Modulation of the JNK Pathway in Liver Affects Insulin Resistance Status *

Yoshihisa Nakatani ‡, Hideaki Kaneto ‡§, Dan Kawamori, Masahiro Hatazaki, Takeshi Miyatsuka, Taka-aki Matsuoka, Yoshitaka Kajimoto, Munehide Matsumasa, Yoshimitu Yamasaki, and Masatsugu Hori

Yoshihisa Nakatani and Hideaki Kaneto contributed equally to this work

Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Address correspondence to: Hideaki Kaneto, MD, PhD
Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan

Tel. (81-6) 6879-3633; Fax (81-6) 6879-3639
e-mail: kaneto@medone.med.osaka-u.ac.jp

Running title: JNK Pathway in Insulin Resistance
ABSTRACT

The c-Jun N-terminal kinase (JNK) pathway is known to be activated under diabetic conditions and to possibly be involved in the progression of insulin resistance. In this study, we examined the effects of modulation of the JNK pathway in liver on insulin resistance and glucose tolerance. Overexpression of dominant-negative type JNK in the liver of obese diabetic mice dramatically improved insulin resistance and markedly decreased blood glucose levels. Conversely, expression of wild type JNK in the liver of normal mice decreased insulin sensitivity. The phosphorylation state of crucial molecules for insulin signaling were altered upon modification of the JNK pathway. Furthermore, suppression of the JNK pathway resulted in a dramatic decrease in the expression levels of the key gluconeogenic enzymes, and endogenous hepatic glucose production was also greatly reduced. Similar effects were observed in high fat, high sucrose diet-induced diabetic mice. Taken together, these findings suggest that suppression of the JNK pathway in liver exerts greatly beneficial effects on insulin resistance status and glucose tolerance in both genetic and dietary models of diabetes.
INTRODUCTION

The hallmark of type 2 diabetes is insulin resistance and pancreatic β-cell dysfunction (1, 2). Normal β-cells can compensate for insulin resistance by increasing insulin secretion, but insufficient compensation leads to the onset of glucose intolerance. Once hyperglycemia becomes apparent, insulin sensitivity is further reduced and β-cell function progressively deteriorates (3). Various studies have demonstrated that hyperglycemia is the direct cause of these phenomena, collectively called “glucose toxicity” (4-8).

It has been reported that the activity of c-Jun N-terminal kinase (JNK) (9-13), which is known to be activated by various stress signals such as cytokines or oxidative stress, is abnormally elevated in various tissues under diabetic conditions (14-16) and that activation of the JNK pathway interferes with insulin action (14, 17). Indeed, it was shown that insulin resistance is substantially decreased in mice homozygous for a targeted mutation in the JNK gene (14). Furthermore, we recently reported that JNK activation is involved in the reduction of insulin gene expression by oxidative stress and that suppression of the JNK pathway can protect β-cells from glucose toxicity (18). Thus, it is likely that JNK is a crucial mediator of the progression of insulin resistance as well as β-cell dysfunction found in type 2 diabetes.

Here we report that suppression of the JNK pathway in the liver improves insulin resistance in the whole body and markedly ameliorates glucose intolerance in diabetic mice.
MATERIALS AND METHODS

Preparation of recombinant adenoviruses.
Recombinant adenoviruses expressing wild type (WT) and dominant-negative type (DN) JNK were prepared using the AdEasy system (kindly provided by Dr. Bert Vogelstein, the Johns Hopkins Oncology Center) (33). In brief, the encoding region of WT- and DN-JNK (K55R) was cloned into a shuttle vector pAdTrack-CMV, and to allow for homologous recombination, the linearized plasmid containing WT- or DN-JNK and the adenoviral backbone plasmid, pAdEasy-1, were introduced into electrocompetent Escherichia coli BJ5183 cells. The linearized plasmids were transfected into the adenovirus packaging cell line 293 using LipofectAMINE (Invitrogen, Carlsbad, CA), and the adenovirus titers were increased up to 1X10^8 plaque forming units (PFU)/ml in the 293 cells. Control adenovirus expressing green fluorescent protein (Ad-GFP) was prepared in the same manner. Adenovirus titers were further increased up to 1X10^{10} PFU/ml using Adeno-X™ Virus Purification Kit (Clontech). Virus titers were estimated using Adeno-X™ Titer Kit (Clontech).

