Hepatitis C Virus Infection and Diabetes: Direct Involvement of the Virus in the Development of Insulin Resistance

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**Background & Aims:** Epidemiological studies have suggested a linkage between type 2 diabetes and chronic hepatitis C virus (HCV) infection. However, the presence of additional factors such as obesity, aging, or cirrhosis prevents the establishment of a definite relationship between these 2 conditions. **Methods:** A mouse model transgenic for the HCV core gene was used. **Results:** In the glucose tolerance test, plasma glucose levels were higher at all time points including in the fasting state in the core gene transgenic mice than in control mice, although the difference was not statistically significant. In contrast, the transgenic mice exhibited a marked insulin resistance as revealed by the insulin tolerance test, as well as significantly higher basal serum insulin levels. Feeding with a high-fat diet led to the development of overt diabetes in the transgenic mice but not in control mice. A high level of tumor necrosis factor-α, which has been also observed in human chronic hepatitis C patients, was considered to be one of the bases of insulin resistance in the transgenic mice, which acts by disturbing tyrosine phosphorylation of insulin receptor substrate-1. Moreover, administration of an anti-tumor necrosis factor-α antibody restored insulin sensitivity. **Conclusions:** The ability of insulin to lower the plasma glucose level in the HCV transgenic mice was impaired, as observed in chronic hepatitis C patients. These results provide a direct experimental evidence for the contribution of HCV in the development of insulin resistance in human HCV infection, which finally leads to the development of type 2 diabetes.

Approximately 200 million people are chronically infected with hepatitis C virus (HCV) in the world. Chronic HCV infection may lead to cirrhosis and hepatocellular carcinoma, thereby being a worldwide problem both in medical and socioeconomical aspects.1,2 In addition, chronic HCV infection is a multifaceted disease, which is associated with numerous clinical manifestations, such as essential mixed cryoglobulinemia, porphyria cutanea tarda, and membranoproliferative glomerulonephritis.3 Recent epidemiological studies have added another clinical condition, type 2 diabetes, to a spectrum of HCV-associated diseases.4–7 However, the establishment of a definite causative relationship between HCV infection and diabetes is hampered by the presence of other factors such as obesity, aging, or liver injury in patients with chronic HCV infection.

Type 2 diabetes is a complex, multisystem disease with a pathophysiology that includes a defect in insulin secretion, increased hepatic glucose production, and resistance to the action of insulin, all of which contribute to the development of overt hyperglycemia.8,9 Although the precise mechanisms whereby these factors interact to produce glucose intolerance and diabetes are uncertain, it has been suggested that the final common pathway responsible for the development of type 2 diabetes is the failure of the pancreatic β-cells to compensate for the insulin resistance. Hyperinsulinemia in the fasting state is observed relatively early in type 2 diabetes, but it is considered to be a secondary response that compensates for the insulin resistance.8,9 Overt diabetes occurs over time when pancreatic β-cells bearing the burden of increased insulin secretion fail to compensate for the insulin resistance.

In this study, to elucidate the role of HCV in a possible association between diabetes and HCV infection, transgenic mice that carry the core gene of HCV10,11 were analyzed. We found that these mice developed insulin resistance. An addition of a high-calorie diet led to the development of type 2 diabetes by dis-

Abbreviations used in this paper: EDL, extensor digitorum longus; ELISA, enzyme-linked immunosorbent assay; FPG, fasting plasma glucose; HCV, hepatitis C virus; IRS, insulin receptor substrate; JNK, c-Jun N-terminal kinase; TNF-α, tumor necrosis factor-α.
rupturing the balance between insulin resistance and secretion.

