Increasing fructose 2,6-bisphosphate overcomes hepatic insulin resistance of type 2 diabetes

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Wu, Chaodong, David A. Okar, Christopher B. Newgard, and Alex J. Lange. Increasing fructose 2,6-bisphosphate overcomes hepatic insulin resistance of type 2 diabetes. Am J Physiol Endocrinol Metab 282: E38–E45, 2002.—Hepatic glucose production is increased as a metabolic consequence of insulin resistance in type 2 diabetes. Because fructose 2,6-bisphosphate is an important regulator of hepatic glucose production, we used adenovirus-mediated enzyme overexpression to increase hepatic fructose 2,6-bisphosphate to determine if the hyperglycemia in KK mice, polygenic models of type 2 diabetes, could be ameliorated by reduction of hepatic glucose production. Seven days after treatment with virus encoding a mutant 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase designed to increase fructose 2,6-bisphosphate levels, plasma glucose, lipids, and insulin were significantly reduced in KK/H1J and KK.Cg-A/J mice. Moreover, high fructose 2,6-bisphosphate levels downregulated glucose-6-phosphatase and upregulated glucokinase gene expression, thereby reversing the insulin-resistant pattern of hepatic gene expression of these two key glucose-metabolic enzymes. The increased hepatic fructose 2,6-bisphosphate also reduced adiposity in both KK mice. These results clearly indicate that increasing hepatic fructose 2,6-bisphosphate overcomes the impairment of insulin in suppressing hepatic glucose production, and it provides a potential therapy for type 2 diabetes.

hepatic glucose production; glucose-6-phosphatase; glucokinase; adenovirus

THE LIVER PLAYS AN IMPORTANT ROLE in maintaining blood glucose homeostasis by controlling hepatic glucose production (HGP; see Refs. 6 and 44). In type 2 diabetes, suppression of HGP by insulin is impaired (13, 34). This, along with decreased insulin-stimulated glucose transport and metabolism in adipocytes and skeletal muscle, is a characteristic of insulin resistance in type 2 diabetes (34). Indeed, insulin resistance in liver contributes to the excessive hepatic output of glucose (9), which is highly correlated with hyperglycemia in the late state of type 2 diabetes (8). At the cellular level, inappropriate HGP involves an increased flux through glucose-6-phosphatase (G-6-Pase) and/or decreased flux through glucokinase (GK; see Refs. 10, 25, 26, 28, 40). G-6-Pase catalyzes the terminal step in HGP from the gluconeogenic and glycogenolytic pathways, and GK catalyzes the phosphorylation of glucose as the first step of glucose utilization (15). In liver, both the gene expression and activities of these enzymes are regulated by insulin (1, 12, 15, 16, 40). Therefore, the imbalance in the expression levels of G-6-Pase and GK may contribute to loss of control of HGP in diabetes and the phenotype of insulin resistance.

Previously, we reported that HGP can be regulated by modulating cellular levels of fructose 2,6-bisphosphate (F-2,6-P2; see Ref. 47), an allosteric activator of 6-phosphofructo-2-kinase and an inhibitor of fructose-2,6-bisphosphatase (31, 32). The bifunctional enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PF-2-K/F-2,6-P2ase), is the only catalyst for both synthesis and degradation of F-2,6-P2 (30). Adenovirus-mediated overexpression of a mutated form of 6PF-2-K/F-2,6-P2ase, which possesses Ser32-Ala and His258-Ala mutations (Ad-Bif-DM) designed to increase F-2,6-P2, produced a blood glucose-lowering effect and partially normalized levels of circulating free fatty acids (FFA) and triglycerides (TG) in streptozotocin (STZ)-induced diabetic mice via suppression of HGP (47). Therefore, we hypothesized that the same treatment would also be effective for type 2 diabetes, which is characterized by increased HGP and hyperlipidemia. To test this hypothesis, we compared two type 2 diabetic mouse models, KK/Hij and KK.Cg-A/J (14, 42), treated with Ad-Bif-DM with those treated with control virus (Ad-gal) and saline-treated normal C57BL/6J mice. The KK/Hij mouse has mild hyperglycemia, hyperinsulinemia, and obesity, partially because of a defect in the leptin receptor (14). The KK.Cg-A/J mouse presents a more severe type 2 diabetic phenotype than that of the KK/Hij mouse because of the association with the A' allele (42) and ectopic expression of agouti (5); the latter leads to obesity via inhibition of the melanocortin pathway (11, 37). Here, we report that increasing hepatic F-2,6-P2 levels in both KK/Hij and KK.Cg-A/J mice decreased blood glucose...
levels and ameliorated many of the metabolic consequences of type 2 diabetes. Additionally, increased hepatic F-2,6-P2 reversed the insulin-resistant pattern of the hepatic G-6-Pase and GK expression.