Animals and administration of recombinant adenoviruses.
Male C57BL6 and C57BL/KsJ-db/db mice were purchased from Japan SLC (Hamamatsu, Japan). Mice (8 weeks old) were injected with Ad-WT-JNK, Ad-DN-JNK, or Ad-GFP (1X10^{10} PFU/ml for Ad-WT-JNK and 2X10^{9} PFU/ml for Ad-DN-JNK) from the cervical vein. After adenovirus injection, blood glucose levels were measured regularly with a portable glucose meter (Glu-test Sensor, Sanwa, Japan) after tail snipping. For measurement of serum insulin levels, blood samples of mice after a 6-h fast were collected into heparinized capillary tubes and serum insulin levels were determined with Insulin-EIA Test Kit (Glazyme).
Glucose tolerance tests.
After a 6-h fast, mice were injected intraperitoneally with glucose (2.0 g/kg body weight). Blood samples were taken at various time points (0-120 min), and blood glucose levels and serum insulin levels were determined as described above.

Insulin tolerance tests.
After a 6-h fast, mice were injected intraperitoneally with insulin (2.0 U/kg for C57BL-KsJ-db/db mice). Blood samples were taken at various time points (0-90 min) and blood glucose levels were measured as described above.

Euglycemic hyperinsulinemic clamp.
Fourteen days before the clamp study, Ad-WT-JNK (1X10^{10} PFU/ml) or Ad-DN-JNK (2\times10^{9} PFU/ml) was injected from the left jugular vein. Three days before the clamp study, a silicon catheter (Phicon Tube, Fuji-Systems, Tokyo, Japan) was inserted into the right jugular vein under general anesthesia with sodium pentobarbital. The catheter, which is required for infusion in the clamp study, was exteriorized at the back of the neck through a subcutaneous tunnel and filled with heparinized saline (200 U/ml). Clamp studies were performed on mice under conscious and unstressed conditions after a 6-h fast. A euglycemic hyperinsulinemic clamp with a tracer dilution method was applied to determine peripheral glucose uptake and endogenous glucose production. Experiments consisted of a 120-min euglycemic hyperinsulinemic clamp period (15 pmol/kg/min of regular human insulin for C57BL6 mice; 27 pmol/kg/min for C57BL/KsJ-db/db mice during the 120 min clamp period). During this period, blood glucose levels were monitored every 5 min and the rate of 50% glucose containing 10% [6,6-\textsuperscript{2}H\textsubscript{2}]-glucose infusion into the jugular vein was adjusted to maintain blood glucose concentrations at 120 \pm 10 mg/dl.
Measurement of endogenous hepatic glucose (HGP) production by stable isotope-labeled glucose enrichment.

To estimate HGP, stable isotope-labeled glucose enrichment was determined. Blood samples were taken at 90, 105, 120 min and 20 μl of each plasma sample were deproteinized with 60 μl of 99.5% ethanol. The supernatant was evaporated, and the residue was derivatized by the following procedure. First, 7.5 μl of MBTFA (N-methyl-bis (trifluoroacetamide), Pierce, Rockford, IL) and 7.5 μl of pyridine were added to the residue, and the mixture was heated for 1 h at 60°C. The reaction product (1 μl) containing trifluoro-acetylated glucose was then analyzed by gas chromatography and mass spectrometry (Model TSQ-700, Finningan-MAT, San Jose, CA) with a silicon SE-30 capillary column (30 m X 0.25 mm I.D., Gasukuro Kogyo, Tokyo, Japan). The trifluoroacetyl derivative of glucose was separated from the other compounds by gas chromatography and was analyzed by electron impact mass spectrometry at 70 eV. The fragment ion peaks of unlabeled and [6,6-2H2]-glucose were measured at a mass per electrical charge (m/e) of 319 and 321, respectively.

Western blot analysis.

