Hepatitis B Virus X Protein Regulates Hepatic Glucose Homeostasis via Activation of Inducible Nitric Oxide Synthase

Hye-Jun Shin, Young-Ho Park, Sun-Uk Kim, Hyung-Bae Moon, Do Sim Park, Ying-Hao Han, Chul-Ho Lee, Dong-Seok Lee, In-Sung Song, Dae Ho Lee, Minhye Kim, Nam-Soon Kim, Dae-Ghon Kim, Jin-Man Kim, Sang-Keun Kim, Yo Na Kim, Su Sung Kim, Cheol Soo Choi, Young-Bum Kim, and Dae-Yeul Yu

From the Disease Model Research Laboratory, Aging Research Center and World Class Institute, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Republic of Korea, the Department of Veterinary Medicine, Chungnam National University, Daejeon 305-764, Republic of Korea, the Department of Functional Genomics, University of Science and Technology, Daejeon 305-333, Republic of Korea, the Department of Pathology and Institute of Medical Science, Wonkwang University College of Medicine, Iksan 570-499, Republic of Korea, the Department of Natural Sciences, Kyungpook National University, Daegu 702-701, Republic of Korea, the Genome Research Center, Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-806, Republic of Korea, the Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts 02215, the Department of Internal Medicine, Chonbuk National University Medical School and Hospital, Jeonju 561-756, Republic of Korea, the College of Medicine, Chungnam National University, Daejeon 305-764, Republic of Korea, and the Lee Gil Ya Cancer and Diabetes Institute and Division of Endocrinology Gil Medical Center, Gachon University of Medicine and Science, Incheon 406-840, Republic of Korea.

Dysregulation of liver functions leads to insulin resistance causing type 2 diabetes mellitus and is often found in chronic liver diseases. However, the mechanisms of hepatic dysfunction leading to hepatic metabolic disorder are still poorly understood in chronic liver diseases. The current work investigated the role of hepatitis B virus X protein (HBx) in regulating glucose metabolism. We studied HBx-overexpressing (HBxTg) mice and HBxTg mice lacking inducible nitric oxide synthase (iNOS). Here we show that gene expressions of the key gluconeogenic enzymes were significantly increased in HepG2 cells expressing HBX (HepG2-HBX) and in non-tumor liver tissues of hepatitis B virus patients with high levels of HBx expression. In the liver of HBxTg mice, the expressions of gluconeogenic genes were also elevated, leading to hyperglycemia by increasing hepatic glucose production. However, this effect was insufficient to cause systemic insulin resistance. Importantly, the actions of HBx on hepatic glucose metabolism are thought to be mediated via iNOS signaling, as evidenced by the fact that deficiency of iNOS restored HBx-induced hyperglycemia by suppressing the gene expression of gluconeogenic enzymes. Treatment of HepG2-HBx cells with nitric oxide (NO) caused a significant increase in the expression of gluconeogenic genes, but JNK1 inhibition was completely normalized. Furthermore, hyperactivation of JNK1 in the liver of HBxTg mice was also suppressed in the absence of iNOS, indicating the critical role for JNK in the mutual regulation of HBx- and iNOS-mediated glucose metabolism. These findings establish a novel mechanism of HBx-driven hepatic metabolic disorder that is modulated by iNOS-mediated activation of JNK.

The liver is a key organ that regulates glucose homeostasis by balancing the storage of glucose and the output of glucose (1, 2). Pre-diabetes describes a condition affecting increasing numbers of the population who have abnormal glucose homeostasis but not diabetes mellitus and who suffer impaired glucose tolerance or fasting glucose (3). Glucose intolerance is common in patients with liver cirrhosis of various etiologies, and ~20% of cirrhotic patients have overt diabetes (4). Recent observational studies have provided consistent evidence on associations of diabetes with increased risk of cancers of the liver (5). In Korea, both elevated fasting serum glucose levels and a diagnosis of diabetes are regarded as independent risk factors for several major cancers, including stomach, pancreatic, lung, and liver cancers, and the risks of these conditions increase with an increased level of fasting serum glucose (6). HBV infection is also strongly associated with type 2 diabetes among Asian, Asian Americans, Pacific Islanders, and Italians (7–10). Dia-