METHODS

Recombinant adenovirus. Adenovirus containing the cDNA encoding a mutated form of rat liver 6PF-2-K/F-2,6-P2ase (Ad-Bif-DM) was prepared as described previously (47). An adenovirus vector coding for \textit{Escherichia coli} β-galactosidase (Ad-gal) was used as a control.

Animal experiments. Eight male KK/H1J mice and nine female KK.Cg-A/+/J mice aged 8–10 wk old were obtained from Jackson Laboratories (Bar Harbor, ME). A mild non-insulin-dependent diabetes mellitus (NIDDM) phenotype is only displayed in male KK/H1J mice, whereas a severe NIDDM phenotype is displayed in both genders of KK.Cg-A/+-/- mice. There was no gender bias in the choice of KK.Cg-A/+/J mice. Animal experiments were designed as described previously (47) with minor modifications. Mice in each group were injected with Ad-gal or Ad-Bif-DM at a dose of 0.3 ml/20 g mouse body wt (1–5 × 10^11 plaque-forming units/ml) via the tail vein. All virus-treated mice were also treated with cyclosporin A and prednisone to suppress the immune response against adenovirus, as described previously (47). Plasma glucose levels were monitored 2 days before and 0, 3, 5, and 7 days after viral infusion. At the end of the experiment, blood samples were collected from the tail vein, and 0.1 M EDTA was used as anticoagulant. Plasma was obtained by centrifugation of collected blood (47). After blood collection, all mice were killed for tissue harvest. Four age-matched C57BL/6J mice from Jackson Laboratories were used as nondiabetic controls (43) and were treated only with saline (47). To determine the effects of cyclosporin A and prednisone treatment on glucose metabolism, another four a/a C57BL/6J mice were treated only with cyclosporin A and prednisone. Analysis of both plasma and liver metabolites indicated that cyclosporin A and prednisone had no effects on glucose metabolism (data not shown), which was consistent with that from cyclosporin A- and prednisone-treated 129J mice (47). The body weight of each mouse was measured the same day that plasma glucose was monitored.

The study protocols were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Minnesota.

Immunoblotting. Immunoblotting analyses for 6PF-2-K/F-2,6-P2ase, GK, and G-6-Pase were performed as described previously (16, 23, 47). Briefly, a total of 50 μg liver extract proteins (for 6PF-2-K/F-2,6-P2ase), 100 μg liver microsomal protein (for G-6-Pase), or 10 μg liver homogenate (for GK) was used for Western blot analyses. Rabbit anti-rat liver 6PF-2-K/F-2,6-P2ase (at 1,000 dilution), anti-rat liver G-6-Pase (at 1:50 dilution), or anti-rat liver GK (at 1:50 dilution) serum was used as primary antibody. The blot was followed by a 1:10,000 dilution of a goat anti-rabbit horseradish peroxidase-conjugated secondary antibody kit (ECL, Amersham Life Science, Buckinghamshire, UK).

F-2,6-P2 Content. F-2,6-P2 was extracted and assayed as described previously (47).

Plasma metabolites and insulin. Plasma levels of glucose, lactate, pyruvate, TG, FFA, and glycogen in liver homogenate were assayed as described previously (47). Plasma insulin was measured by a rat insulin ELISA kit (Crystal, Chicago, IL). Reactivity of the kit to mouse insulin is 105%. Mouse insulin was used as a standard.

Liver metabolites and glycogen. The concentrations of lactate, pyruvate, TG, FFA, and glycogen in liver homogenate were measured as described previously (47). The hepatic contents of glucose 6-phosphate (G-6-P) and fructose 6-phosphate (F-6-P) were assayed using G-6-P dehydrogenase and phosphoglucone isomerase (19).

"Liver metabolites and glycogen. The concentrations of lactate, pyruvate, TG, FFA, and glycogen in liver homogenate were assayed as described previously (47). The hepatic contents of glucose 6-phosphate (G-6-P) and fructose 6-phosphate (F-6-P) were assayed using G-6-P dehydrogenase and phosphoglucone isomerase (19)."