Whole cell extracts obtained from liver were fractionated by 10% SDS-PAGE and transferred to reinforced cellulose nitrate membrane (Optitran BA-S85, Schleicher & Schuell). After blocking, the membranes were incubated at 4 °C overnight in TBS buffer (50 mM Tris-HCl, 150 mM NaCl) containing a 1:1000 dilution of rabbit anti-IRS-1, anti-IRS-1-pSer307 antibody (Upstate Biotechnology), anti-Akt, or anti-Akt-pSer473 antibody (Cell Signaling, Beverly, MA), and then incubated for 1 h at room temperature in TBS containing a 1:1000 dilution of anti-rabbit IgG antibody coupled to horseradish peroxidase (Bio-Rad). Immunoreactive bands were visualized by incubation with LumiGLO (Cell Signaling) and exposed to light-sensitive film.

Northern blot analysis.
Ten micrograms of total RNA isolated from freeze-clamped liver tissues were electrophoresed on 1.0% formaldehyde-denatured agarose gel in 1 X MOPS running buffer, and then transferred overnight to a Hybond-N⁺ membrane (Amersham, Arlington Heights, IL, USA). The PEPCK, G6Pase, and GK cDNA probes were labeled with [α-³²P] dCTP using the Rediprime labeling system kit (Amersham). After overnight hybridization with a ³²P-labeled probe at 42°C, the membranes were washed in 2 X SSPE, 0.1% SDS at 42°C. The probed membranes were exposed to an imaging plate, BAS-MS 2040 (Fujifilm, Tokyo, Japan), and the hybridization intensity was quantified using a BAS2500 system (Fujifilm).

**Diet study.**

Six-week male mice (C57BL6) were divided at random into 2 groups. One group was fed a high-fat, high-sucrose diet (AIN93G, Oriental Yeast, Suita, Japan) and the other was fed standard laboratory mouse chow (Clea Japan, Tokyo, Japan) for 4 weeks.

**Statistical analysis.**

Results are expressed as mean ± S.E. Differences between groups were examined for statistical significance using the Student’s t test or analysis of variance (ANOVA) with the Fisher’s PLSD test.
RESULTS

Activation of the JNK pathway in the liver decreases insulin sensitivity in C57BL/6 mice.

To examine the effects of JNK overexpression in the liver on insulin sensitivity and glucose tolerance in non-diabetic animals, we prepared WT-JNK expressing adenovirus (Ad-WT-JNK) and control adenovirus (Ad-GFP), and delivered each adenovirus to 8 week-old C57BL6 mice from the cervical vein. It is noted that this Ad-WT-JNK express GFP as well as WT-JNK. As shown in Fig. 1A, a marked increase in JNK expression was observed in the liver, but not in other tissues such as muscle and adipose tissue. The left panel of Fig. 1A shows a representative liver after exposure to Ad-WT-JNK. As seen by the GFP fluorescence, many cells in the liver were infected with the adenovirus. There was no difference in body weight and food intake between the two groups (data not shown), and nonfasting blood glucose concentrations in Ad-WT-JNK-treated mice were also comparable to those in Ad-GFP-treated mice (Fig. 1B). Next, to examine the effect of WT-JNK overexpression in the liver on glucose tolerance, we performed the intraperitoneal glucose tolerance test (IPGTT). As shown in Fig. 1C, there was no difference in glucose tolerance between Ad-GFP-treated and Ad-WT-JNK-treated mice. Fasting serum insulin concentrations in Ad-WT-JNK-treated mice were higher than those in Ad-GFP-treated mice at the compatible fasting blood glucose levels (Fig. 1D), indicating that JNK overexpression increases insulin resistance. To investigate this point further, we performed the euglycemic hyperinsulinemic clamp test. As shown in the left panel of Fig. 1E, in the clamp study, the steady-state glucose infusion rate (GIR) in Ad-WT-JNK-treated C57BL6 mice was significantly lower than that in Ad-GFP-treated mice (58.0 ± 6.8 versus 73.7 ± 3.2 mg/kg/min, p<0.05, n = 6), indicating that activation of the JNK pathway in the liver reduces insulin sensitivity in C57BL6 mice. Furthermore, we evaluated endogenous hepatic glucose production (HGP) in Ad-WT-JNK-treated mice using tracer methods. As shown in the right panel of Fig. 1E, HGP in Ad-WT-JNK-treated mice was significantly greater than that in
Ad-GFP-treated mice (18.2 ± 3.1 versus 6.4 ± 3.3 μmol/kg/min, p<0.05, n = 6). In contrast, there was no difference in glucose disappearance rate between the two groups, which reflects glucose utilization in the peripheral tissues (data not shown). These results indicate that overexpression of JNK decreases insulin sensitivity mainly by increasing HGP in control mice. On the other hand, although JNK is well known to be involved in the immune response and regulation of apoptosis, HE staining revealed no morphological changes in the liver after WT-JNK overexpression (data not shown), indicating that the increase in insulin resistance induced by WT-JNK overexpression is not due to the induction of apoptosis in the liver.