**Materials and Methods**

**Transgenic Mice**

The production of HCV core gene transgenic mice has been described previously. Briefly, the core gene from HCV of genotype 1b, which is placed downstream of a transcriptional regulatory region from the hepatitis B virus, was introduced into C57BL/6 mouse embryos (Clea Japan, Tokyo, Japan). The mice were cared for according to institutional guidelines, fed an ordinary chow diet (Funabashi Farms, Funabashi, Japan), and maintained in a specific pathogen-free state. At an indicated time, the mice were fed a high-fat diet (Oriental Yeast Co., Ltd., Tokyo, Japan) for up to 2 months. Caloric content of food was 4.70 kcal/g for high-fat diet and 3.56 kcal/g for ordinary diet. The high-fat diet contains 18.5% protein, 22.1% fat (4.7% vegetable fat and 17.4% animal fat), 5.4% ash, 2.5% fiber, 6.5% moisture, and 43.0% carbohydrate, and the ordinary diet contains 22.4% protein, 5.7% fat, 6.6% ash, 3.1% fiber, 7.7% moisture, and 54.5% carbohydrate. Because there is a sex preference in the development of liver lesion in the transgenic mice, we used only male mice that were heterozygously transgenic for the core gene, and as controls we used nontransgenic litter mates of the transgenic mice. Transgenic mice carrying the HCV envelope genes under the same regulatory region as that in the core gene transgenic mice were also used as controls. At least 5 mice were used in each experiment and the data were subjected to statistical analysis.

**Glucose Tolerance Test**

The mice were fasted for >16 hours before the study. D-Glucose (1g/kg body weight) was administered by intraperitoneally (IP) injection to conscious mice. Blood was drawn at different time points from the orbital sinus, and plasma glucose concentrations were measured by using an automatic biochemical analyzer DRI-CHEM 3000V (Fuji Film, Tokyo, Japan). The levels of serum insulin were determined by radioimmunoassay (BIOTRAK; Amersham Pharmacia Biotech, Piscataway, NJ) with rat insulin as a standard.

**Insulin Tolerance Test**

The mice were fed freely and then fasted during the study period. Human insulin (1 U/kg body weight) (Humulin; Novo Nordisk, Denmark) was administered by IP injection to fasted conscious mice, and glucose concentrations were determined at the time points indicated. Values were normalized to the baseline glucose concentration at the administration of insulin.

**Morphometric Analysis**

Sections of the pancreas were prepared and evaluated for morphometry after H&E staining or immunostaining. Relative islet area and islet number were determined with an image analyzer (QUE-2; Olympus Optical Co., Tokyo, Japan).

**Enzyme-Linked Immunosorbent Assay**

ELISA for mouse tumor necrosis factor (TNF)-α was performed using a commercially available mouse TNF-α ELISA kit (BioSource International, Camarillo, CA). Samples were prepared as reported previously. Briefly, the liver of transgenic and control mice were lysed with a buffer containing 1% Tween 80, 10 mmol/L Tris-HCl [pH 7.4], 1 mmol/L EDTA, 0.05% sodium azide, 2 mmol/L PMSF, and the Protease Inhibitor Cocktail (Complete; Roche Molecular Biochemicals, Indianapolis, IN) and homogenized on ice for 20 seconds. The homogenates were centrifuged at 11,000 × g for 10 minutes at 4°C, and the supernatants were collected and assayed. ELISA was performed in triplicate for each sample. The concentrations of the cytokines in the liver were normalized by determining the amount of total protein in each sample using the BCA Protein Assay Kit (Pierce, Rockford, IL).

**Immunoprecipitation and Western Blotting**

For immunoprecipitation studies, liver tissues were homogenized in lysis buffer (10 mmol/L Tris-HCl at pH 7.5, 150 mmol/L NaCl, 10 mmol/L sodium pyrophosphate, 1.0 mmol/L β-glycerophosphate, 1.0 mmol/L sodium orthovanadate [Na3VO4], 50 mmol/L sodium fluoride [NaF], the Protease Inhibitor Cocktail [Complete, Roche Molecular Biochemicals], and 1.0% Triton X-100), and homogenates were precipitated with an anti–insulin receptor substrate (IRS)-1 or anti–IRS-2 rabbit polyclonal antibody (UBI, Lake Placid, NY) and then with Sepharose 4B beads (Amersham Biosciences). Resulting pellets were washed 3 times and then subjected to Western blotting. Pellets were resuspended in Western sample buffer (5% β-mercaptoethanol, 2% sodium dodecyl sulfate, 62.5 mmol/L Tris-HCl, 1 mmol/L EDTA, 10% glycerol), and then subjected to 2%–15% gradient sodium dodecyl sulfate/ PAGE (PAG Mini “DAIICHI” 2/15 (13W), Daiichi Diagnostics, Tokyo, Japan), and electrotransferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Bedford, MA). The filter was then reacted with antiphosphorylated tyrosine (Santa Cruz Biotechology Inc., Santa Cruz, CA), antiphosphorylated serine (Cell Signaling Technology, Inc., Beverly, MA), anti–IRS-1 or anti–IRS-2 mouse monoclonal antibody (BD Biosciences, Lexington, KY), followed by immunostaining with secondary biotinylated IgG (Vector Labs, Inc., Burlingame, CA) and visualization using an ECL kit (Amersham Intl., Buckinghamshire, UK).

