The Farnesoid X Receptor Modulates Hepatic Carbohydrate Metabolism during the Fasting-Refeeding Transition*

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The liver plays a central role in the control of blood glucose homeostasis by maintaining a balance between glucose production and utilization. The farnesoid X receptor (FXR) is a bile acid-activated nuclear receptor. Hepatic FXR expression is regulated by glucose and insulin. Here we identify a role for FXR in the control of hepatic carbohydrate metabolism. When submitted to a controlled fasting-refeeding schedule, FXR−/− mice displayed an accelerated response to high carbohydrate refeeding with an accelerated induction of glycolytic and lipogenic genes and a more pronounced repression of gluconeogenic genes. Plasma insulin and glucose levels were lower in FXR−/− mice upon refeeding the high-carbohydrate diet. These alterations were paralleled by decreased hepatic glycogen content. Hepatic insulin sensitivity was unchanged in FXR−/− mice. Treatment of isolated primary hepatocytes with a synthetic FXR agonist attenuated glucose-induced mRNA expression as well as promoter activity of L-type pyruvate kinase, acetyl-CoA carboxylase 1, and Spot14. Moreover, activated FXR interfered negatively with the carbohydrate response elements regions. These results identify a novel role for FXR as a modulator of hepatic carbohydrate metabolism.

The liver plays a major role in maintaining plasma glucose homeostasis by controlling a delicate balance between hepatic glucose uptake/utilization and hepatic glucose production. In the fed state, the liver stores energy from glucose by synthesizing glycogen and fat. Conversely, when plasma glucose concentrations decrease during fasting, the liver produces glucose by the glycogenolytic and gluconeogenic pathways. This fasting-refeeding transition involves a highly coordinated adaptation of the expression of genes encoding key metabolic enzymes that is orchestrated by hormones and nutrients.

In the fed state, insulin and glucose act in concert to promote the expression of genes controlling glucose utilization and fatty acid (FA) synthesis, including glucokinase (hexokinase type IV), L-type pyruvate kinase (Lpk), acetyl-coenzyme A carboxylase-1 (Acc-1), and fatty acid synthase (Fas) (1). A primary action of insulin appears to be the induction of glucokinase expression, which catalyzes the first step of intracellular hepatic glucose metabolism and signaling (2). The sterol regulatory element-binding protein-1c (Srebp-1c) has emerged as a major mediator of this insulin action (3). On the other hand, glucose also directly affects gene transcription via carbohydrate-response elements (ChoREs) that have been identified in both glycolytic (Lpk) and lipogenic (Acc, Fas, and Spot14) genes (4, 5). Recently, the transcription factor carbohydrate response element-binding protein (ChrebP) was identified based on its ability to bind the ChoRE of the Lpk promoter (6, 7). Additionally, hepatocyte nuclear factor 4α (HNF4α) acts in concert with proteins that bind to ChoREs to elicit maximal Lpk promoter activity (8, 9).

In the fasting state, the production of glucose by the liver is crucial for tissues unable to use FA as a source of energy, such as the brain and red blood cells. Phosphoenolpyruvate carboxykinase (Pepck) is considered to be a rate-controlling enzyme of gluconeogenesis (10). Inhibition of Pepck activity by insulin is considered an important physiological mechanism to repress hepatic glucose production in the early post-prandial state (11).

The farnesoid X receptor (FXR) is a nuclear receptor that is activated by bile acids (BAs) (12–14). FXR regulates BA synthesis from cholesterol by controlling the expression of several key enzymes (15, 16). FXR also influences blood lipids because FXR-deficient (FXR−/−) mice display elevated serum levels of triglycerides and cholesterol (17). Recently, we found that FXR expression in rat liver is regulated by glucose and insulin (18), suggesting that this nuclear receptor may also play a role in carbohydrate metabolism. In accordance with this hypothesis, FXR has been shown to regulate hepatic Pepck expression (19–21). Nevertheless, the physiological relevance of this regulation rests to be determined.

* This work was supported in part by the FEDER-Conseil Régional Nord-Pas de Calais Génépôle 01360124 and the Fondation Leducq. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¶ Holds a scholarship from the Ministerio de Hacienda del Gobierno de Chile.

