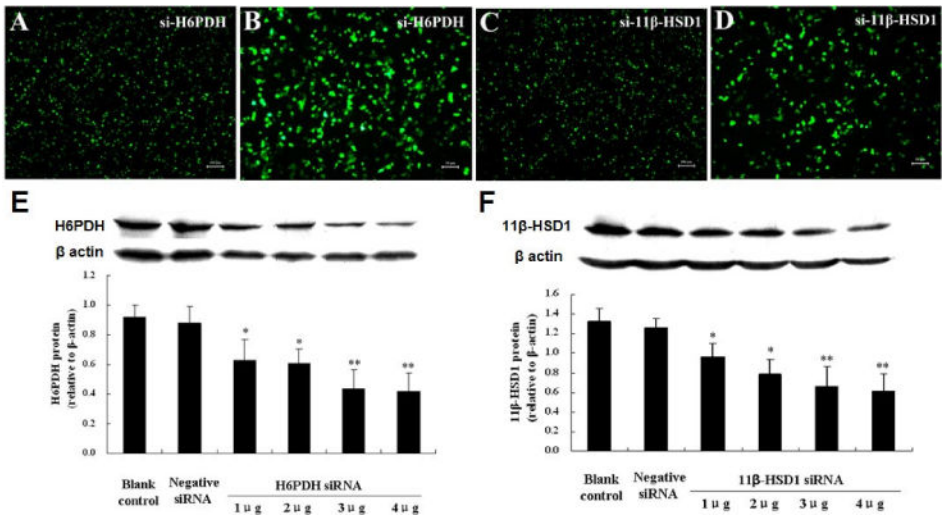

Fig. 2. The transfection ratio and concentration screening of H6PDH and 11β-HSD1 siRNA in rat hepatic cells

A–D. After H6PDH or 11β-HSD1 siRNA transfection for 48h, CBRH-7919 could be observed by fluorescence microscope with 100 times and 200 times. E. After H6PDH siRNA transfection in different concentrations for 72h, H6PDH expression was shown. F. After 11β-HSD1 siRNA transfection in different concentration for 72h, 11β-HSD1 expression was shown. *p<0.05, **p<0.01 compared with negative control.

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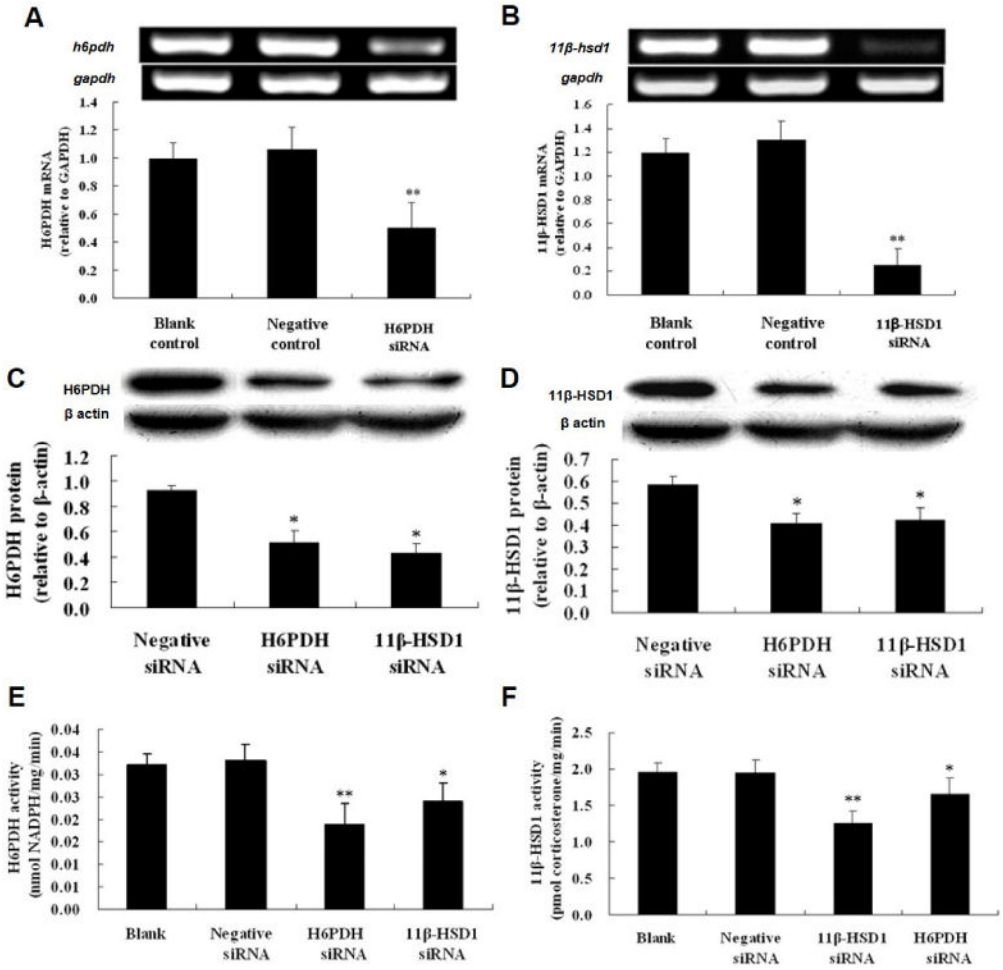

Fig. 3. The interplay of H6PDH and 11β-HSD1

A. After H6PDH siRNA transfection for 48h, *h6pdh* mRNA was measured. B. After 11β-HSD1 siRNA transfection for 48h, *11β-hsd1* mRNA was measured. C–D. H6PDH or 11β-HSD1 expressions in CBRH-7919 were measured after H6PDH and 11β-HSD1 siRNA transfection for 72h. E–F. After H6PDH and 11β-HSD1 siRNA transfection for 72h, H6PDH or 11β-HSD1 activities were recorded. *p<0.05, **p<0.01 compared with negative control.