Statistical analysis. The statistical comparison between groups was carried out using Student's t-test. P < 0.05 were considered significant.

RESULTS

Overexpression of 6PF-2-K/F-2,6-P2ase and changes in hepatic F-2,6-P2 content. Mice of the KK/H1J and KK.Cg-A/+/J strains develop obesity-related type 2 diabetes. To test the effect of increasing hepatic F-2,6-P2 levels, mice of either strain were given a single intravenous injection of an adenovirus vector expressing a mutant form of 6PF-2-K/F-2,6-P2ase (Ad-Bif-DM). Diabetic control mice were injected with an Ad-gal. Because Ad-gal produced no effect on any metabolite (47), the normal control mice, C57BL/6J, were only treated with saline. In the livers of both KK/H1J and KK.Cg-A/+/J mice, overexpression of the mutant 6PF-2-K/F-2,6-P2ase was evident by immunoblotting analysis 7 days after treatment with Ad-Bif-DM (Fig. 1A).

Hepatic F-2,6-P2 content was higher in Ad-gal-treated KK.Cg-A/+/J mice (11.92 ± 1.91 nmol/g) than that in either C57BL/6J (5.56 ± 0.70 nmol/g) or Ad-gal-treated KK/H1J (6.25 ± 1.71 nmol/g; P < 0.05; Fig. 1B) mice. This is probably the result of glucose per se stimulating the kinase activity of 6PF-2-K/F-2,6-P2ase via xylulose 5-phosphate-dependent protein phosphatase (27), which will tend to increase hepatic F-2,6-P2.

The Ad-Bif-DM treatment produced a 116 or 54% increase in hepatic F-2,6-P2 content in KK/H1J (13.54 ± 2.24 nmol/g) and in KK.Cg-A/+/J (18.38 ± 1.71 nmol/g) mice, respectively (P < 0.05 vs. Ad-gal). Hence, the adenoviral therapy was effective at increasing cellular hepatic F-2,6-P2 content.

Effects of 6PF-2-K/F-2,6-P2ase overexpression on plasma metabolites and insulin. Compared with C57BL/6J mice, both Ad-gal-treated KK/H1J and KK.Cg-A/+/J mice were hyperglycemic and hyperlipidemic (Fig. 2A and Table 1) and had elevated plasma insulin as well (Fig. 2B), confirming type 2 diabetes and insulin resistance in these mice. Treatment of diabetic mice with Ad-Bif-DM produced effects on plasma metabolites that were consistent with overcoming insulin resistance at the metabolic level. The levels of plasma glucose started to decrease in both Ad-Bif-DM-treated KK/H1J and KK.Cg-A/+/J mice on day 1 (Fig. 2A, P < 0.05 vs. Ad-gal and P < 0.01 vs. day 0), and, by day 7, plasma glucose had decreased 35 and 28%, respectively (P < 0.01 vs. Ad-gal on day 0). Concomitant with the decreased plasma glucose, plasma lactate and pyruvate levels were increased significantly in both Ad-Bif-DM-treated KK/H1J (P < 0.05 vs. Ad-gal) and KK.Cg-A/+/J (P < 0.05 vs. Ad-gal) mice. These data are consistent with increased glycolysis in the liver of Ad-Bif-DM-treated mice. After treatment (7 days), plasma TG and FFA had decreased 43 and 55%,
Fig. 1. Overexpression of 6-phosphofructo-2-kinase (6PF-2-K)/fructose-2,6-bisphosphatase (F-2,6-P2ase) in the liver of KK mice and changes in hepatic fructose 2,6-bisphosphate (F-2,6-P2). Salinetreated C57BL/6J mice served as normal controls (n = 4). Both KK/H1J and KK.Cg-Ay/J mice were treated with control virus [adenovirus vector coding for E. coli β-galactosidase (Ad-gal)] on day 0 and were used as diabetic controls (n = 4). Adenovirus coding the mutated 6PF-2-K/F-2,6-P2ase [Ser242Ala and His246Ala] mutations (Ad-Bif-DM) was infused to both KK/H1J (n = 4) and KK.Cg-Ay/J (n = 5) mice on day 0 as the treatment. All mice were killed for tissue collection on day 7. A: immunoblotting for 6-phosphofructo-2-kinase (6PF-2-K/F-2,6-P2ase). Pooled proteins (50 μg) were from saline-treated C57BL/6J (lane 1), Ad-gal-treated KK/H1J (lane 2), Ad-Bif-DM-treated KK/H1J (lane 3), Ad-gal-treated KK.Cg-Ay/J (lane 4), and Ad-Bif-DM-treated KK.Cg-Ay/J (lane 5) mice. B: changes in hepatic F-2,6-P2. Hepatic F-2,6-P2 was extracted from liver and measured as described in MATERIALS AND METHODS. Data are means ± SE. *P < 0.05 vs. C57BL/6J; †P < 0.05 vs. Ad-gal; Table 1. After Ad-Bif-DM treatment, levels of plasma insulin were lowered in both KK/H1J (3,242.55 ± 781.69 pg/ml) and KK.Cg-Ay/J (5,241.40 ± 463.13 pg/ml; P < 0.05 vs. Ad-gal; Fig. 2B) mice. These effects were all consistent with an amelioration of the metabolic effects of type 2 diabetes resulting from elevated hepatic F-2,6-P2 content.