This work was supported by the 21st Century Frontier Program in the Functional Human Genome Project (Grant HGMO200934), by the International Collaboration Program of Science and Technology (Grant FGM0600914), by the World Class Institute (WCI) Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology of Korea (MEST), by a grant from the National R&D Program for Cancer Control, Ministry for Health and Welfare (Grant BCM0061011), by the KRIBB Research Initiative Program Grant (Grant KGM3320911), by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare and Family Affairs, Korea (Grant A084651) (to C. S. C.), and by National Institutes of Health Grant R01DK083567 (to Y. B. K.) and American Diabetes Association Grant 1-09-RA-87 (to Y. B. K.).

The on-line version of this article (available at http://www.jbc.org) contains supplemental methods and Tables 1 and 2.

1 These authors contributed equally to this work.
2 To whom correspondence may be addressed: 330 Brookline Ave., CLS-736, Boston, MA. Fax: 617-735-3323; E-mail: ykim2@bidmc.harvard.edu.
3 To whom correspondence may be addressed: 52 Oun-dong, Yuseong-gu, Daejeon 305-806, Republic of Korea. Fax: 82-42-860-4609; E-mail: dyyu10@kribb.re.kr.

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Betic hyperglycemia is associated with serum alanine amino-transferase activity in patients with hepatitis B infection (11). However, the mechanisms by which HBV leads to glucose metabolism are not elucidated at all. HBV is a hepatotrophic virus that causes acute and chronic hepatocellular injury. HBV X protein (HBx), one of four open reading frames in the HBV genome, has been implicated in regulating apoptosis, inflammation, and tumorigenesis (12, 13) by acting as a coactivator of transcription factors, including nuclear factor-κB (NF-κB) (14), cAMP-response element-binding protein (CREB) (15), and mitogen-activated protein kinases (MAPKs) (16). Our previous studies demonstrated that HBx

**FIGURE 1.** HBx regulates the gene expression of glucose metabolism-related molecules in hepatocytes. A, expression profiles of metabolic genes in HepG2 cells. B, expression profiles of metabolic genes of HepG2-HBx cells infected with lentiviruses expressing HBx shRNA. mRNA levels were analyzed by qRT-PCR. C, increased expression of gluconeogenic genes in HBV-infected patient livers. mRNA was purified from the non-tumor regions of patient livers with HBx expression and cancer (n= 15–22). Data are presented as the mean ± S.E. *, p < 0.05; **, p < 0.01; ***, p < 0.001 versus control.

**FIGURE 2.** HBx expression causes impaired hepatic glucose metabolism in mice. A, growth curves were constructed by plotting mean ± S.E. body weight of WT and HBxTg mice (n= 11–16). B, comparison of liver and epididymal fat weight/body weight between WT (n = 7) and HBxTg (n = 8) mice at 6 months of age. C, glucose concentration in blood derived from mouse tail vein in 12-h fasted and ad libitum-fed mice at 6 months of age. D and E, insulin sensitivity was examined by a glucose tolerance test (D) and insulin tolerance test (E). Mice were 6 months of age (n= 10–13). F, glycogen content was measured in liver of 12-h fasted and ad libitum-fed mice (n = 6). Periodic acid-Schiff stained paraffin-embedded liver sections of HBxTg mice at 6 months of age. The scale bar represents 100 μm. G, glucose amount secreted in media from cultured primary hepatocytes of WT and HBxTg mice. Data are presented as the mean ± S.E. (*, p < 0.05; **, p < 0.01; ***, p < 0.001).
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causes hepatic steatosis through the transcriptional activation of sterol regulatory element-binding protein 1 (SREBP1) and peroxisome proliferator-activated receptor (PPARγ) transcripts (17), suggesting the involvement of HBx in the transcriptional regulation of glucose metabolism-related genes.