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This paper is available on line at http://www.jbc.org

29971

The Journal of Biological Chemistry Vol. 280, No. 33, Issue of August 19, pp. 29971–29979, 2005

Printed in U.S.A.
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To address this issue, we performed fasting-refeeding experiments in FXR wild-type (FXR+/+) and FXR−/− mice. Our results indicate that FXR interferes with glycolytic and lipogenic pathways in liver by controlling, among other genes, Lpk, Acc-1, and Spot14 transcription. These data suggest that FXR also functions as a molecular modulator of hepatic carbohydrate and lipid metabolism, which uncovers a novel physiological response to the enterohepatic circulation of bile acids.

**EXPERIMENTAL PROCEDURES**

Materials—The LPK promoter plasmids (−3014LPKpGL3 and −184LPKpGL3) were constructed by cloning the fragments (−3014 to +4 or −184 to +4 relative to the transcriptional start site of rat Lpk, respectively, into pGL3 vector (Promega). L3L4-LPK-TpkGL3 was constructed by cloning 4 copies of fragment −168 to −146 of rat Lpk into TpkGL3 vector. pCDNA3-FXR and FXRE-pTALuc plasmids were kindly provided by Prof. P. Young. ACC-1pGL3 and (CholERX2-Spot14−pGL3 were kindly provided by Prof. H. Towle. Chemicals were from Sigma, GW4064 from Genfit SA (Loos, France), rabbit polyclonal (22, 23).

Tris-HCl, pH 7.5, 150 mmol/liter NaCl, 5 mmol/liter EDTA, 30 mmol/liter nitrogen. Liver proteins were extracted in lysis buffer (20 mmol/liter mammalian anti-IRβ antibodies from Santa Cruz Biotechnology, mouse monoclonal anti-phosphotyrosine (4G10) and rabbit polyclonal anti-phospho-astro-kinase p85α and anti-IRS-2 antibodies from Upstate Biotechnology. Rabbit polyclonal phospho-Akt (Ser473 and Thr308) and Akt antibodies were purchased from Cell Signaling.

**Animals and Diets—**All studies were approved by the institutional review boards for the care and use of experimental animals. Homozygous FXR-deficient (FXR−/−) mice (17) and sex- and age-matched wild-type mice were perfused on the C57BL/6 background. Mice were housed in a pathogen-free barrier facility with a 12-h light/12-h dark cycle, were maintained on a standard laboratory chow diet (UAR AO3, Villemoisson, Orge, France). For the fasting-refeeding experiments, 8–12-week-old female mice were divided into three groups: non-fasted (NF), fasted (F), and re-fed (RF). The non-fasted group was fed standard Chow diet ad libitum and killed at 9:00 a.m. The fasted group was fasted during 24 h from 2 a.m. to 9:00 a.m. The re-fed group was subjected to an additional refeeding with a high carbohydrate/low fat diet (Harlan Teklad TD88122, Madison, WI) for 6 or 24 h. The high carbohydrate diet contained 48.6% sucrose, 16.6% corn starch, 22.2% casein, 5.5% cellulose, 1% corn oil, 3.9% mineral, 1% vitamins.

Blood and Tissue Sampling—Plasma glucose concentrations were determined using Glucotrend 2 (Roche Diagnostics); plasma insulin levels using a solid phase two-site enzyme immunoassay (Mercodia AB, Sweden); plasma triglyceride, free fatty acids, lactate, and β-hydroxybutyrate concentrations using commercially available kits (BioMerieux, France; Wako Chemicals, Germany; Sigma Diagnostics; Random Laboratories). Liver triglyceride, glycogen concentrations, and in vivo hepatic triglyceride production were measured as described (22).

**In Vivo Insulin Stimulation and Analysis of Insulin Signaling—**Mice were anesthetized with pentobarbital, and injected with 1 IU/kg of human insulin (Actrapid, Novo Nordisk) into the portal vein. Livers were removed 5 min after injection and frozen in liquid nitrogen. Liver proteins were extracted in lysis buffer (20 mmol/liter Tris-HCI, pH 7.5, 150 mmol/liter NaCl, 5 mmol/liter EDTA, 30 mmol/liter sodium pyrophosphate, 50 mmol/liter NaF, 1% Triton X-100, 1 μg/ml pepstatin, 2 μg/ml leupeptin, 5 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate). For immunoprecipitation experiments, proteins were incubated overnight at 4 °C with the indicated antibodies in the presence of protein A-agarose. The immunoprecipitates were washed in lysis buffer, subjected to SDS-PAGE, and transferred to nitrocellulose. Membranes were subjected to immunodetection with the indicated antibodies. Immunoreactive bands were revealed using an ECL detection kit (Amersham Biosciences).