Effects of 6PF-2-K/F-2,6-P2ase overexpression on liver metabolites and glycogen. The data in Table 2 show liver metabolites and glycogen. Pyruvate increased significantly (P < 0.05 vs. Ad-gal) by day 7 in both Ad-Bif-DM-treated KK/H1J and KK.Cg-Ay/J mice, whereas lactate and TG were not changed. Surprisingly, liver glycogen content was very low in Ad-Bif-DM-treated KK.Cg-Ay/J mice. It also led to a 23% decrease in liver F-6-P in KK/H1J mice and a 9% increase in liver G-6-P in KK.Cg-Ay/J mice (P < 0.05 vs. Ad-gal). G-6-P was not changed in Ad-Bif-DM-treated KK/H1J mice, and F-6-P was not changed in Ad-Bif-DM-treated KK.Cg-Ay/J mice.

Fig. 2. Changes in levels of plasma glucose and insulin in C57BL/6J and KK mice. Plasma glucose levels of mice from saline-treated C57BL/6J (n = 4), Ad-gal-treated KK/H1J (n = 4), Ad-Bif-DM-treated KK/H1J (n = 4), Ad-gal-treated KK.Cg-Ay/J (n = 4), and Ad-Bif-DM-treated KK.Cg-Ay/J (n = 5) groups were monitored on days 2, 4, and 7. Plasma glucose was assayed from blood samples collected on day 7. Data are mean ± SE; A: changes in levels of plasma glucose. All KK mice (before virus infusion and after Ad-gal infusion) showed hyperglycemia (P < 0.01 vs. C57BL/6J). Ad-Bif-DM treatment brought about glucose-lowering effects in both KK/H1J and KK.Cg-Ay/J mice, which started from day 3 through day 7 (P < 0.05 or P < 0.01 vs. Ad-gal or before adenovirus infusion, respectively). B: changes in levels of plasma insulin. Diabetic control mice showed hyperinsulinemia. *P < 0.01 vs. C57BL/6J. After Ad-Bif-DM treatment, levels of plasma insulin were lowered in both KK/H1J and KK.Cg-Ay/J mice. †P < 0.05 and ‡P < 0.01 vs. Ad-gal-treated KK/H1J and KK.Cg-Ay/J, respectively.
protein in both KK/H1J (1.8-fold) and KK.Cg-Av/J (1.4-fold; \( P < 0.01 \) vs. Ad-gal-treated KK/H1J and \( P < 0.05 \) vs. Ad-gal-treated KK.Cg-Av/J, respectively) mice. The Western blots for G-6-Pase or GK do not directly indicate the difference in absolute amounts between G-6-Pase and GK protein. However, after densitometry and normalization of the G-6-Pase-to-GK ratio from the average of normal control mice, it is clear that the normalization of the G-6-Pase-to-GK ratio from the treatment resulted in decreased epididymal fat mass in both KK/H1J and KK.Cg-Av/J mice. To increase hepatic F-2,6-Pase, we introduced a mutated form of 6PF-2-K/F-2,6-P2ase, which was designed to increase hepatic F-2,6-P (47), to KK/H1J and KK.Cg-Av/J mice via an adenovirus (Ad-Bif-DM). After this treatment (7 days), the successful overexpression of the mutant 6PF-2-K/F-2,6-P2ase in the livers of both KK/H1J and KK.Cg-Av/J mice resulted in increased levels of hepatic F-2,6-P and brought about the glucose-lowering effects. These results demonstrate that increasing hepatic F-2,6-P2 is a “metabolic fix” for diabetes mellitus that overcomes the metabolic consequences of hepatic insulin resistance in type 2 diabetes.