Inflammation is an important pathogenic factor of metabolic liver diseases, including non-alcoholic steatohepatitis (18) and hepatopathies (19). Inflammatory mediators, such as nitric oxide and TNF-α, have been shown to impair the metabolic action of insulin in the liver, which ultimately results in hepatic dysfunction causing insulin resistance. Indeed, expression of inducible nitric oxide synthase (iNOS) was elevated in the liver of chronic HBV-infected patients (20). During acute or chronic HBV infection, HBx plays a pivotal role in the induction of hepatic inflammatory responses, as evidenced by the fact that HBx transactivates the promoter of the iNOS gene through the proximal NF-κB-binding site (21). Moreover, TNF-α synthesis induced by HBV is transcriptionally up-regulated by HBx in hepatocytes (22). These results imply that HBx may act as a key component connecting a link between hepatic glucose metabolism and inflammation.

In the current study, we investigated the role of HBx in glucose metabolism by studying transgenic mice that carry the HBx gene in vivo and cultured hepatic cell lines in vitro, with particular emphasis on transcriptional regulation of gluconegonic enzymes and whole body glucose homeostasis. We also sought to determine whether activation of iNOS is required for HBx-mediated metabolic dysregulation. Here we show that increased HBx expression is sufficient to cause hyperglycemia and impaired glucose tolerance by enhancing gene expression of hepatic gluconegonic enzymes and hepatic glucose production. These effects are thought to be regulated through nitric oxide (NO)/JNK signaling. Our data establish a novel function of HBx in the regulation of hepatic glucose homeostasis in chronic hepatitis-related metabolic diseases.

**TABLE 1**

**Serum parameters measured in 6-month-old mice fasted overnight**

Fasting serum parameters measured in 6-month-old mice fasted overnight. *p < 0.05; ***, p < 0.01; ****, p < 0.001 for WT versus HBx Tg mice. $, p < 0.05; $, p < 0.01; $$$, p < 0.001 for HBx Tg versus HBx Tg/ iNOS−/− mice. Data are expressed as means ± S.E.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>WT</th>
<th>HBx Tg</th>
<th>HBx Tg/ iNOS−/−</th>
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<tbody>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>78 ± 1.1</td>
<td>113 ± 2.6***</td>
<td>95 ± 5.6#</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>120 ± 2.8</td>
<td>196 ± 3.7***</td>
<td>161 ± 5.2##</td>
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<td>FFA (µmol/liter)</td>
<td>1.15 ± 0.046</td>
<td>1.76 ± 0.043**</td>
<td>1.96 ± 0.107</td>
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<tr>
<td>Serum insulin (ng/ml)</td>
<td>1.101 ± 0.182</td>
<td>0.340 ± 0.070</td>
<td>0.280 ± 0.015</td>
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<tr>
<td>Fed</td>
<td>1.842 ± 0.366</td>
<td>1.095 ± 0.235</td>
<td>1.010 ± 0.344</td>
</tr>
<tr>
<td>Serum glucagon (pg/ml)</td>
<td>414 ± 26</td>
<td>535 ± 27*</td>
<td>458 ± 27</td>
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<tr>
<td>ALT (unit/liter)</td>
<td>41 ± 3.8</td>
<td>51 ± 2.8**</td>
<td>43 ± 3.6</td>
</tr>
<tr>
<td>AST (unit/liter)</td>
<td>51 ± 5.1</td>
<td>83 ± 7.2**</td>
<td>73 ± 5.6</td>
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<tr>
<td>HDL (mg/dl)</td>
<td>74 ± 4</td>
<td>89 ± 7**</td>
<td>ND</td>
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<tr>
<td>LDL (mg/dl)</td>
<td>7 ± 2</td>
<td>12 ± 3*</td>
<td>ND</td>
</tr>
<tr>
<td>Adiponectin (µg/ml)</td>
<td>23.3 ± 0.35</td>
<td>15.1 ± 0.23*</td>
<td>18.9 ± 0.18#</td>
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</table>

**FIGURE 3. Whole body glucose metabolism during hyperinsulinemic-euglycemic clamp in HBx Tg mice.** A and B, basal and clamp hepatic glucose production (HGP) (A) and basal glucose clearance (B) during the clamp of WT and HBx Tg mice. C and D, plasma glucose concentrations (C) and glucose infusion rate (D) during the clamps. E and F, whole body glucose turnover rate, whole body glycolysis rate, whole body glycogen synthesis (E), and insulin-stimulated muscle (gastrocnemius) glucose uptake (F) were determined. 2DG, 2-deoxy-D-[1-14C]glucose. All data are presented as mean ± S.E. (*, p < 0.05, t test, n = 10–11).
cient (C57BL/6-NOS2tm/Lau) mice were obtained from the Jackson Laboratory. Double mutant (HBxTg/iNOS−/−) mice were produced by crossing HBxTg mice with iNOS−/− mice. The mice were housed in a pathogen-free animal facility under a standard 12-h light/dark cycle. All animal procedures were conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee, Korea Research Institute of Bioscience and Biotechnology (KRIBB, Daejeon, Korea).