**Hyperinsulinemic-Euglycemic Clamp**

Mice underwent a hyperinsulinemic-euglycemic clamp using D-[3-3H]glucose (NEN Life Science, Boston, MA) to measure the rate of glucose appearance and hepatic glucose production (HGP) as described previously. Three days after jugular catheter placement, a hyperinsulinemic-euglycemic clamp was conducted with a continuous infusion of human
insulin to raise serum insulin within a physiological range. Blood samples were drawn at intervals for the immediate measurement of blood glucose concentration, and 20% glucose was infused at variable rates to maintain blood glucose at ca. 125 mg/dL. All infusions were done using microdialysis pumps (KD Scientific Inc., Boston, MA). The rate of glucose appearance (mg/kg per minute), which equals the rate of total body glucose utilization during steady state, was calculated as the ratio of the rate of infusion of [3-3H]glucose and the steady state plasma [3H]-glucose specific activity. HGP (mg/kg/min) during clamps was determined by subtracting the glucose infusion rate from the rate of glucose appearance.

**Glucose Uptake by Skeletal Muscle**

The extensor digitorum longus (EDL) or soleus muscle was excised from 2-month-old mice and exposed to insulin at the indicated concentrations. 2-Deoxyglucose uptake was determined as described previously.16

**Treatment With Anti–TNF-α Antibody**

To suppress TNF-α, a dose of 200 μg/mouse of neutralizing hamster monoclonal antibody (TN3-19.12, Santa Cruz Biotechnology Inc.) was administered by IP injection on days 1 and 4, and plasma glucose and insulin levels were determined at day 5.17

**Statistical Analysis**

The results are expressed as means ± standard error. The significance of the difference in means was determined by Student t test or Mann–Whitney U test whenever appropriate. P < 0.05 was considered significant.

**Results**

**Hyperinsulinemia and Insulin Resistance in Transgenic Mice**

At the age between 1 and 12 months, there was no significant difference in body weight between the core gene transgenic mice and control mice. Figure 1A shows body weight of 2-month-old mice. Fasting plasma glucose (FPG) levels were slightly elevated in the core gene transgenic mice compared with control mice, but the difference was not significant (P = 0.79, Figure 1B). In contrast, there was a marked increase in the level of serum insulin in the core gene transgenic mice than control mice (P < 0.001, Figure 1C). Hyperinsulinemia was observed in the core gene transgenic mice as early as 1 month old. These findings suggest that decreased responsiveness to the hormone may have resulted in compensatory hyperinsulinemia. Administration of glucose to 2-month-old core gene transgenic mice revealed mild glucose intolerance compared with control mice of the same age, but the difference was not statistically significant at any time points measured (Figure 2A). HCV envelope gene transgenic mice of the same age, in which the envelope genes were expressed under the same transcriptional regulatory region as the core gene transgenic mice, did not manifest hyperinsulinemia or elevated FPG levels, indicating that not the transcriptional regulatory region used but the expressed gene itself is essential in this phenotype.

The insulin tolerance test conducted at the age of 2 months revealed that the reduction in plasma glucose concentration after IP insulin injection was impaired in the core gene transgenic mice, displaying higher plasma glucose levels than those in control mice at all time points measured (Figure 2B). At 40 and 60 minutes, the difference was statistically significant between transgenic
and control mice (39.6 ± 1.3 vs. 24.4 ± 1.1 and 43.7 ± 2.1 vs. 26.4 ± 2.3, P < 0.05). These data are consistent with a defect in the actions of insulin on glucose disposal and/or production in the core gene transgenic mice.

