Perturbation of glucose flux in the liver by decreasing F26P2 levels causes hepatic insulin resistance and hyperglycemia

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Wu, Chaodong, Salmaan A. Khan, Li-Jen Peng, Honggui Li, Steven G. Carmella, and Alex J. Lange. Perturbation of glucose flux in the liver by decreasing F26P2 levels causes hepatic insulin resistance and hyperglycemia. Am J Physiol Endocrinol Metab 291: E536–E543, 2006.—Hepatic insulin resistance is one of the characteristics of type 2 diabetes and contributes to the development of hyperglycemia. How changes in hepatic glucose flux lead to insulin resistance is not clearly defined. We determined the effects of decreasing the levels of hepatic fructose 2,6-bisphosphate (F26P2), a key regulator of glucose metabolism, on hepatic glucose flux in the normal 129J mice. Upon adenoviral overexpression of a kinase activity-deficient 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase, the enzyme that determines F26P2 level, hepatic F26P2 levels were decreased twofold compared with those of control virus-treated mice in basal state. In addition, under hyperinsulinemic conditions, hepatic F26P2 levels were much lower than those of the control. The decrease in F26P2 leads to the elevation of basal and insulin-suppressed hepatic glucose production. Also, the efficiency of insulin to suppress hepatic glucose production was decreased (63.3 vs. 95.5% suppression of the control). At the molecular level, a decrease in insulin-stimulated Akt phosphorylation was consistent with hepatic insulin resistance. In the low hepatic F26P2 states, increases in both gluconeogenesis and glycogenolysis in the liver are responsible for elevations of hepatic glucose production and thereby contribute to the development of hyperglycemia. Additionally, the increased hepatic gluconeogenesis was associated with the elevated mRNA levels of peroxisome proliferator-activated receptor-γ coactivator-1α and phosphoenolpyruvate carboxykinase. This study provides the first in vivo demonstration showing that decreasing hepatic F26P2 levels leads to increased gluconeogenesis in the liver. Taken together, the present study demonstrates that perturbation of glucose flux in the liver plays a predominant role in the development of a diabetic phenotype, as characterized by hepatic insulin resistance.

fructose 2,6-bisphosphate; hepatic glucose production; diabetes; glycolysis; gluconeogenesis

HEPATIC INSULIN RESISTANCE is characterized by the inefficiency of insulin to suppress hepatic glucose production (HGP). This impairment, along with peripheral insulin resistance, characterized by reductions of insulin-stimulated glucose uptake in skeletal muscle and adipose tissues, contributes to the development of hyperglycemia of type 2 diabetes. To explore the molecular mechanism of hepatic insulin resistance, components of insulin signaling pathways, such as insulin receptor, insulin receptor substrate (IRS)-1, and IRS-2, have been specifically knocked out or knocked down in mouse liver (12, 29). These alterations produced hepatic insulin resistance as well as deranged glucose homeostasis. These studies provide convincing evidence for the involvement of insulin-signaling pathway in the development of hepatic insulin resistance.

Given the polygenic nature underlying the causes of insulin resistance, understanding how nutritional and environmental factors induce hepatic insulin resistance is also relevant to the nature of the defect. An advantage could be gained by understanding the role that nutritional factors play in a given tissue by defining how hepatic glucose metabolism is involved in the development of insulin resistance. In fact, several studies have addressed this issue (28, 30). One study of particular interest demonstrates that transgenic overexpression of phosphoenolpyruvate carboxykinase (PEPCK) in the liver of mice caused an increase in HGP and a decrease in hepatic insulin sensitivity. These defects were associated with decreases in protein amount and phosphorylation states of IRS-2 (28). However, the manipulation failed to produce hyperglycemia. More importantly, the relationship between altered glucose flux and insulin resistance remained undefined.