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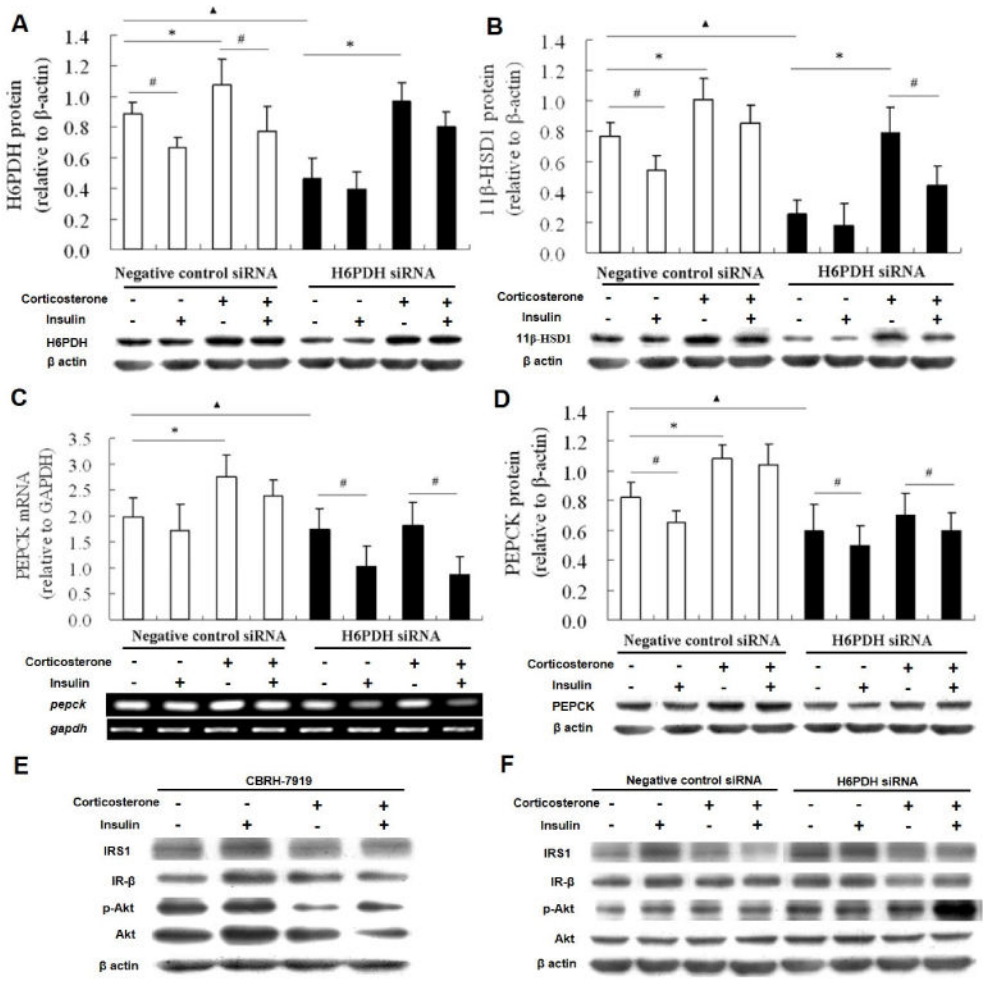

Fig. 4. The influence of hepatocyte gluconeogenesis by H6PDH and 11β-HSD1 siRNA and the influence of hepatocyte insulin signaling pathway stimulated by H6PDH siRNA

A–B. After H6PDH transfection for 24h, with 10^{-6}M corticosterone cultured for 48h and insulin stimulated for 30min, H6PDH and 11β-HSD1 expressions were shown. C–D. After H6PDH transfection for 24h, with 10^{-6}M corticosterone cultured for 48h and insulin stimulated for 30min, PEPCK mRNA and protein expressions were shown. E. After 10^{-6}M corticosterone cultured for 48h and insulin stimulated for 30min, protein expressions of CBRH-7919 were shown, including IR-β, IRS1, Akt and p-Akt, with β actin as standard reference. F. After H6PDH transfection for 24h, with 10^{-6}M corticosterone cultured for 48h and insulin stimulated for 30min, protein expressions of CBRH-7919 were shown, including IR-β, IRS1, Akt, p-Akt and β actin. ▲$p<0.05$ compared between H6PDH siRNA and negative control, *$p<0.05$ compared between with and without corticosterone cultured group, #$p<0.05$ compared between with and without insulin stimulation group.

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Table 1

Body weight and biochemical parameters of type 2 diabetes mellitus rats

Group	CON	DM
Weight(g)	363.9±6.02	376.6±7.25
FBG(mmol/L)	4.63±0.25	22.03±1.29*
Insulin(mIU/L)	13.50±1.39	6.96±0.75*
TC(mmol/L)	1.62±0.11	2.95±0.54*
TG(mmol/L)	0.44±0.08	2.43±0.27*

*$p<0.01$ compared with CON group ($n\geq10$)

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Table 2

The amount of serum corticosterone and liver corticosterone in CON group and DM group

Group	Serum Corticosterone (ng/ml)	Liver Corticosterone (ng/100mg)
CON	114.56±12.60	179.22±17.53
DM	119.48±15.48	261.27±20.81*

n≥5,

*p<0.01 compared with CON group