Morphology of Pancreatic Islet Cells

Because a critical factor contributing to whether insulin resistance progresses to diabetes is the capacity of the pancreatic β-cells to respond to increased demands for insulin secretion, we evaluated the morphology of pancreatic islet cells by histologic examination. In the pancreas of HCV core gene transgenic mice, an approximately 3-fold increase in islet mass was observed (Figure 3, P < 0.05), which is consistent with β-cell compensation to insulin resistance. There was no infiltration of inflammatory cells within or surrounding the islets.

Feeding Transgenic Mice a High-Fat Diet

Leads to Overt Diabetes

Thus, an insulin resistance is present but no apparent glucose intolerance (overt diabetes) in the HCV core gene transgenic mice. This is probably because of the genetic background of C57BL/6 mice, which has been shown to maintain either normal or mildly elevated glucose levels despite insulin resistance.14 To determine whether a high-fat diet exacerbates the prediabetic phenotype, 2-month-old HCV core gene transgenic mice were fed a high-fat diet for up to 8 weeks. Both the transgenic and control mice showed a similar increase (about 30%) in body weight (Figure 4A). After 8 weeks on this diet, 100% (10 out of 10) of the transgenic mice exhibited casual (fed) plasma glucose levels >250 mg/dL, whereas none of the 10 control mice fed the same diet exhibited levels >250 mg/dL (325.0 ± 66.6 vs. 179.0 ± 17.4 mg/dL, P < 0.01, Figure 4B). Insulin levels were significantly higher in the core gene transgenic mice than in control mice both at fasting and fed state (Figure 4C, P < 0.01 and P < 0.001). In control mice, serum insulin levels in high-fat diet state were significantly higher than those in normal diet state at fed state (Figures 1C and 4C, P < 0.01). Although FPG levels were not significantly different between the transgenic and control mice, these results indicate that feeding a high-fat diet leads to the development of overt diabetes in a mouse model for HCV infection. Body weight gain, particularly with high levels of lipid, may trigger the process leading to overt diabetes in an insulin resistance model mouse with compensatory hyperplasia of islet cells.

Insulin Resistance in the Core Gene Transgenic Mice Is Chiefly Caused by Hepatic Insulin Resistance

We then investigated the mechanism of insulin resistance in the core gene transgenic mice. There was no

Figure 3. Analysis of pancreatic islet mass in the core gene transgenic and control mice. (A and B) Morphology of representative islets (H&E staining) from normal control mice (A) or the core gene transgenic mice (B). (C) Relative islet area, expressed as a percentage of the total stained pancreatic section, for control mice (nTg) and the core gene transgenic mice (Tg) (n = 10 in each group). Values are mean ± standard error; *P < 0.05.

Figure 4. Body weight and glucose homeostasis after a high-fat diet. Control and transgenic mice were fed a high-fat diet for 8 weeks; thereafter, body weight and blood parameters were determined. (A) Body weight at the end of the high-fat diet (n = 10 in each group). (B) Plasma glucose levels determined in a fasting or fed state (n = 10 in each group). (C) Serum insulin levels in a fasting or fed state (n = 10 in each group). Values are mean ± standard error; NS, statistically not significant; **P < 0.01; ***P < 0.001; nTg, nontransgenic mice; Tg, transgenic mice.
significant difference in body weight between the transgenic and control mice as already shown in Figure 1A. After the age of 3 months, the core gene transgenic mice developed hepatic steatosis, which is known to be one of the causes of insulin resistance in humans. However, in 1-month-old mouse livers that were used in the analysis of insulin resistance, no hepatic steatosis was noted. No difference was observed in the levels of free fatty acids in the sera between the transgenic and control mice (0.56 ± 0.33 vs. 0.50 ± 0.21 mmol/L, n = 7 in each group, \( P = 0.65 \)).

Then, we explored the role of the liver in pathogenesis of insulin resistance in the core gene transgenic mice. To directly measure HGP, the hyperinsulinemic-euglycemic clamp technique was conducted as described in Materials and Methods. The core gene transgenic mice showed a normal or slightly lower rate of HGP during the basal period as compared with control mice (Figure 5A). Although insulin infusion during the clamp suppressed HGP by 60% in the control mice, insulin induced little effect on HGP of the core gene mice (Figure 5A). This is consistent with the notion that insulin resistance in the core gene transgenic mice is chiefly depending on the shortage of insulin action on the liver.