In the liver, fructose 2,6-bisphosphate (F26P2) is a key regulator of glucose metabolism. The single enzyme, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PFK2/FBP2), makes F26P2 from fructose 6-phosphate (F-6-P) and ATP or breaks F26P2 to F-6-P and inorganic phosphate (15). Under physiological conditions, elevated levels of plasma glucose and/or insulin cause dephosphorylation of 6PFK2/FBP2 through activation of protein phosphatase (PP)2A and/or other unknown PP (16), leading to increases in the kinase activity and decreases in bisphosphatase activity of 6PFK2/2B2 and thereby generating F26P2. In contrast, glucagon, at elevated levels, activates protein kinase A and causes phosphorylation of 6PFK2/2B2, leading to decreases in the kinase activity and increases in bisphosphatase activity of 6PFK2/2B2 and thereby degrading F26P2 (16). At high levels, F26P2 favors glycolysis and suppresses gluconeogenesis through allosteric activation of 6-phosphofructo-1-kinase (6PFK1) and inhibition of fructose-1,6-bisphosphatase (FBPase), respectively, in the liver (6, 19). In addition to these well-established allosteric effects, recent studies (33, 35) have shown that high levels of F26P2 also favor glycolysis and suppress gluconeogenesis at gene expression levels, i.e., upregulation of glucokinase (GK) and downregulation of glucose-6-phosphatase (G-6-Pase) gene expression. In diabetic states, hepatic F26P2 levels are absolutely low due to insulin deficiency or relatively low due to insulin resistance, which is closely associated with hyperglycemia (33, 34). In the present study, we used adenoviral overexpression of the kinase activity-deficient 6PFK2/2B2 to
create the low hepatic F26P2 state and characterized changes in glucose flux using the euglycemic hyperinsulinemic clamp technique. We also related alterations in glucose flux to the development of hepatic insulin resistance and hyperglycemia.

RESEARCH DESIGN AND METHODS

Adenovirus preparation. Adenoviruses containing the cDNA encoding kinase activity-deficient rat liver 6PFK2/FBP2 (Ad-Bif-KD) were prepared as described previously (35). The kinase activity-deficient 6PFK2/FBP2 is designed to decrease F26P2 levels. Viruses containing Escherichia coli β-galactosidase (Ad-gal) or green fluorescent protein (Ad-GFP) were used as the control. Previous experiments (32, 34) have shown that either Ad-gal or Ad-GFP treatment has no effect on levels of any hepatic and plasma metabolites. For this reason, Ad-gal- and Ad-GFP-treated mice were considered the same as control.

Animal treatments. Male 129J mice were 12–14 wk old (~25 g) and obtained from the Jackson Laboratory (Bar Harbor, ME). All mice were fed ad libitum. Before adenovirus treatment, immunosuppressants were given to 129J mice starting on day −2 and ending on day 3, as described previously (34). Adenoviruses (Ad-gal/Ad-GFP or Ad-Bif-KD) were infused through the tail vein at day 0 and maintained for the rest of the experiment. Plasma glucose levels were monitored on days −2, 0, 3, 5, and 7. On day 7 (7 days after virus infection), virus-treated mice were fasted for 4 h before blood and liver samples were collected. Three additional sets of mice with identical viral treatments were used separately for euglycemic hyperinsulinemic clamp studies, glucose tolerance test (GTT), or insulin sensitivity test (IST). The study protocols were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Minnesota.

Euglycemic hyperinsulinemic clamp studies. Clamp studies were conducted in awake, unrestrained, chronically catheterized mice as previously described (13, 21, 26, 27, 32). On day 7, mice were fasted for 4 h and followed by a 170-min in vivo protocol, which included an 80-min basal period for assessment of the rates of glucose turnover for 4 h and followed by a 170-min in vivo protocol, which included measurements at 20% glucose was initiated at time 0 and maintained for the rest of the experiment. Plasma glucose levels were monitored on days −2, 0, 3, 5, and 7. On day 7 (7 days after virus infection), virus-treated mice were fasted for 4 h before blood and liver samples were collected. Three additional sets of mice with identical viral treatments were used separately for euglycemic hyperinsulinemic clamp studies, glucose tolerance test (GTT), or insulin sensitivity test (IST). The study protocols were reviewed and approved by the Institutional Animal Care and Use Committees of the University of Minnesota.

Measurement of metabolic parameters. Plasma levels of insulin were measured using enzyme immunoassay kits (American Laboratory Products, Windham, NH). Plasma levels of glucose, lactate, triglycerides, and free fatty acids (FFA) were measured using metabolic assay kits (Thermo Clinical Diagnostics, Louisville, KY; Sigma, St. Louis, MO; and Wako Chemicals, Neuss, Germany). All measurements were the same as previously described (32, 33, 35). For F26P2 measurement, frozen liver tissue was homogenized in 10 vol of 50 mM NaOH and kept at 80°C for 5 min. The extract was cooled and neutralized at 0°C by addition of ice-cold 1 M acetic acid in the presence of 20 mM HEPES. After centrifugation at 8,000 g for 10 min, supernatant was collected and assayed for F26P2 by 6PFK1 activation method (31, 34).