Patients—The paired peritumoral and tumoral hepatocellular carcinoma (HCC) tissues of HBV patients were collected from the hospital of Chonbuk National University. The HBV patients only reacted to antibodies of HBV. All patients participating in this study gave informed consent before surgery.

Hyperinsulinemic-Euglycemic Clamp Study—Seven days prior to the hyperinsulinemic-euglycemic clamp studies, indwelling catheters were placed into the right internal jugular vein. After an overnight fast, [3-3H]glucose (HPLC-purified; PerkinElmer Life Sciences) was infused at a rate of 0.05 Ci/min to assess the basal hepatic glucose production. Following the basal period, hyperinsulinemic-euglycemic clamp was conducted for 140 min with a primed/continuous infusion of human insulin (126 pmol/kg of prime, 18 pmol/kg/min of infusion) (Novo Nordisk). Blood samples (10 μl) were collected at 10–20-min intervals, plasma glucose was immediately analyzed during the clamps by a glucose oxidase method (GM9 Analyzer; Analox Instruments), and 20% dextrose was infused at variable rates to maintain plasma glucose at basal concentrations (~6.7 mM). To estimate insulin-stimulated whole body glucose fluxes, [3-3H]glucose was infused at a rate of 0.1 Ci/min throughout the clamps, and 2-deoxy-δ-[1-14C]glucose (PerkinElmer Life Sciences) was injected as a bolus at the 85th min of the clamp to estimate the rate of insulin-stimulated tissue glucose uptake, as described previously (23). Blood samples (10 μl) for the measurement of plasma3H and 14C activities were taken at the end of the basal period and during the last 45 min of the clamp. Rates of basal and insulin-stimulated whole body glucose fluxes and tissue glucose uptake were determined as described previously (23).

Statistical Analysis—Data were analyzed using the SigmaStat 3.1 software. Quantitative data are presented as the mean ± the S.E. from at least three independent experiments. Comparisons between groups were analyzed by the unpaired two-tailed Student’s t test or one-way or two-way analysis of variance with post hoc analyses, when appropriate, as indicated.

RESULTS

HBx Regulates the Expression of Gluconeogenic Genes—We investigated the effect of HBx on hepatic glucose metabolism by assessing gene expression of the key gluconeogenic enzymes in HepG2-HBx cells. Overexpression of HBx caused a significant increase in gene expression of gluconeogenic molecules (G6Pase, PEPCK, and PGC1α) and of FOXO1 and its target genes, such as insulin-like growth factor-binding protein-1 (IGFBP1) (24) and insulin receptor substrate 2 (IRS2) (25) in HepG2 cells (Fig. 1A). However, the glucose transporter 2 (GLUT2), insulin receptor (INSR), carnitine palmitoyltrans-
with high or low levels of gluconeogenic enzymes in non-tumor liver tissues of HBV patients (Fig. 1B). We also investigated gene expression of the key gluconeogenic enzymes in HepG2 cells as well as in HBV patients.