To study the involvement of muscles in the development of insulin resistance in the core gene transgenic mice, we then examined whether or not insulin-stimulated glucose uptake is impaired in the skeletal muscles. The extensor digitorum longus muscle (EDL) from 2-month-old core gene transgenic and control mice were excised and exposed to insulin at the intermediate (0.30 nmol/L) and maximal (10.0 nmol/L) concentrations. There was no significant difference in 2-deoxyglucose uptake in the EDL muscle between the core gene transgenic mice and control mice at either insulin concentration (Figure 5B, at 0.30 nmol/L, \( P = 0.23 \) and at 10.0 nmol/L, \( P = 0.76 \)). As another representative muscle that differs from EDL in metabolic properties, the soleus muscle was examined in the same manner as EDL. 2-Deoxyglucose uptake by the soleus muscle was not significantly different between the core gene transgenic and control mice (Figure 5C, at 0.30 nmol/L, \( P = 0.49 \) and at 10.0 nmol/L, \( P = 0.49 \)). Thus, in the core gene transgenic mice, contribution of the peripheral skeletal muscle in the development of insulin resistance is negligible. This is in agreement with the observation that the core protein was exclusively present in the liver as detected by Western blotting, which was confirmed by a sensitive enzyme immunoassay (Tsutsumi T. et al., unpublished data, December 2002).

Elevated TNF-\( \alpha \) Level and Altered Tyrosine Phosphorylation of Insulin Receptor Substrate-1 in the Liver and Insulin Resistance

We have noted an increase in TNF-\( \alpha \) levels in the liver of HCV core gene transgenic mice, which has also been documented in the sera of human hepatitis C patients. On the other hand, TNF-\( \alpha \) has been shown to induce insulin resistance in experimental animals and cultured cells. Therefore, we next determined the protein expression level of TNF-\( \alpha \) by ELISA in the liver of these mice that were used in the current study. The TNF-\( \alpha \) levels in the liver of 2-month-old transgenic mice were 702.2 ± 283.3 pg/mg protein and 313.5 ±
113.6 pg/mg protein in that of 2-month-old control mice (n = 10 in each group, P < 0.001). Thus, the levels of TNF-α exhibited a more than 2-fold increase in the HCV core gene transgenic mice compared with the control mice, which may be associated with insulin resistance.

Suppression of tyrosine phosphorylation of IRS-1 and -2 is one of the mechanisms by which a high level of TNF-α causes insulin resistance. We, therefore, examined the suppression of tyrosine phosphorylation of IRS-1 in response to insulin action in the core gene transgenic mice. Twenty minutes after the administration of human insulin (1 U/kg body weight), when the plasma glucose levels decreased (Figure 2B), IRS-1 in the liver of control mice exhibited a marked phosphorylation of its tyrosine. In contrast, phosphorylation level of tyrosine in IRS-1 in the liver of core gene transgenic mice manifested apparently no increase compared with the basal level after the administration of insulin (Figure 6). In contrast, there was no difference in the time course of tyrosine phosphorylation of IRS-2 between the core gene transgenic and control mice (data not shown). These results indicate that a suppression of tyrosine phosphorylation of IRS-1, that is, a suppression of the insulin action in the liver, is at least one of the mechanisms of insulin resistance in HCV transgenic mice, whereas pathways other than IRS-1 may also be involved.

The c-Jun N-terminal kinase (JNK) pathway has been shown to mediate the inhibitory effect of TNF-α on insulin action through the phosphorylation of serine in IRS-1. Because an activation of the JNK pathway was observed in the liver of core gene transgenic mice, phosphorylation of serine residues in IRS-1 was examined using antiphosphorylated serine monoclonal antibodies (Ser^307 and Ser^612). However, there was no difference in the time course of serine phosphorylation after insulin stimulation between the core gene transgenic and control mice (data not shown).