Quantitation of mRNA by real-time RT-PCR. The mRNA levels were measured using real-time PCR, as previously described (31, 34). Total RNA was isolated from frozen liver or primary hepatocytes using STAT 60 reagent according to the manufacturer’s protocol (TEL-TEST B, Friendswood, TX). RT-PCR reactions were performed using the One-Step RT-PCR kit, using 250 ng of total RNA (Roche Diagnostics, Indianapolis, IN). A control cDNA was used for interplate calibration, and the variability in the initial quantities of cDNA was normalized using ribosomal 18S RNA amplifications. Results were obtained from individual mice from three different groups, each tested in triplicate. Results were expressed as arbitrary units, indicating relative expression normalized to 18S RNA.

Western blot analysis. Frozen livers were homogenized in lysis buffer (50 mM HEPES, pH 7.4, 1% Triton X-100, 50 mM sodium pyrophosphate, 0.1 M sodium fluoride, 10 mM EDTA, 10 mM sodium orthovanadate, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 2 mM benzamidine, and 2 mM PMSF). Akt and phospho-Akt were analyzed by Western blot as previously described (35). Antibodies against Akt and phospho-Akt were purchased from Cell Signaling Technology (Beverly, MA).

RESULTS

Overexpression of kinase activity-deficient 6PFK2/FBP2 decreases hepatic F26P2 levels. By catalyzing both synthesis and degradation of F26P2, 6PFK2/FBP2 is its sole determinant. The kinase activity deficient-6PFK2/FBP2, which has a Ser52-aspasate mutation and a Thr55-valine mutation, is markedly diminished with respect to kinase activity. This form of the enzyme is engineered to only degrade F26P2 (35) and was therefore introduced into the liver of normal mice via adenovirus treatment. Control viruses were either Ad-gal or Ad-GFP and were used interchangeably because they did not alter the

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levels of any hepatic plasma metabolites, including hepatic F26P2 levels (32–35). Upon virus treatment for 7 days, hepatic F26P2 levels of Ad-Bif-KD-treated mice were decreased two-fold compared with those of Ad-gal-treated mice control [2.60 ± 0.23 vs. 4.96 ± 0.78 nmol/g, P < 0.05; Fig. 1 (basal)]. These data indicate that hepatic F26P2 levels were successfully manipulated in vivo.

We also measured hepatic F26P2 levels of adeno-virus-treated mice after hyperinsulinemic clamp. In response to hyperinsulinemia, we observed an expected increase in F26P2, because insulin is able to stimulate endogenous 6PFK/FBP2 to produce more F26P2. However, Ad-Bif-KD-treated mice exhibited a much smaller increase in F26P2 levels than control under the same conditions [Fig. 1 (insulin)]. These data demonstrate that overexpression of the kinase activity-deficient 6PFK2/FBP2 is able to circumvent insulin stimulation increase in F26P2 by degrading F26P2 generated by endogenous 6PFK/FBP2.

Decreasing F26P2 levels in the liver causes an elevation of HGP. Because elevated HGP is the main contributor to hyperglycemia of diabetes (2, 4, 11, 25), we determined the effects of decreasing hepatic F26P2 levels on basal and insulin-suppressed HGP using the euglycemic hyperinsulinemic clamp technique. During the clamp study, plasma glucose levels were monitored to maintain the steady states of glucose, which were also confirmed by plasma [3H]glucose specific activities (data not shown). Under the basal condition, HGP of Ad-Bif-KD-treated mice was significantly higher than that of Ad-GFP-treated mice (16.73 ± 0.64 vs. 12.78 ± 0.38 mg·kg\(^{-1}\)·min\(^{-1}\), P < 0.001; Fig. 2A). Under the hyperinsulinemic condition, the insulin-suppressed HGP of Ad-Bif-KD-treated mice was also significantly higher than that of Ad-GFP-treated mice (6.73 ± 1.19 vs. 0.56 ± 0.06 mg·kg\(^{-1}\)·min\(^{-1}\), P < 0.01). In addition, the efficiency of insulin to suppress HGP was markedly lower in Ad-Bif-KD-treated mice than in Ad-GFP-treated mice (63.3 vs. 95.5% suppression, P < 0.01). Consistent with changes in HGP under the hyperinsulinemic condition, TGO was maintained unchanged. In primary hepatocytes, decreasing F26P2 levels caused a similar change in mRNA levels of PGC-1α (5.7 vs. 0.7 fold, 0.05 and 0.21 vs. 0.05 of basal, respectively, P < 0.01; Fig. 2C). These data suggested that decreasing F26P2 levels caused changes in the mRNA levels of PGC-1α and PEPCK, contributing to an elevation of gluconeogenesis.