**Suppression of the JNK pathway in the liver markedly reduces insulin resistance and ameliorates glucose tolerance in C57BL/KsJ-db/db mice.**

To examine the effect of suppression of the JNK pathway in the liver on insulin resistance and glucose intolerance in diabetic animals, we prepared DN-JNK expressing adenovirus (Ad-DN-JNK) and control adenovirus (Ad-GFP), and delivered each adenovirus via the cervical vein to 8 week-old C57BL/KsJ-db/db obese diabetic mice. There was no difference in body weight and food intake between the two groups (data not shown). As shown in Fig. 2A, when C57BL/KsJ-db/db mice were treated with Ad-DN-JNK, nonfasting blood glucose levels were markedly reduced from 405 ± 42 mg/dl to 211 ± 30 mg/dl (2 weeks after adenovirus injection), whereas no such effect was observed in Ad-GFP-treated mice. Fasting blood glucose concentrations (after a 6-h fast) were also lower in Ad-DN-JNK-treated mice compared to Ad-GFP-treated mice, although there was no difference in serum insulin concentrations between these two groups (Fig. 2B). To examine the effect of suppression of JNK activity in the liver on insulin resistance, we performed intraperitoneal insulin tolerance test (IPITT). As shown in Fig. 2C, the hypoglycemic response to insulin was larger in Ad-DN-JNK-treated C57BL/KsJ-db/db mice compared to Ad-GFP-treated mice. To investigate this point further, we performed the euglycemic hyperinsulinemic clamp test. As shown in Fig. 2D, in the
clamp study, GIR in Ad-DN-JNK-treated mice was around 2-fold higher than that in Ad-GFP-treated mice (20.8 ± 2.4 versus 10.1 ± 2.6 mg/kg/min, p<0.05, n = 6), indicating that suppression of the JNK pathway in the liver reduces insulin resistance and thus ameliorates glucose tolerance in C57BL/KsJ-db/db mice. Furthermore, hepatic glucose production was significantly lower in Ad-DN-JNK-treated mice compared to Ad-GFP-treated mice (24.0 ± 4.2 versus 38.0 ± 3.9 μmol/kg/min, p<0.05, n = 6) (Fig. 2D). In contrast, there was no difference in the glucose disappearance rate between these two groups (data not shown). These results indicate that reduction of insulin resistance and amelioration of glucose tolerance by DN-JNK overexpression are mainly due to suppression of hepatic glucose production.