In normal physiological circumstances, like the fasting-to-fed transition, the F-2,6-P2 level responds to glucose and/or insulin (27, 2). However, the F-2,6-P2 content in hepatocytes isolated from obese Zucker rats, which was already higher than control, was not increased further in response to insulin (36). In our study, when plasma insulin was 30-fold higher than control, there was no increase with the Ad-gal-treated KK/H1J mice or doubling with the Ad-gal-treated KK.Cg-Av/J mice in hepatic F-2,6-P2. Moreover, the amounts of 6PF-2-K/F-2,6-P2ase did not change significantly in any diabetic control relative to normal controls (Fig. 1A). The relatively low levels of hepatic F-2,6-P2 in diabetic liver might contribute to the glucose intolerance in type 2 diabetes.

### Table 1. Plasma metabolites from control and diabetic mice treated with adenovirus

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>C57BL/6J</th>
<th>KK/H1J</th>
<th>KK.Cg-Av/J</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lac, ( \mu \text{mol/g} )</td>
<td>9.63 ± 0.41</td>
<td>6.46 ± 0.84*</td>
<td>7.82 ± 0.57</td>
</tr>
<tr>
<td>Pyr, ( \mu \text{mol/g} )</td>
<td>0.35 ± 0.05</td>
<td>0.19 ± 0.02*</td>
<td>0.34 ± 0.06†</td>
</tr>
<tr>
<td>TG, ( \mu \text{mol/g} )</td>
<td>28.75 ± 1.41</td>
<td>28.63 ± 0.59</td>
<td>29.57 ± 0.82</td>
</tr>
<tr>
<td>Gly, mg/g</td>
<td>7.81 ± 2.08</td>
<td>6.95 ± 1.52</td>
<td>11.24 ± 1.48†</td>
</tr>
<tr>
<td>G-6-P, ( \mu \text{mol/g} )</td>
<td>0.49 ± 0.02</td>
<td>0.55 ± 0.01*</td>
<td>0.57 ± 0.01</td>
</tr>
<tr>
<td>F-6-P, ( \mu \text{mol/g} )</td>
<td>0.22 ± 0.01</td>
<td>0.22 ± 0.01</td>
<td>0.17 ± 0.01†</td>
</tr>
</tbody>
</table>

Data are means ± SE; \( n = 4–5 \) mice in each group. Gly, glycogen; G-6-P, glucose 6-phosphate; F-6-P, fructose 6-phosphate. Liver metabolites were assayed 7 days after adenovirus infusion. *\( P < 0.05 \) vs. C57BL/6J saline. †\( P < 0.05 \) vs. Ad-gal. ‡\( P < 0.01 \) vs. Ad-gal.
6PF-2-K/F-2,6-P\textsubscript{2}ase and F-2,6-P\textsubscript{2}, despite very high plasma insulin, reflect the insulin resistance of the liver (36). In other words, the observed impairment of insulin action on liver 6PF-2-K/F-2,6-P\textsubscript{2}ase and F-2,6-P\textsubscript{2} may lead to a failure to suppress HGP, contributing to the diabetes. The relatively low F-2,6-P\textsubscript{2} is an indicator of the metabolic consequence of hepatic insulin resistance, which exacerbates the existent hyperglycemia and insulin resistance (35). Insulin resistance at the hepatic level was also evident from increased G-6-Pase and/or decreased GK proteins in diabetic controls.