Decreasing F26P2 levels in the liver impairs Akt phosphorylation. Insulin resistance in the liver was evidenced by the fact that the efficiency with which insulin was able to suppress HGP was decreased in the low hepatic F26P2 state. To substantiate hepatic insulin resistance, we measured changes in protein amount and phosphorylation of Akt, a key component of insulin-signaling pathway, under both basal and hyperinsulinemic (clamp) conditions. Compared with Ad-GFP treatment, Ad-Bif-KD treatment caused a significant decrease in insulin-induced Akt phosphorylation in the liver (Fig. 3), whereas the amount of Akt was not affected. These data confirmed the existence of hepatic insulin resistance.

Decreasing F26P2 levels in the liver impairs whole body glucose turnover. To know the changes of whole body glucose turnover, we determined the effects of decreasing hepatic F26P2 levels on the rates of basal and insulin-stimulated glucose turnover in the virus-treated mice. Under the basal condition, whole body glucose uptake (disposal), calculated as Rd (7, 10, 13), was higher in Ad-Bif-KD-treated mice than in Ad-GFP-treated mice (36.8 vs. 141.7% increase, P < 0.01). In addition, a lower GIR was required to keep Ad-Bif-KD-treated mice euglycemic during the hyperinsulinemic clamp study (Fig. 4B). These data
demonstrate that decreasing hepatic F26P2 levels caused impairment of whole body glucose turnover.

We next measured changes in whole body glycolysis. Compared with that of Ad-GFP-treated mice, whole body glycolysis of Ad-Bif-KD-treated mice was significantly decreased (Fig. 4C). This decrease is consistent with the reduced levels of hepatic lactate in Ad-Bif-KD-treated mice, indicating a reduction of glycolysis in the liver. However, it should be noted that an increase in gluconeogenesis also contributes to the reduced levels of hepatic lactate.

Decreasing F26P2 levels in the liver causes hyperglycemia. In response to Ad-Bif-KD treatment, plasma glucose levels of the treated mice were significantly elevated on day 3 and climbed higher throughout day 7 (P < 0.05, day 3 vs. day 0; P < 0.01, days 5 and 7 vs. day 0; Fig. 5A and Table 1). On day 7, mild hyperglycemia (154.61 ± 5.64 and 129.40 ± 8.50 mg/dl before and after 4 h of fasting, respectively) was observed in Ad-Bif-KD-treated mice. Upon Ad-gal treatment, the levels of plasma glucose, in contrast, remained stable throughout the entire experiment period (Fig. 5A and Table 1). Con-
sistent with the elevated levels of plasma glucose, the levels of plasma lactate of Ad-Bif-KD-treated mice were decreased compared with those of Ad-gal-treated mice (Table 1), indicating a decrease in glycolysis. The decrease in glycolysis was consistent with the rates of glycolysis measured during a euglycemic hyperinsulinemic clamp experiment (see above). Upon Ad-Bif-KD treatment, the plasma levels of triglycerides and FFA were not changed (Table 1), indicating that lipid metabolism is not affected by decreasing hepatic F26P2 levels. In this regard, FFAs may not be involved in the generation of hepatic insulin resistance.

Decreasing F26P2 levels in the liver leads to glucose intolerance and reduced insulin sensitivity. Because decreasing hepatic F26P2 levels impaired the ability of insulin to stimulate in vivo glucose turnover, we conducted GTTs and ISTs to confirm the existence of impairment of whole body glucose homeostasis. Upon glucose challenge, Ad-Bif-KD-treated mice exhibited an elevation of glucose clearance curve compared with Ad-gal-treated mice. This change indicates glucose intolerance (Fig. 5B). Upon insulin challenge, Ad-Bif-KD-treated mice also displayed an elevation of glucose clearance curve, indicating a reduction of insulin sensitivity compared with Ad-gal-treated mice (Fig. 5C).