Next, we examined whether Ad-DN-JNK exerts some effect on glucose tolerance and insulin sensitivity in non-diabetic C57BL6 mice. In contrast to C57BLKsJ-db/db mice, there was no difference in fasting blood glucose levels between Ad-GFP-treated and Ad-DN-JNK-treated C57BL6 mice; after a 6-h fast, blood glucose levels in Ad-GFP-treated and Ad-DN-JNK-treated C57BL6 mice were 148 ± 9 mg/dl and 151 ± 8 mg/dl, respectively. Also there was no difference in the intraperitoneal glucose tolerance test (IPGTT) between Ad-GFP-treated and Ad-DN-JNK-treated C57BL6 mice. Next, to evaluate the effect on insulin sensitivity, we measured fasting serum insulin levels. After a 6-h fast, serum insulin levels in Ad-GFP-treated and Ad-DN-JNK-treated C57BL6 mice were almost same: 0.7 ± 0.4 ng/ml and 0.8 ± 0.4 ng/ml, respectively. Furthermore, to evaluate the effect of Ad-DN-JNK on insulin sensitivity in non-diabetic mice, we performed euglycemic hyperinsulinemic glucose clamp test in C57BL6 mice. In contrast to C57BLKsJ-db/db mice, there was no difference in glucose infusion rate (GIR) between Ad-GFP-treated and Ad-DN-JNK-treated C57BL6 mice (73.7 ± 3.2 versus 73.4 ± 4.7 mg/kg/min, n = 5). These results are consistent with the idea that Ad-DN-JNK suppresses the JNK pathway which is activated under diabetic conditions and thus leads to decrease of insulin resistance and amelioration of glucose tolerance.
Alteration of insulin action in the liver via the JNK pathway is associated with the phosphorylation status of insulin signaling molecules and expression of gluconeogenic enzymes.

It has been reported that serine phosphorylation of insulin receptor substrate-1 (IRS-1) inhibits insulin-stimulated tyrosine phosphorylation of IRS-1, leading to an increase in insulin resistance (14, 17). To explore the molecular mechanisms involved in the alteration of insulin actions via the JNK pathway, we evaluated IRS-1 serine 307 phosphorylation in the liver of Ad-WT-JNK-treated C57BL6 mice. As shown in Fig. 3A, IRS-1 serine 307 phosphorylation was increased in Ad-WT-JNK-treated mice compared to Ad-GFP-treated mice. We also found a decrease in IRS-1 tyrosine phosphorylation in Ad-WT-JNK-treated mice compared to Ad-GFP-treated mice. Furthermore, a decrease in Akt serine 473 phosphorylation was observed in Ad-WT-JNK-treated C57BL6 mice compared to Ad-GFP-treated mice. Similarly, we examined the molecular mechanisms involved in the reduction of insulin resistance by DN-JNK overexpression in the liver. As shown in Fig. 3A, IRS-1 serine 307 phosphorylation was markedly decreased in Ad-DN-JNK-treated mice compared to Ad-GFP-treated mice. We also found an increase in IRS-1 tyrosine phosphorylation in Ad-DN-JNK-treated mice compared to control mice. Reduction of Akt serine 473 phosphorylation was observed in Ad-DN-JNK-treated C57BL/KsJ-db/db mice compared to Ad-GFP-treated mice. Therefore, an increase in IRS-1 serine phosphorylation may be closely associated with the development of insulin resistance induced by JNK overexpression.

Next, we examined the expression levels of the key gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), both of which are known to be regulated by insulin signaling. As shown in Fig. 3B, expression levels of both enzymes, G6Pase and PEPCK, were significantly increased by Ad-WT-JNK treatment in C57BL6 mice. In contrast, expression levels of both enzymes were markedly decreased by Ad-DN-JNK treatment in C57BL/KsJ-db/db mice. These results indicate that suppression of the JNK pathway
enhances insulin signaling which leads to a decrease in gluconeogenesis and amelioration of glucose tolerance.

**Suppression of the JNK pathway in the liver improves high-fat, high-sucrose diet-induced insulin resistance and glucose tolerance.**

Since it is well known that a high-fat and high-sucrose diet induces insulin resistance, we fed C57BL6 mice a high-fat, high-sucrose diet and examined whether suppression of the JNK pathway has any effect on high-fat, high-sucrose diet-induced insulin resistance. There was no difference in body weight and food intake between the two groups (data not shown). As shown in Fig. 4A, nonfasting blood glucose gradually became lower 2-3 weeks after the challenge in the DN-JNK-treated mice. Four weeks after starting the high-fat, high-sucrose diet, nonfasting blood glucose and insulin concentrations were significantly lower in Ad-DN-JNK-treated mice compared to Ad-GFP-treated mice (nonfasting blood glucose: 115 ± 12 versus 157 ± 7 mg/dl, p< 0.05; insulin concentration: 8.2 ± 1.5 versus 10.0 ± 1.2 ng/ml) (Fig. 4B). To further investigate this point, we performed the intraperitoneal glucose tolerance test (IPGTT). As shown in Fig. 4C, blood glucose levels in Ad-DN-JNK-treated mice were significantly lower compared to Ad-GFP-treated mice. These results indicate that suppression of the JNK pathway in the liver improves insulin resistance and glucose tolerance induced by a high-fat, high-sucrose diet. Taken together, suppression of the JNK pathway in the liver improves insulin resistance and ameliorates glucose tolerance in two different diabetic animal models with insulin resistance: C57BL/KsJ-db/db diabetic mice (Fig. 2) and high-fat, high-sucrose diet-induced diabetic mice (Fig. 4).
DISCUSSION