HBx Expression Causes Impaired Glucose Tolerance in Mice—We next examined the physiological role of HBx in the regulation of glucose homeostasis and insulin sensitivity in vivo. HBxTg mice had normal body weight until 6 months of age; thereafter they did not gain weight when compared with wild-type (WT) mice (Fig. 2A). Liver weight in HBxTg mice was increased ~25% when compared with WT mice, whereas adipose tissue mass decreased significantly (~70%) (Fig. 2B). Furthermore, HBxTg mice showed several hepatic histopathological changes, including fatty changes, inflammation and dysplasia (supplemental Table 1), and an increase in serum parameters, such as triglyceride, cholesterol, and free fatty acid (Table 1). These observations indicate possible involvement of HBx in complicated liver metabolic diseases. Notably, HBxTg mice had a high risk for glucose intolerance, as evidenced by higher glucose levels in fasted and fed states (Fig. 2C). As expected, blood glucose levels at all times during the glucose tolerance tests were also markedly higher than controls (Fig. 2D). However, HBxTg mice showed insulin tolerance (Fig. 2E). After injection of insulin, blood glucose levels dropped to a similar rate in HBxTg mice and WT. Except at the starting point, blood glucose levels were virtually identical in the WT and HBxTg mice at various time points after insulin injection. However, unexpectedly serum insulin level was significantly lowered in HBxTg mice when compared with WT mice, regardless of feeding states, whereas serum glucagon level was greatly elevated in HBxTg mice (Table 1). Consistent with the results, hepatic glycogen level was significantly reduced in HBxTg livers in concert with an increase in hepatic glucose production (Fig. 2, F and G). These results suggest that the impaired glucose metabolism in HBxTg livers may be associated with the changes in hormones regulating serum glucose level.

HBxTg Mice Increase Glucose Production—To further determine the role of HBx on fasting hyperglycemia and insulin resistance, we assessed basal hepatic glucose production and insulin-stimulated whole body and tissue-specific glucose fluxes in HBxTg using hyperinsulinemic-euglycemic clamp studies with radioisotope-labeled glucose infusion. The basal hepatic glucose production was significantly increased by 23% in HBxTg mice when compared with the WT mice (Fig. 3A). These data clearly indicate that fasting hyperglycemia observed in HBxTg mice was mostly attributed to increased hepatic glucose production because there was no difference in basal glucose clearance (Fig. 3B). However, whole body insulin sensitivity did not differ, as reflected by no difference in glucose infusion rate (Fig. 3, C and D). Moreover, there was no differen-
ence in insulin-induced suppression of hepatic glucose production and whole body (Fig. 3E) and muscle glucose uptake (Fig. 3F). These data suggest that HBx expression results in fasting hyperglycemia by increasing hepatic glucose production in vivo.

Changes in Glucose Metabolism and Insulin Signaling in HBxTg Mice—Gene expressions involved in glucose metabolism and insulin signaling were examined by qRT-PCR analysis (see supplemental Table 2) using the livers. Consistent with in vitro data, gene expressions of glucose metabolism-related molecules, including PEPCK, G6Pase, PGC1α, FOXO1, and IGFBP1, were significantly increased in the liver of HBxTg mice when compared with WT mice (Fig. 4A). These effects are independent of changes in nutritional states. HBxTg mice also had increased protein levels of PEPCK, G6Pase, and PGC1α in the liver (Fig. 4B).

Basal Akt and FOXO1 phosphorylation was significantly reduced in the liver of HBxTg mice, whereas basal JNK phosphorylation was increased (Fig. 4C). The ability of insulin to increase PI3K (phosphatidylinositol 3-kinase) activity associated with IRS-1 but not IRS-2 was impaired in HBxTg mice when compared with WT mice (Fig. 4, D and E) without change in insulin receptor phosphorylation. Interestingly, insulin-stimulated Akt phosphorylation was normal, suggesting that maximal stimulation of Akt may not require the full activation of PI3K activity. Collectively, these data demonstrate that liver dysfunction induced by HBx may be associated with impaired PI3K activation.

Reciprocal Regulation in Expression between HBx and iNOS in Induction of Gluconeogenesis—Studies revealed that HBx leads to hepatic inflammation by activating iNOS, which can produce NO (26). It is thus important to investigate whether increased levels of NO are sufficient to increase gene expression involved in gluconeogenic pathways in the presence of HBx. Our data demonstrated that iNOS mRNA was significantly increased in the liver of HBxTg mice when compared with WT mice, and its expression pattern was greatly similar to that of the HBx gene in nutritional states (Fig. 5, A and B). It is there-
fore likely that HBx and iNOS expression is mutually regulated under a certain condition and that hyperactivation of iNOS may contribute to the pathogenesis of HBx-induced hepatic gluconeogenesis. Furthermore, increased HBx mRNA levels were seen in HepG2-HBx cells treated with sodium nitroprusside (SNP), an NO donor, indicating NO-mediated expression of HBx. In control cells, gene expression of HBx seen in HepG2-HBx neogenesis. Furthermore, increased NO could induce gluconeogenesis through activation of iNOS—To investigate whether HBx/NO-mediated JNK activation leads to increased expression of gluconeogenic genes, we measured JNK1 expression and phosphorylation were elevated in response to SNP, whereas gene expression of G6Pase, PEPCK, and PGC1α was up-regulated. However, treatment of HepG2-HBx cells with SNP further increased the gene expression of G6Pase, PEPCK, PGC1α, and FOXO1 when compared with those of control HepG2 cells (Fig. 5C). Thus, our data suggest that gluconeogenesis genes were significantly up-regulated in HBxTg mice and HepG2-HBx cells, indicating that NO could induce gluconeogenesis through activation of HBx transcription.