**Electrophoretic Mobility Shift Assays—**Electrophoretic mobility shift assays were performed as described (25) using a radiolabeled IR 1–268 probe (Promega). FXR wild-type (FXR+/+) and FXR−/− mice were sacrificed by cervical dislocation. Livers were removed 5 min after injection and frozen in liquid nitrogen. Liver proteins were extracted in lysis buffer (20 mmol/liter Tris-HCI, pH 7.5, 150 mmol/liter NaCl, 5 mmol/liter EDTA, 30 mmol/liter sodium pyrophosphate, 50 mmol/liter NaF, 1% Triton X-100, 1 μg/ml pepstatin, 2 μg/ml leupeptin, 5 μg/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride, 1 mM sodium orthovanadate). For immunoprecipitation experiments, proteins were incubated overnight at 4 °C with the indicated antibodies in the presence of protein A-agarose. The immunoprecipitates were washed in lysis buffer, subjected to SDS-PAGE, and transferred to nitrocellulose. Membranes were subjected to immunodetection with the indicated antibodies. Immunoreactive bands were revealed using an ECL detection kit (Amersham Biosciences).
cose absorption in FXR−/− mice, because an early blood glucose excursion curve after an oral glucose tolerance test was similar between FXR−/− and FXR+/+ (data not shown). Insulin concentrations in non-fasted and fasted states were not different between the two genotypes. However, plasma insulin levels were significantly lower in FXR−/− mice upon refeeding, reflecting either a pancreatic adaptative response to the relative hypoglycemia or an increased insulin sensitivity. Lactate concentrations were higher in non-fasted and refed FXR−/− mice, suggesting an increase of glucose utilization in these animals. In contrast, plasma concentrations of β-hydroxybutyrate were not statistically different between both groups. Whereas plasma FFA concentrations were slightly higher in FXR−/− during the non-fasted state, FFAs did not differ in both fasted and refed states between both genotypes.

To evaluate whether FXR plays a role in hepatic glucose metabolism, mRNA levels of several key enzymes of glycolysis, lipogenesis, and gluconeogenesis were measured. Hepatic mRNA levels of Lpk, a major enzyme of the glycolytic pathway, increased more rapidly in FXR−/− than in FXR+/+ mice in response to refeeding (Fig. 1A), being approximately 3-fold higher in FXR−/− after 6 h refeeding. However, the maximal increase of Lpk mRNA obtained after 24 h of refeeding did not differ between the two genotypes. On the other hand, the expression of glucokinase was not significantly different between both strains (data not shown). In the absence of FXR, increased glycolysis may direct substrate flow toward hepatic de novo lipogenesis, because both Acc-1 and Fas mRNAs were induced more rapidly upon refeeding (Fig. 1, B and C). Concerning the gluconeogenesis pathway, the expression of Pepck appeared to be more rapidly down-regulated upon refeeding in FXR−/− mice, being already returned to its basal value after 6 h of refeeding (Fig. 1D). In contrast, the expression of the catalytic subunit of glucose-6-phosphatase (Glc-6-Phase) did not differ between both strains (data not shown).

To determine the metabolic consequences of Fxr deficiency in the liver in vivo, glycogen and TG contents were measured after fasting-refeeding. Liver glycogen content was significantly reduced in both fed and refed FXR−/− mice (Fig. 2A). Hepatic expression of glycogen synthase and glycogen phosphorylase, two key enzymes of glycogen metabolism, were similar in both groups (data not shown). As previously described, FXR−/− mice displayed a higher liver TG content in the non-fasted state. Nevertheless, hepatic TG content did not differ after 6 (Fig. 2B) and 24 h (data not shown) of refeeding in both strains. Under fasting conditions, however, hepatic TG concentrations rose to a similar extent in FXR−/− and FXR+/+ mice. Therefore, hepatic TG concentrations followed the plasma FFA increase during fasting (Table 1), likely reflecting the changes in adipose tissue lipolysis. As expected (17), plasma TG concentrations were increased in FXR−/− mice in the non-fasted state (Fig. 2C). Whereas plasma TG concentrations decreased to a similar level in the two groups during fasting, they were significantly higher in FXR−/− mice upon refeeding. Plasma TG concentrations in FXR−/− mice upon refeeding is associated with increased export, because hepatic very low density lipoprotein production was significantly increased in FXR−/− mice after 6 h of refeeding (Fig. 2D). Taken together, these results suggest that increased mRNA expression of lipogenic enzymes observed in FXR−/− mice during refeeding leads to increased triglyceride synthesis.