**DISCUSSION**

Hepatic insulin resistance is a hallmark of type 2 diabetes contributing to the development of hyperglycemia. This impairment has been successfully produced in mice via interruption of the components of insulin-signaling pathways (12, 29). On the other hand, perturbation of glucose metabolism in the liver has also been shown to induce hepatic insulin resistance (28, 30). However, how glucose flux perturbation causes hepatic insulin resistance is not clear. In the present study, we studied the effects of decreasing hepatic F26P2 levels on hepatic glucose flux in the normal 129J mice. Here, we provide the first in vivo evidence that decreasing F26P2 levels promotes gluconeogenesis in the liver. We also found that the elevated flux through gluconeogenesis and glycogenolysis is responsible for hepatic insulin resistance, as well as elevated HGP, and thereby contributes to the development of hyperglycemia. These results highlight the central role of liver in the control of whole body glucose homeostasis.

To characterize how hepatic glucose flux is altered in the low F26P2 state, we introduced a kinase activity-deficient 6PFK2/FBP2 to normal 129J mice via adenovirus-mediated overexpression. This manipulation caused a decrease in hepatic F26P2 levels. In addition, this manipulation impaired normal response of endogenous 6PFK2/FPB2 to hyperinsulinemia in terms of generating F26P2. However, caution should be taken because it is impossible to distinguish the activities of endogenous 6PFK2/FPB2 from those of the overexpressed enzyme in vivo. In this regard, we relied solely on the measurement of hepatic levels of F26P2 to estimate the consequences of the net or combined activities of endogenous and overexpressed 6PFK2/FPB2. Nevertheless, decreasing F26P2 levels in the liver clearly caused elevations in HGP under both basal and hyperinsulinemic conditions. In addition, decreasing hepatic

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**Fig. 3.** Impairment of insulin-stimulated Akt phosphorylation. Normal mice were treated with adenoviruses on day 0 and followed for 7 days, as described in RESEARCH DESIGN AND METHODS. On day 7, virus-treated mice were fasted for 4 h followed by sample collection (basal) or euglycemic hyperglycemic clamping study (clamp).

**Fig. 4.** Impairment of whole body glucose turnover. Normal mice were treated with adenoviruses on day 0 and followed for 7 days, as described in RESEARCH DESIGN AND METHODS. On day 7, virus-treated mice were fasted for 4 h followed by euglycemic hyperinsulinemic clamp. Data are means ± SE (n = 6–8). *P < 0.05, **P < 0.01, or ***P < 0.001 vs. Ad-GFP. A: basal and insulin-stimulated whole body glucose uptake. B: glucose infusion rate (GIR). C: glycolysis.

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F26P2 levels brought about insulin resistance in the liver, which is evidenced by a decrease in the efficiency of insulin to suppress HGP. At the molecular level, hepatic insulin resistance was associated with a dramatic decrease in hyperinsulinemia-induced Akt phosphorylation in mice having low hepatic F26P2 levels. In light of the fact that F26P2 is a regulator of glucose metabolism, this study confirms the concept that manipulating glucose metabolism can alter insulin sensitivity of a given tissue (3, 5, 35). In terms of mechanism, increases in both gluconeogenesis and glycogenolysis are responsible for the generation of hepatic insulin resistance induced by decreasing F26P2 levels. Previously, the in vivo effects of F26P2 on inhibition of the flux through gluconeogenic pathway have not been established, although F26P2 has been identified as an inhibitor of the gluconeogenic enzyme FBPase for over 25 years (20). In fact, there was evidence to the contrary where Jin et al. (8) showed that high levels of F26P2 did not suppress gluconeogenesis in rats after an oral glucose load. Here, the present data are the first to demonstrate that low levels of F26P2 lead to an increase in gluconeogenesis in vivo.