In this study, we show that activation of the JNK pathway in the liver using WT-JNK adenovirus increases insulin resistance and, in contrast, suppression of the JNK pathway using DN-JNK adenovirus decreases insulin resistance and markedly improves glucose tolerance in two different diabetic animal models with insulin resistance. These results suggest that the JNK pathway could be a potential therapeutic target for diabetes (Fig. 5). In addition, while it is well known that various insulin target tissues such as adipose tissue and muscle as well as the liver are involved in the progression of insulin resistance, in our study insulin resistance of the whole body in db/db mice was markedly improved by DN-JNK overexpression only in the liver, although there was no difference in glucose disappearance rate. These results suggest that JNK activation in the liver plays a pivotal role in causing insulin resistance and glucose tolerance in the whole body.

The JNK pathway is known to be activated by several factors such as oxidative stress, free fatty acids (FFAs), tumor necrosis factor-α (TNF-α), all of which are known to be increased under diabetic conditions. Under diabetic conditions, reactive oxygen species (ROS) (19) are produced in various tissues (20-23) and are involved in the development of insulin resistance (24-26) as well as the progression of β-cell deterioration (18, 27-32). FFAs and TNF-α are also likely to be involved in the development of insulin resistance; levels of FFAs and TNF-α are increased under obese diabetic conditions with insulin resistance, which leads to a further increase in insulin resistance. Thus, although not examined in our study, we assume that improvement of insulin resistance by the suppression of the JNK pathway was at least in part counterbalancing the deleterious effects of several factors such as oxidative stress, FFAs and TNF-α.

Here we report the dramatic improvement of insulin resistance by suppression of the JNK pathway, indicating the novel important role of the JNK pathway in insulin resistance. So far, the importance of the Ikappa kinase (IKK) pathway in insulin resistance has been reported (34, 35);
salicylate which inhibits the IKK pathway improves insulin resistance in skeletal muscle and decreases blood glucose levels in type 2 diabetic patients. Our present data suggest that specific inhibitors of the JNK pathway for the liver are potential therapeutic agents for insulin resistance in diabetic patients (36).

In conclusion, activation of the JNK pathway as seen under diabetic conditions increases insulin resistance, and in contrast, suppression of the JNK pathway decreases insulin resistance and markedly improves glucose tolerance in diabetic animal models.
ACKNOWLEDGMENTS

We thank Dr. Bert Vogelstein (Johns Hopkins Oncology Center) for kindly providing the AdEasy system. We also thank Ms. Yuko Sasaki for her excellent technical assistance and Ms. Chikayo Yokogawa for her efficient secretary assistance.
FOOTNOTES

* This work was supported in part by grants from the Ministry of Education of Japan (to Y.Y.).

‡ These two authors contributed equally to this work.

§ To whom corresponding should be addressed : Department of Internal Medicine and Therapeutics, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita, Osaka 565-0871, Japan. Tel. 81-6-6879-3633; Fax 81-6-6879-3639; E-mail: kaneto@medone.med.osaka-u.ac.jp.