**Table I**

|                      | FXR+/+ | FXR−/− |
|----------------------|--------|--------|
|                       | NF (n = 12) | F (n = 13) | RF (n = 8) | NF (n = 14) | F (n = 15) | RF (n = 7) |
| Glucose (mg/dL)      | 161 ± 4  | 103 ± 4  | 177 ± 4  | 157 ± 4  | 100 ± 5  | 148 ± 12*  |
| Insulin (µg/liter)   | 1.24 ± 0.27 | 0.26 ± 0.04 | 1.14 ± 0.17 | 1.57 ± 0.40 | 0.18 ± 0.03 | 0.59 ± 0.10*  |
| Lactate (mg/dL)      | 285 ± 31 | 230 ± 17 | 413 ± 37 | 427 ± 56* | 163 ± 18* | 553 ± 55* |
| Lipases (s/mmol)     | 0.13 ± 0.02 | 1.96 ± 0.11 | 0.30 ± 0.07 | 0.22 ± 0.07 | 2.17 ± 0.18 | 0.16 ± 0.05* |
| Free fatty acid (mmol/liter) | 0.52 ± 0.03 | 1.09 ± 0.06 | 0.41 ± 0.13 | 0.82 ± 0.06* | 1.26 ± 0.11 | 0.38 ± 0.08 |

**Fig. 1.** FXR−/− mice display an altered hepatic gene expression profile of glycolytic and lipogenic enzymes during the fasting-refeeding schedule. Non-fasted (NF) FXR−/− and age-matched FXR+/+ mice were subjected to 24-h fasting (F), and then refed for 6 h (RF) with a high carbohydrate/low fat diet, as described under “Experimental Procedures.” Results are expressed as the mean ± S.E. Statistical significant differences between FXR−/− and FXR+/+ mice are indicated by the asterisk (*, p < 0.05).
upon refeeding (Fig. 3C). A similar, albeit less pronounced, response was observed for Chrebp (Fig. 3D). However, both genes responded similarly in FXR+/− and FXR−/− mice. Taken together, these results suggest that the observed differences in glycolytic (Pfk) and lipogenic (Fas and Acc-I) gene expression in FXR−/− mice are not mediated via alterations in the transcriptional regulation of Srebp-1c and Chrebp.

Conversely, peroxisome proliferator-activated receptor γ-coactivator 1α (PGC-1α) expression increased upon fasting and decreased upon refeeding, in line with previous reports [28]. Pgc-1α mRNA levels were significantly lower in the basal (non-fasted) state, slightly increased during fasting, and more rapidly decreased after 6 h of refeeding in FXR−/− mice (Fig. 3E). Hnf4α mRNA also decreased more rapidly after 6 h of refeeding in FXR−/− mice (Fig. 3E). Finally, Foxo1 mRNA levels displayed a similar expression profile as Pgc-1α and Hnf4α, with a faster decrease upon refeeding in FXR−/− mice (Fig. 3F). However, the level of serine 256 phosphorylation of endogenous Foxo1 protein did not differ in liver of both strains after 6 h of refeeding (data not shown).

**Fxr Deficiency Does Not Alter Hepatic Insulin Signaling**—Because insulin and glucose act synergistically to induce the transcription of lipogenic genes, we examined whether insulin or glucose signaling or both were altered in the liver of FXR−/− mice. Basal hepatic glucose production, assessed during an euglycemic, hyperinsulinemic clamp, after a moderate fasting period of 9 h, was lower in FXR−/− compared with FXR+/− mice (136 versus 103 μmol/kg/min; p < 0.05). In contrast, in the steady state condition, clamped hepatic glucose production was suppressed to a similar extent by insulin in both groups (FXR+/−, 54 ± 7 μmol/kg/min; FXR−/−, 48 ± 7 μmol/kg/min), indicating that hepatic insulin sensitivity was not altered in FXR−/− mice. To further investigate insulin receptor (IR)-mediated signaling in the liver, mice were fasted overnight and injected with either recombinant human insulin (1 IU/kg) or saline via the portal vein. Insulin-stimulated tyrosine phosphorylation of IR and insulin receptor substrate 2 (IRS-2), the main IR-docking substrate in the regulatory subunit of phosphatidylinositol 3-kinase to IRS-2 (IRS-2), the main IR-docking substrate in