When gluconeogenesis is elevated, increases in expression and/or activity of PEPCK and/or G-6-Pase, the two key gluconeogenic enzymes, are expected (14). In the present study, we observed an increase in the mRNA levels of PEPCK, as well as PGC-1α, in the low F26P2 state. These observations are novel with respect to the effects of F26P2 (at low levels) and also confirmed the key role of PEPCK in the control of hepatic gluconeogenesis. However, we (9) did not observe an increase in the mRNA levels of G-6-Pase, although the elevated TGO suggested an increase in the flux through G-6-Pase. Previously, we (1) have shown that increasing hepatic F26P2 levels suppresses G-6-Pase gene expression through effects secondary to lowering plasma glucose levels, whereas increasing F26P2 levels in rat hepatoma Fao cells stimulate G-6-Pase gene expression. These discrepancies suggest that the regulation of G-6-Pase is a complex process. In addition, the reason for the increase in the mRNA levels of PEPCK and PGC-1α in the low hepatic F26P2 state remains unclear. Because Akt is involved in the effects of insulin on suppression of PGC-1α-stimulated gluconeogenesis (22), it is likely that the impairment of insulin-induced Akt phosphorylation was associated with the increased PGC-1α as well as PEPCK gene expression in the low hepatic F26P2 state.

An increase in hepatic glycogenolysis also contributed to an elevation of HGP in response to decreasing hepatic F26P2 levels. The underlying mechanism for increased glycogenolysis is not clear. However, in the low hepatic F26P2 state, flux through 6PFK1 was likely reduced, whereas flux through

### Table 1. Plasma metabolic profile of adenovirus-treated mice

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ad-gal</th>
<th>Ad-Bif-KD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dl</td>
<td>110.16±8.52</td>
<td>154.61±5.64†</td>
</tr>
<tr>
<td></td>
<td>92.67±6.51</td>
<td>129.40±8.50†</td>
</tr>
<tr>
<td>Insulin, pg/ml</td>
<td>313.62±69.08</td>
<td>506.34±41.35*</td>
</tr>
<tr>
<td></td>
<td>5.08±0.27</td>
<td>3.13±0.50*</td>
</tr>
<tr>
<td>Triglycerides, mM</td>
<td>1.76±0.28</td>
<td>1.74±0.04</td>
</tr>
<tr>
<td>Free fatty acids, mM</td>
<td>1.13±0.02</td>
<td>1.08±0.01</td>
</tr>
</tbody>
</table>

Data are means ± SE, n = 6–8. Ad-gal, viruses containing *Escherichia coli* β-galactosidase; Ad-Bif-KD, adenoviruses containing the cDNA encoding kinase activity-deficient rat liver 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (6PFK/FBP2). Normal 129J mice were treated with adenoviruses on day 0 and followed for 7 days. On day 7, plasma glucose was monitored before and after 4 h of fasting. Plasma levels of insulin, lactate, and triglycerides were measured only after 4 h of fasting. *P < 0.05 or †P < 0.01 vs. Ad-gal.
glycogenolysis. In combination, increases in glycogenolysis over is expected, which would explain an increase in hepatic glycogen synthesis through both direct and indirect pathways. Under such circumstance, an increase in liver glycogen turnover is expected, which would explain an increase in hepatic glycogen repletion that perturbing glucose metabolism occurred in the liver (32), the present study demonstrates a central role that is played by the liver in the development of hyperglycemia.

In response to decreasing hepatic F26P2 levels, whole body glycolysis was decreased. This change also contributed to the development of hyperglycemia. In addition, hepatic lactate levels were reduced. For these reasons, it is very likely that hepatic glycolysis was also decreased, which could be attributable to a decrease in allosteric activation of 6PFK1 brought about by decreasing hepatic F26P2 levels. Because hepatic F26P2 at high levels stimulates GK gene expression (17, 35), a decrease in the mRNA levels of GK was expected upon decreasing hepatic F26P2 levels. However, this is not the case. There was no change in the mRNA levels of GK. It is possible that the overexpressed 6PFK2/FBP2 protein itself, which has been shown to potentiate GK gene expression (17), offsets the decrease in GK gene expression that occurs upon decreasing hepatic F26P2 levels. Further study is required to clarify this.

In conclusion, the perturbation of glucose flux brought about by decreasing hepatic F26P2 levels caused hepatic insulin resistance. This impairment is attributable to decreased flux through glycolysis and increased flux through the pathways of gluconeogenesis and glycogenolysis in the liver. In addition, the development of hepatic insulin resistance is associated with a decrease in insulin-induced Akt phosphorylation. The observation that perturbing glucose metabolism in the liver causes hepatic insulin resistance, as well as mild hyperglycemia, in the treated mice demonstrates that the liver plays a central role in the regulation of whole body glucose homeostasis.

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GRANTS

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