The abbreviation used are: JNK, c-Jun N-terminal kinase; WT, wild type; DN, dominant-negative type; Ad, adenovirus; GFP, green fluorescent protein; PFU, plaque forming unit; GIR, glucose infusion rate; HGP, hepatic glucose production; IRS-1, insulin receptor substrate-1; PEPCK, phosphoenolpyruvate carboxykinase; G6Pase, glucose-6-phosphatase; FFAs, free fatty acids; TNF-α, tumor necrosis factor-α; ROS, reactive oxygen species; IKK, Ikappa kinase.
REFERENCES


FIGURE LEGENDS

Fig. 1. Effects of adenoviral WT-JNK overexpression in the liver on insulin resistance and glucose tolerance in C57BL6 mice.

(A) Ad-WT-JNK or Ad-GFP (1X10^{10} PFU/ml) was injected into the cervical vein of male C57BL/6 mice. After injection with the adenovirus, total protein was obtained from the liver and Western blot analysis was performed with an antibody for JNK. Induction of JNK expression is clearly observed in the liver, but not in muscle or adipose tissue (left panel). In addition, the GFP fluorescence in the right panel shows that many cells in the liver are infected with the adenovirus after treatment with Ad-JNK.

(B) Nonfasting blood glucose levels in C57BL6 mice treated with Ad-WT-JNK or Ad-GFP. After infection with the adenovirus, nonfasting blood glucose levels were measured for 25 days.

(C) Glucose tolerance in C57BL6 mice treated with Ad-WT-JNK or Ad-GFP. Two weeks after infection with Ad-WT-JNK or Ad-GFP, intraperitoneal glucose tolerance tests (IPGTT) were performed. After a 6-h fast, glucose was injected intraperitoneally at a dose of 2.0 g/kg body weight, and blood glucose levels were measured.

(D) Fasting blood glucose and serum insulin levels 2 weeks after infection with Ad-WT-JNK or Ad-GFP.

(E) Glucose infusion rate (GIR) and endogenous hepatic glucose production (HGP) in C57BL6 mice treated with Ad-WT-JNK or Ad-GFP. Two weeks after the adenovirus infection, GIR and HGP were estimated with an euglycemic hyperinsulinemic clamp. *, p<0.05, n = 6.

Fig. 2. Effects of adenoviral DN-JNK overexpression in the liver on insulin resistance and glucose tolerance in C57BL/KsJ-db/db mice.
(A) Nonfasting blood glucose levels in C57BLKsJ-db/db mice treated with Ad-DN-JNK or Ad-GFP (2X10^9 PFU/ml). After infection with the adenovirus, nonfasting blood glucose levels were measured for 25 days.

(B) Fasting blood glucose and serum insulin levels 2 weeks after infection with Ad-DN-JNK or Ad-GFP.

(C) Insulin resistance in C57BL6 mice treated with Ad-DN-JNK or Ad-GFP. Two weeks after Ad-DN-JNK or Ad-GFP treatment, intraperitoneal insulin tolerance tests (IPITT) were performed. After a 6-h fast, insulin was injected intraperitoneally at a dose of 2.0 U/kg body weight, and blood glucose levels were measured.

(D) Glucose infusion rate (GIR) and endogenous hepatic glucose production (HGP) in C57BL6 mice treated with Ad-DN-JNK or Ad-GFP. Two weeks after the adenovirus infection, GIR and HGP were estimated with an euglycemic hyperinsulinemic clamp. *, p<0.05, n = 6.

Fig. 3. Effects of WT- and DN-JNK overexpression on insulin signaling and gluconeogenesis in the liver.

(A) Ad-WT-JNK or Ad-GFP was injected into C57BL6 mice, and Ad-DN-JNK or Ad-GFP was injected into C57BL6 mice. Two weeks after the adenovirus infection, expressions of total and serine-phosphorylated forms (Ser 307) of IRS-1 were examined with Western blot analysis, and tyrosine-phosphorylated form of IRS-1 was examined with immunoprecipitation. Expressions of total and serine-phosphorylated forms of Akt (Ser 473) were also examined with Western blot analysis. Similar results were obtained at least three independent experiments.

(B) Two weeks after the adenovirus infection, mRNA levels of the key gluconeogenetic enzymes, PEPCK and G6Pase, were examined by Northern blot analysis. *, p<0.05, n = 3.
Fig. 4. Effects of adenoviral DN-JNK overexpression in liver on insulin resistance and glucose tolerance in C57BL6 mice treated with a high-fat / high-sucrose diet.