**Fig. 2. FXR−/− mice display altered hepatic carbohydrate metabolism during fasting-refeeding transition.** Glycogen (A) and triglyceride (B) contents as well as plasma triglyceride levels (C) were measured in FXR−/− (filled bars) and age-matched FXR+/− (empty bars) mice (n = 4–6 mice/group) submitted to the fasting-refeeding schedule. D, plasma triglyceride accumulation and hepatic triglyceride secretion (inner plot) after lipolysis inhibition was measured in both strains after 6 h of HCHO refeeding (n = 5–6 mice/group). Data are expressed as mean ± S.E. Statistically significant differences between FXR+/− and FXR−/− mice are indicated (*, p < 0.05; ***, p < 0.001). NF, non-fasted; F, fasted; RF, refed.

**Fig. 3. Hepatic gene expression levels of different transcription factors in livers from FXR+/− and FXR−/− mice upon fasting-refeeding.** Analysis was performed as described in the legend to Fig. 1. Values are normalized relative to 36B4 mRNA and are expressed (mean ± S.E.) relative to those of ad libitum non-fasted FXR+/− mice, which are arbitrarily set at 1. Statistical differences were indicated: §, p < 0.05; §§, p < 0.01 compared with fed mice. *, p < 0.05; **, p < 0.01; ***, p < 0.001 compared with FXR−/− mice (n = 5–7 mice/group).
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FIG. 4. Hepatic insulin signaling is not altered in FXR−/− mice (A–C). 12-h fasted FXR−/− and FXR+/+ mice were infused with either saline (−) or recombinant human insulin (1 IU/kg) (+) via the portal vein. Liver protein extracts were subjected to immunoprecipitation (IP) using antibodies directed against insulin receptor-β (IR-β) (A), IRS-2 (B) followed by Western blotting with anti-phosphotyrosine (PY), -IR-β, -IRS-2, or -p85 regulatory subunit of phosphatidylinositol 3-kinase antibodies. The same liver samples were subjected to Western blot analysis using Thr308- or Ser473-phosphorylated Akt or Akt total antibodies. The blots are representative of two different experiments (n = 6 mice/group).

analog 8-CPT-cAMP, and dropped significantly upon incubation with insulin. Pre-treatment of the cells with GW4064 did not alter the insulin-induced repression of Pealc mRNA expression (Fig. 5A), whereas it efficiently increased the expression of the Fxr-target gene Shp under the same conditions (data not shown). Because glucose is able to inhibit Pealc expression in the absence of insulin (29, 30), it was then investigated whether Fxr activation by GW4064 could alter this effect. Pealc expression was decreased by ~50% in the presence of a high (25 mmol/liter) glucose concentration (Fig. 5B). Interestingly, pre-treatment with GW4064 abolished the inhibitory action of glucose on Pealc expression.

To determine whether activated Fxr also affects the activation of other glucose-responsive genes, primary rat hepatocytes were pre-treated with GW4064 (5 μmol/liter) and subsequently cultured in low or high glucose concentrations. Lpk expression was strongly induced by high glucose (Fig. 5C). Glucose-dependent induction of Lpk expression was reduced upon treatment with GW4064 (Fig. 5C). Moreover, the glucose-mediated induction of lipogenic genes such as Spot14 (S14) and Acc-1 was also reduced by GW4064 (Fig. 5, D and E). Glucose induction of Glc-6-Pase, another gene positively regulated by glucose (31), was also significantly blunted by Fxr activation with GW4064 (Fig. 5F). This suggests that Fxr modulates a broad panel of glucose-responsive genes, including glycolytic, lipogenic, and gluconeogenic enzymes. To verify the efficiency of Fxr activation, expression of the Fxr-target gene Shp was measured (Fig. 5G). As expected, GW4064 strongly induced the expression of Shp, independent of the prevailing glucose concentration. These results support the concept that activated Fxr interferes with glucose signaling independent of changes in Shp expression.