Deficiency of iNOS Normalizes HBx-induced Impaired Glucose Tolerance in Mice—We further determined whether blockade of iNOS signaling could prevent the ability of HBx to induce hyperglycemia and impaired glucose tolerance in mice. Toward this end, we generated HBxTg mice that lacked iNOS gene expression by breeding HBxTg mice with iNOS−/− mice (Fig. 7A). Elevated glucose levels in HBxTg mice were normalized to the level of WT mice by a deficiency of iNOS in HBxTg/iNOS−/− mice (Fig. 7B). Aligned with these findings, iNOS deficiency completely rescued hyperglycemia and impaired glucose tolerances that were seen in HBxTg mice (Fig. 7, C and D). However, plasma insulin levels in HBxTg mice were unaltered by loss of iNOS, suggesting the specificity of iNOS action, and serum glucagon levels were lowered in HBxTg/iNOS−/− mice in the fasted state when compared with HBxTg mice (Table 1). Moreover, hepatic glycogen levels and serum levels of triglyceride and cholesterol in HBxTg/iNOS−/− mice were restored to those of WT mice (Fig. 7E and Table 1). These data strongly suggest that iNOS signaling is required for HBx-induced hepatic impaired glucose tolerance in mice.

Reduced Expression of Metabolic Genes in HBxTg/iNOS−/− Mice—The fact that deficiency of iNOS normalizes impaired glucose tolerance in HBxTg mice promptly led us to investigate gene expression of glucose metabolism-related molecules in the liver of HBxTg/iNOS−/− mice. Hepatic HBx mRNA levels were significantly decreased in the fasted state in HBxTg/iNOS−/− mice when compared with HBxTg mice (Fig. 8A), indicating NO-dependent HBx up-regulation. Deficiency of iNOS led to a significant decrease in gene expression levels of glucose metabolism-related molecules, including PEPCK, G6Pase, PGC1α, FOXO1, IRS1, and IRS2, all of which were elevated in the liver of HBxTg mice (Fig. 8B). In addition, hepatic JNK1 mRNA levels were significantly decreased when iNOS was deleted in HBxTg mice (Fig. 8C). Collectively, these
results suggest that iNOS plays an essential role in regulating HBx-induced expression of hepatic gluconeogenic key enzymes, which could contribute to the pathogenesis of hepatic metabolic dysfunction.

DISCUSSION

The current study investigated the role of HBx in the regulation of hepatic glucose metabolism, specifically the impact of gluconeogenesis. Hepatic glucose production was greatly increased in HBx Tg liver, which is most likely due to the increased expression of gluconeogenic genes (PEPCK, PGC1α, and G6Pase). Ultimately, the enhanced gluconeogenesis further caused hyperglycemia in HBx Tg mice with age. Interestingly, nitric oxide-induced JNK activation is closely associated with the disturbed hepatic glucose metabolism. Thus, our data provide compelling evidences that HBx is an important player in regulating hepatic glucose homeostasis during the progression of chronic hepatitis-related metabolic diseases.

Inflammation has been implicated as an important etiological factor in the development of both hyperglycemia and type 2 diabetes. HBx induces the intrahepatic inflammatory process during acute and chronic hepatitis progression and HCC developments (22). Recently, we notified that HBx transactivates the iNOS gene promoter through the proximal NF-κB-binding site (21). Here we showed that the level of iNOS transcript was significantly increased in the liver of HBx Tg mice. Interestingly, HBx expression was also increased by treatment of HepG2-HBx cells with a NO donor. Moreover, the NO donor triggers induction of gluconeogenic genes in these cells. Based on these results, we suggest that the impairment in regulation of gluconeogenesis may be accelerated by the synergistic action between HBx and iNOS.