(A) Nonfasting blood glucose levels in C57BLKsJ-db/db mice treated with Ad-DN-JNK or Ad-GFP (2X10^9 PFU/ml). After infection with the adenovirus, nonfasting blood glucose levels were measured for 4 weeks.

(B) Fasting blood glucose and serum insulin levels 4 weeks after infection with Ad-DN-JNK or Ad-GFP.

(C) Glucose tolerance in C57BL6 mice treated with Ad-DN-JNK or Ad-GFP. Four weeks after Ad-DN-JNK or Ad-GFP treatment, intraperitoneal glucose tolerance tests (IPGTT) were performed. After a 6-h fast, glucose was injected intraperitoneally at a dose of 2.0 g/kg body weight, and blood glucose levels were measured. *, p<0.05, n = 6.

Fig. 5. Possible mechanism for JNK-mediated insulin resistance and glucose tolerance.
Fig. 1

A

<table>
<thead>
<tr>
<th>Liver</th>
<th>Muscle</th>
<th>Fat</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ad-GFP</td>
<td>Ad-GFP</td>
<td>Ad-WT-JNK</td>
</tr>
<tr>
<td>Ad-GFP</td>
<td>Ad-WT-JNK</td>
<td>Ad-GFP</td>
</tr>
</tbody>
</table>

B

Nonfasting Blood Glucose (mg/dl) vs. (days)

Adenovirus Injection

0 5 10 15 20 25

C

Blood Glucose (mg/dl) vs. (min)

0 30 60 120
Fig. 2

A

Nonfasting Blood Glucose (mg/dl)

Adenovirus Injection

(days)

B

Fasting Blood Glucose (mg/dl)

Fasting Serum Insulin (ng/ml)

Ad-GFP

Ad-DN-JNK

Ad-GFP

Ad-DN-JNK

*
Fig. 2

C

![Graph showing Blood Glucose (% initial glucose) over time. The graph compares Ad-GFP and Ad-DN-JNK groups. There are error bars indicating variability.](image)

D

![Bar charts showing Glucose Infusion Rate and Hepatic Glucose Production. The charts compare Ad-GFP and Ad-DN-JNK groups, with error bars indicating variability.](image)
Fig. 3

A

<table>
<thead>
<tr>
<th></th>
<th>C57BL6</th>
<th>C57BL/KsJ- db/db</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ad-GFP</td>
<td>Ad-DN-JNK</td>
</tr>
<tr>
<td>IRS-1-P (Ser 307)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IRS-1-P (Tyr)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total IRS-1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Akt-P (Ser 473)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>total Akt</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th></th>
<th>C57BL6</th>
<th>C57BL/KsJ- db/db</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ad-GFP</td>
<td>Ad-DN-JNK</td>
</tr>
<tr>
<td>G6Pase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEPCK</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-actin</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

![Graph showing relative ratios for G6Pase and PEPCK](https://example.com/graph.png)
Fig. 5

Diabetic Conditions

<table>
<thead>
<tr>
<th>IRS1 (Ser)</th>
<th>Tyr</th>
<th>JNK</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ Akt-P ↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Insulin Resistance ↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Deterioration of Glucose Tolerance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Ad-DN-JNK

<table>
<thead>
<tr>
<th>IRS1 (Ser)</th>
<th>Tyr</th>
<th>JNK</th>
</tr>
</thead>
<tbody>
<tr>
<td>↓ Akt-P ↑</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Insulin Resistance ↓</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Amelioration of Glucose Tolerance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Modulation of the JNK pathway in liver affects insulin resistance status
Yoshihisa Nakatani, Hideaki Kaneto, Dan Kawamori, Masahiro Hatazaki, Takeshi Miyatsuka, Taka-aki Matsuoka, Yoshitaka Kajimoto, Munehide Matsuhisa, Yoshimitsu Yamasaki and Masatsugu Hori

*J. Biol. Chem.* published online August 24, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M406963200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/early/2004/08/24/jbc.M406963200.citation.full.html#ref-list-1