Blockade of TNF-α Action Restores Insulin Sensitivity

Then the anti-TNF-α antibody was administered to block the in vivo activity of TNF-α in mice as described in the Materials and Methods section. Twenty-four hours after the second administration of the anti-TNF-α antibody (200 μg/mouse), serum insulin levels in transgenic mice became significantly lower than those of control mice (data not shown).

Figure 6. Phosphorylation of tyrosine in IRS-1 in response to insulin stimulation. Liver tissues from control mice and core gene transgenic mice with or without anti-TNF-α antibody treatment were analyzed before and 20 and 40 minutes after insulin administration. The samples were subjected to immunoprecipitation with anti-IRS-1 antibody and subsequently immunoblotted with antibodies as indicated. Experiments were performed in triplicate, and a representative picture is shown. (A) Immunoblot with antiphosphotyrosine antibody. There was no augmentation of phosphorylation of tyrosine in IRS-1 after insulin stimulation in the core gene transgenic mice, whereas tyrosine phosphorylation was markedly enhanced in control mice. Insulin-stimulated tyrosine phosphorylation was restored 40 minutes after anti-TNF-α antibody treatment. (B) Immunoblotting with anti-IRS-1 antibody as a control of IRS-1 load. nTg, nontransgenic mice; Tg, transgenic mice; anti-PY, antiphosphotyrosine antibody; anti-PS, antiphosphoserine antibody. IP, immunoprecipitation.

Figure 7. Serum insulin levels and insulin tolerance test after anti-TNF-α antibody treatment. (A) Serum insulin levels were determined in the fasting state with or without anti-TNF-α antibody treatment as described in the Materials and Methods section. Insulin levels decreased significantly after anti-TNF-α antibody treatment in the core gene transgenic mice (n = 5 in each group). (B) Insulin tolerance test (n = 5 in each group). Human insulin was administered by IP injection to fasted conscious mice and glucose concentrations were determined 24 hours after the second administration of anti-TNF-α antibody. As control, mice were injected with hamster IgG instead of anti-TNF-α antibody. Values were normalized to the baseline glucose concentration at the time of insulin administration. Values are mean ± standard error; *P < 0.05 when compared with Tg control; nTg, nontransgenic mice; Tg, transgenic mice.
sitivity of the core gene transgenic mice to insulin activity. At this time point, phosphorylation of tyrosine in IRS-1 in the liver of transgenic mice in response to insulin action was restored to a similar level to that in control mice (Figure 6A, 40 minutes after insulin administration). These results strongly support the notion that the increased level of TNF-α is one of the bases for insulin resistance in the HCV core gene transgenic mice.

Taken together, these data indicate that the presence of the HCV core protein in the liver, at a level similar to that in human chronic hepatitis C patients, confers insulin resistance to the mice by affecting the liver, by disturbing the insulin-induced suppression of hepatic glucose production.

**Discussion**

Since Allison et al. reported an association between HCV infection and diabetes, evidence has been accumulating connecting these 2 conditions. In such studies, HCV infection has a significantly stronger association with diabetes than hepatitis B viral infection. The variables other than HCV infection that are associated with diabetes are cirrhosis, male sex, and aging. In addition to these clinic-based, case-control studies, Mehta et al. have reported the result of investigation at population level. In this cross-sectional national survey, persons 40 years or older with HCV infection were more than 3 times more likely to have type 2 diabetes than those without HCV infection. Thus, the association of HCV infection with diabetes has become closer as shown by epidemiological studies. However, there are some difficulties in establishing a definite relationship between HCV infection and diabetes on the basis of epidemiological studies; in patients, there are other numerous factors perturbing the verification of the definite relationship, such as obesity, aging, or particularly advanced liver injuries. Moreover, the biological mechanism underlying diabetes or insulin resistance in HCV infection is unknown. In vitro or cultured cell studies have a very limited utility for the study of insulin resistance or diabetes because insulin resistance is a condition that involves multiple organs, such as the skeletal muscles and liver. Thus, the use of good experimental animal model systems may be useful both in establishing a definite relationship between diabetes and HCV infection and in elucidating the role of HCV in the development of insulin resistance.