The basal levels of F-2,6-P\textsubscript{2} in KK.Cg-A\textsuperscript{a/\textit{j}} mice were higher than those in KK/H1J mice. The reason for this is not clear. However, it indicated that the genetic background (42) in these mice may be responsible for the difference in the hepatic content of F-2,6-P\textsubscript{2}. Regardless of the basal levels, Ad-Bif-DM treatment increased hepatic F-2,6-P\textsubscript{2} content 2- and 1.5-fold, respectively, in both KK/H1J and KK.Cg-A\textsuperscript{a/\textit{j}} mice. Interestingly, after Ad-Bif-DM treatment, levels of plasma insulin decreased in all diabetic mice. This is probably caused by the lower blood glucose, which will reduce the stimulatory effect on insulin secretion from pancreatic β-cells. Additionally, we observed that the greater amounts of overexpressed 6PF-2-K/F-2,6-P\textsubscript{2}ase resulted in a 1.5-fold increase in hepatic F-2,6-P\textsubscript{2} in KK.Cg-A\textsuperscript{a/\textit{j}} mice, whereas relatively less of the overexpressed protein led to a twofold increase in KK/H1J mice. This is consistent with inhibition of 6PF-2-K by citrate, which is presumably increased by stimulated glycolysis and TCA flux (30). Alternatively, this may be related to the ratio of overexpressed enzyme to endogenous enzyme; the latter may be subject to different phosphorylation/dephosphorylation regulation.

As indicated by levels of lactate and pyruvate, hepatic glycolysis is not significantly increased in diabetic controls under hyperinsulinemic conditions. Indeed, the basal levels of pyruvate were decreased in both diabetic models, which suggested an increase in glu-

**Fig. 3.** Immunoblotting for glucose 6-phosphatase (G-6-Pase) and glucokinase (GK). Mouse liver was collected on day 7 immediately after death. Whole liver homogenate (for GK) and liver microsomes (for G-6-Pase) were prepared from mice of saline-treated C57BL/6J (n = 4), Ad-gal-treated KK/H1J (n = 4), Ad-Bif-DM-treated KK/H1J (n = 4), Ad-gal-treated KK.Cg-A\textsuperscript{a/\textit{j}} (n = 4), and Ad-Bif-DM-treated KK.Cg-A\textsuperscript{a/\textit{j}} (n = 5) groups. The blots were scanned by densitometry to quantitate G-6-Pase and GK proteins. Individual densitometry values were normalized to corresponding values from C57BL/6J mice. Data are means ± SE. *Immunoblotting for G-6-Pase and GK. A total of 100 μg microsomal proteins (for G-6-Pase) or 10 μg homogenate proteins (for GK) were loaded for electrophoresis. B: quantitation of G-6-Pase protein. \( ^{\text{aP}} < 0.01 \) vs. C57BL/6J; \( ^{\text{bP}} < 0.05 \) vs. Ad-gal-treated KK/H1J. C: quantitation of GK protein. \( ^{\text{aP}} < 0.01 \) and \( ^{\text{bP}} < 0.05 \) vs. C57BL/6J; \( ^{\text{cP}} < 0.01 \) or \( ^{\text{dP}} < 0.05 \) vs. Ad-gal-treated KK/H1J or KK.Cg-A\textsuperscript{a/\textit{j}}. D: relative G-6-Pase-to-GK ratio. \( ^{\text{aP}} < 0.01 \) vs. C57BL/6J; \( ^{\text{bP}} < 0.01 \) vs. Ad-gal-treated KK/H1J and KK.Cg-A\textsuperscript{a/\textit{j}}.
that insulin prevents the accumulation of lactate in the tricarboxylic acid cycle. Therefore, it was possible that the F-2,6-P_2 dehydrogenase complex (41) that directs pyruvate to determine the conversion of pyruvate to lactate in (47). It indicated that the presence of insulin might have increased hepatic lactate instead of pyruvate showing increased hepatic lactate content obtained from STZ-treated 129J mice, which were not increased in liver after Ad-Bif-DM treatment and F-2,6-P_2 were significantly increased, whereas levels of lactate were not increased in liver after Ad-Bif-DM treatment in either model. These results were also in opposition to data obtained from STZ-treated 129J mice, which showed increased hepatic lactate instead of pyruvate (47). It indicated that the presence of insulin might determine the conversion of pyruvate to lactate in liver. In fact, insulin activates the hepatic pyruvate dehydrogenase complex (41) that directs pyruvate to the tricarboxylic acid cycle. Therefore, it was possible that insulin prevents the accumulation of lactate in liver through this mechanism. Further study is necessary to clarify this. The effect of increased hepatic F-2,6-P_2 on glycogen metabolism was not clear, nor were its relations with G-6-P and F-6-P content. However, when viewed in combination with our other data, it suggests that F-2,6-P_2 regulates glycogen metabolism only via an indirect pathway, which is closely related to the levels of blood glucose, basal glycogen content (47), and fed or fasted status (7). It is important to point out that suppression of HGP by insulin is impaired in the presence of insulin resistance. Also, HGP is increased in diabetes as a result of dysregulation of glucose metabolism resulting from both peripheral and hepatic insulin resistance (13, 38, 45). Our treatment brought about metabolic effects that insulin would have, were there no insulin resistance in liver. Thus, at the metabolic level, our treatment overcame hepatic insulin resistance to achieve an amelioration of the effects of type 2 diabetes. In addition, in preliminary data obtained from cultured cell lines, we have shown an improvement in insulin action by increasing F-2,6-P_2, at least at the level of insulin signal transduction as it affected Akt phosphorylation (18 and unpublished data).