Gluconeogenesis is strongly stimulated during fasting and is aberrantly activated in type 2 diabetes. The transcriptional coactivator PGC1α was strongly induced in the liver in fasting mice and in mouse models of insulin action deficiency (27). Likewise, PGC1α mRNA in HBx Tg mice was significantly increased in the fasting state when compared with the ad libitum-fed state. It is interesting that PGC1α mRNA was more highly increased in HBx Tg mice than in control animals even in the state of ad libitum, suggesting that HBx modulates PGC1α transcription in mice.

Akt signaling has been implicated in the regulation of hepatic glucose metabolism (28). HBx-induced inflammatory stress is associated with impairment in Akt phosphorylation, which was partially mediated by the JNK pathway in hepatocytes (29). JNK is known to induce serine phosphorylation of IRS-1 and thereby reduce the activity of IRS-1 and also its downstream target, Akt. Similarly, the current study has shown that in HBx Tg liver,
decreased Akt phosphorylation and increased JNK1 phosphorylation were observed, whereas JNK1 phosphorylation was alleviated by null mutation of iNOS. In addition, it is known that FOXO1 is mainly determined by Akt phosphorylation status (30). In general, the function of FOXO1 is closely associated with hepatic glucose homeostasis as a transcription factor governing the expression of gluconeogenic genes. Thus, impairment in regulation of FOXO1 signaling may ultimately cause imbalance of hepatic glucose metabolism. Consistent with this, FOXO1 phosphorylation was greatly reduced in HBx Tg liver when compared with WT mice (Fig. 4C). Based on these observations, we suggest that HBx-driven impairment in hepatic glucose metabolism may be dependent on the cross-talk between the IRS-1/Akt/FOXO1 and JNK1 signaling pathways.

It is known that NO-mediated increase in TNF-α or free fatty acid caused suppression of insulin secretion and insulin resistance (31) and that low levels of adiponectin were associated with impaired glucose tolerance and the development of type 2 diabetes (32). Our studies found that serum adiponectin concentrations were significantly decreased in HBx Tg mice when compared with WT mice (Table 1), whereas TNF-α and free fatty acid were significantly increased (data not shown). Interestingly, deficiency of iNOS normalized the levels of serum adiponectin but not TNF-α and free fatty acid levels in HBx Tg mice, suggesting that adiponectin may be involved in the development of HBx-induced glucose intolerance (Table 1). However, it is unclear at this time whether an elevated serum insulin level is due to the direct action of HBx on insulin secretion or secondary effects. It must be further studied in the future.

Experimental evidence revealed that NO donor impaired the ability of insulin to stimulate glucose uptake in muscle cell (33) and glycogen synthesis in hepatocytes (34), indicating the inhibitory role for iNOS on insulin action. Support for this comes from the findings that NO donor administration caused hyperglycemia in mice (35). Here we demonstrated that deficiency of iNOS in HBx Tg mice selectively normalized serum glucose levels but not insulin levels. These effects are most likely due to the inhibition of gluconeogenesis, as indicated by the fact that increased gene expression of gluconeogenic enzymes was restored to normal levels by deletion of iNOS. Thus, iNOS plays a critical role in regulating glucose homeostasis and functions as a key determinant of HBx-induced hyperglycemia.

Based on our studies, we propose a hypothesis for the involvement of HBx in regulating hepatic glucose metabolism. Increased HBx expression by HBV causes hyperglycemia through the activation of the gluconeogenic signaling pathway. This is thought to be mediated via iNOS signaling (Fig. 9). Thus, HBx functions as an important regulator of hepatic glucose metabolism that is closely associated with inflammation in hepatitis-induced liver diseases. Finally, these findings will be useful for discovery of potential treatment targets for dysregulated metabolism in HBV patients and future anti-HBV therapy.

REFERENCES

HBx Regulates Hepatic Glucose Homeostasis via iNOS Activation