In the current study, the HCV core gene transgenic mice exhibited insulin resistance as early as 1-month old, despite an apparent absence of glucose intolerance. Development of insulin resistance without any liver injury or excessive body weight gain, as shown in the current study, clearly indicates that infection of HCV per se is a cause of the development of insulin resistance. Although only the core protein is expressed in these mice instead of HCV replication in humans, the fact that the intrahepatic core protein levels are similar between the core gene transgenic mice and chronic hepatitis C patients warrants extrapolating the result into hepatitis C patients. Certainly, dispersion in the intrahepatic core protein levels in human chronic hepatitis C patients compared with the constant amount of the core protein must be taken into account. The occurrence of insulin resistance in the core gene transgenic mice as early as 1-month old also excluded the possibility that aging is a cause of insulin resistance. Nonetheless, aging could be an aggravating factor for insulin resistance. Thus, the current analysis shows a definite causal relationship between HCV infection and the development of insulin resistance.

Our earlier studies have shown the development of hepatic steatosis in these HCV core gene transgenic mice after the age of 3 months. However, insulin resistance invariably preceded the occurrence of hepatic steatosis, indicating that insulin resistance is not a consequence of hepatic steatosis in these mice. Certainly, it is possible that insulin resistance in the core gene transgenic mice may be affected or aggravated after the occurrence of hepatic steatosis. On the other hand, insulin resistance may be one of the factors that cause hepatic steatosis, whereas the impairment of very-low-density lipoprotein (VLDL) secretion from the liver and hypo-β-oxidation of fatty acids are considered to be the bases of development of hepatic steatosis in the core gene transgenic mice.

The general mechanism underlying insulin resistance is not precisely understood and is considered to be multifactorial. Chiefly, it involves glucose consumption by the skeletal muscle and glucose production in the liver. Our current analysis revealed a failure of insulin in the suppression of HPG in the liver and an absence of suppression of glucose uptake by the muscles in the core gene transgenic mice. Combined, these results indicate the insulin resistance in the core gene transgenic mice is chiefly due to hepatic insulin resistance. An elevated intrahepatic TNF-α level plays one of the roles in causing insulin resistance through suppressing insulin-induced tyrosine phosphorylation of IRS-1. It should be noted that TNF-α levels are invariably elevated in the sera of patients with HCV infection. Moreover, restoration of insulin sensitivity after anti-TNF-α antibody administration strongly supports the notion that TNF-α
is, at least in this animal model, a major factor for the development of insulin resistance in HCV infection. Taken together, insulin resistance in the core gene transgenic mice mainly depends on suppression of the inhibitory effect of insulin on hepatic glucose production. This is consistent with the observation that the core protein is present only in the liver but absent in the skeletal muscle of the core gene transgenic mice (Tsutsumi T., unpublished data, December 2002). Impairment in other undetermined pathways may also be responsible for the development of insulin resistance in HCV infection.

Insulin resistance alone does not always lead to the development of overt diabetes in humans or murine models. Particularly, in the models with the C57/BL6 strain, hyperplasia of the islets of Langerhans in the pancreas compensates for insulin resistance by secreting higher amounts of insulin. Along with a gain in body weight by being fed a high-calorie diet, the core gene transgenic mice but no control mice developed overt diabetes, showing that obesity is a risk factor for diabetes as observed in patients or as shown in animal models for diabetes unrelated to HCV infection. This observation would suggest that HCV infection confers insulin resistance and additional factors such as obesity, aging, or possibly inflammation may contribute to the complete development of overt diabetes. The effect of high-fat diet on control C57/BL6 mice may be milder in the current study compared with a previous study. However, there was a substantial increase in FPG levels in high-fat-diet-fed control mice compared with normal-diet-fed control mice (Figures 1B and 4B). In addition, at fed-state, serum insulin levels in high-fat-diet-fed control mice were significantly increased compared with those in normal-diet-fed control mice (Figures 1B and 4B). It is unclear why plasma glucose levels were not very high at baseline and insulin-stimulated whole body glucose disposal in conscious mice. J Clin Invest 1995;95:429–432.

In conclusion, the HCV core protein induces insulin resistance in transgenic mice without gain in body weight at young age. These results indicate a direct involvement of HCV per se in the pathogenesis of diabetes in patients with HCV infection and provide a molecular basis for insulin resistance in such a condition.

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