It is well known that elevated levels of plasma TG and FFA are characteristic of type 2 diabetes (24, 33). In this study, we observed a lipid-lowering effect of Ad-Bif-DM treatment in KK mice. This effect was accompanied by loss of body weight and epididymal fat mass. The result was similar to that observed in metamorf-in-treated ob/ob mice (21). It indicates that F-2,6-P_2 might have a similar effect to metformin in balancing energy homeostasis between liver and adipose tissue (21). Adipose tissue stores lipids via lipogenesis and uptake of lipoprotein-derived fatty acids (17). The latter are partly derived from very low density lipoprotein 1 (VLDL1) that is released from the liver. In type 2 diabetes, the inhibition of VLDL1 release from liver by insulin is also impaired and contributes to hypertriglyceridemia (22). Thus the increased hepatic F-2,6-P_2 may also inhibit the release of VLDL1 and lower circulating TG. Thereby, the supply of TG to adipose tissue was reduced and resulted in loss of epididymal fat mass. In addition, hyperglycemia stimulates lipogenesis in adipose tissue and initiates increased hepatic lipogenesis, both of which contribute to the preexisting obesity (20); therefore, it is possible that increased F-2,6-P_2 reversed this effect by decreasing blood glucose, which, in turn, reduced adiposity. Alternatively, lipolysis in adipose tissue is increased as a result of lowered plasma insulin (17). Also, we cannot rule out transduction of the adipose tissue by the adenovirus vectors, which may contribute to the effect of reducing epididymal fat mass, even though it has been shown that adenoviral infection in adipose tissue was undetectable (29), and little is known about how the F-2,6-P_2 level affects lipid metabolism in adipose tissue. Adipocyte transduction experiments to directly determine the role of the F-2,6-P_2 level are underway. It is also possible that food intake was reduced in...
Ad-Bif-DM-treated mice, which would contribute to loss of body weight and reduction in adiposity.

As described above, our metabolic fix treatment was able to reduce HGP and overcome insulin resistance at the metabolic level in liver and to reverse many effects of type 2 diabetes. Not only did this treatment enhance glycolysis and/or inhibit gluconeogenesis; it also tended to normalize the G-6-Pase-to-GK ratio. The hepatic gene expression of these enzymes is repressed (G-6-Pase; see Refs. 1 and 40) or stimulated (GK; see Refs. 12, 15, 16) by insulin. Therefore, the increased G-6-Pase and decreased GK proteins under the hyperinsulinemic condition of the Ad-gal-treated diabetic KK mice are, again, indicative of impaired insulin action on the liver. Because both G-6-Pase and GK participate in the cellular mechanism by which insulin suppresses HGP (26, 28), the distal effects of F-2,6-P2 on down-regulation of G-6-Pase and upregulation of GK expression strongly suggest that increasing hepatic F-2,6-P2 overrides hepatic insulin resistance and regulates these key enzymes of glucose metabolism. However, we are not certain whether increased hepatic F-2,6-P2 brings about the gene-regulatory effects to affect metabolic change or whether increased hepatic F-2,6-P2 brings about metabolic changes to regulate gene expression (via glucose metabolites). It is also not clear whether the metabolic fix will increase insulin sensitivity in Ad-Bif-DM-treated KK mice; however, this issue is currently being addressed in ongoing experiments.

Additionally, it has been suggested that the following other mechanisms contribute to the pathogenesis of insulin resistance: 1) increased flux from F-6-P to hexosamine in liver signals a shift toward fuel storage, resulting in obesity and hepatic insulin resistance (46); 2) the elevated circulating levels of FFA cause insulin resistance in both periphery and liver (3, 4); and 3) increased release of hormones (i.e., tumor necrosis factor-α, resistin) from expanded adipose tissue promotes insulin resistance (17, 39). Potentially, the metabolic fix treatment impacted all of these mechanisms.

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