To further confirm the role of Fxr in the regulation of Lpk by glucose, hepatocytes were isolated from FXR−/− and FXR+/+ mice. A 2-fold induction of Lpk mRNA levels was observed in wild-type mice hepatocytes in response to high glucose concentrations, similarly as previously reported (2, 32). Treatment with GW4064 abolished almost completely the glucose induction of Lpk mRNA (Fig. 6A, left panel). In hepatocytes isolated from FXR−/− mice, Lpk mRNA levels were drastically in-
activity, it was determined whether FXR binds physically to
activated Fxr modulates the glucose response via negative in-
consensus FXRE site (Fig. 7E, lanes 2 and 4). FXR bound to
the L3-Lpk site as monomer (Fig. 7E, lane 7), because the
presence of FXR did not modify FXR complex mobility (Fig. 7E,
lane 8). The complex was specific, because the addition of an
antibody against FXR was able to eliminate FXR binding (Fig.
7E, lanes 11 and 12). These results suggest that FXR also bind
to the ChoRE region of the Lpk promoter, an effect that may
contribute to the modulation of its glucose response.

**DISCUSSION**

FXR regulates genes controlling biological pathways such as
the synthesis and transport of BAs as well as lipid and lipopro-
tein metabolism. Here, we demonstrate that FXR also plays a
role in regulating hepatic carbohydrate metabolism. In vivo
analysis of the phenotype of FXR−/− mice revealed an exagger-
ated response to HCHO refeeding, resulting in an earlier, more
pronounced induction of expression of glycolytic and lipogenic
genes. Conversely, ligand-activated FXR decreased the expres-
sion of the same genes in response to high glucose concentra-
tions in primary rodent hepatocytes. In addition, Lpk mRNA
expression was up-regulated in hepatocytes isolated from
FXR−/− mice. At the molecular level, FXR decreased the tran-
scription of the Lpk promoter induced by glucose acting via its
ChoRE. Absence of Fxr therefore leads to an enhanced glyco-
lytic flux, providing substrates for lipogenesis that may con-
tribute to the hypertriglyceridemia observed in FXR−/− mice.

The liver is a major site of carbohydrate metabolism and de
novo lipogenesis. Under HCHO refeeding conditions, glucose
provides the main source of substrates for FA synthesis via
production of acetil coenzyme A. The observation that hepatic
Fxr expression varies during nutritional changes (18, 26) led us
to hypothesize that Fxr may play a role in the control of car-
bohydrate metabolism. Several lines of evidence suggest that
glucose utilization is increased in Fxr−/− mice: (i) plasma

glucose and insulin levels were significantly lower upon refeed-
ing in Fxr−/− mice even though food consumption and glucose
absorption were similar in Fxr−/− and Fxr+/+ mice, (ii) he-

patic expression of Lpk, Fas, and Acc-1 was more rapidly in-
duced upon refeeding in Fxr−/− mice, (iii) the changes in gene
expression of these glycolytic and lipogenic enzymes correlated
with increased hepatic de novo triglyceride synthesis.

FA from the lipogenic pathway are partly used to synthesiz
TG, which can either be stored in the liver or exported as very
low density lipoprotein particles. Whereas hepatic TG content
was significantly higher in non-fasted Fxr−/− mice, it rose to a
similar extent under HCHO refeeding in both Fxr−/− and
Fxr+/+ mice. Furthermore, hepatic TG secretion was in-
creased in Fxr−/− mice upon refeeding, which may contribute
to the elevated plasma TG levels observed under these condi-
tions. FA from de novo lipogenesis efficiently mobilize apoli-
ipoprotein B and induce very low density lipoprotein particle
assembly (33, 34). Moreover, Fxr−/− mice produce larger very
low density lipoprotein particles than wild-type mice (35).

Because both insulin and glucose act synergistically to regu-
late glycolytic and lipogenic gene expression (1), the exagger-
ated response to refeeding in Fxr−/− mice may reflect either an
enhanced hepatic insulin sensitivity, a potentiation of car-
bohydrate-induced signaling, or a combination of both.

Whereas a recent study reported that BAs may interfere with
insulin signaling in hepatocytes (36), we were unable to detect
any modification of hepatic insulin sensitivity in Fxr−/− mice.
This finding is in accordance with the similar mRNA levels of
glucoinase in livers of Fxr−/− and wild-type mice. Therefore,
we determined whether changes in carbohydrate metabolism

**FIG. 6.** Fxr mediates the inhibition of glucose-induced Fxr
gene expression by GW4064. Hepatocytes isolated from Fxr+/+ and
Fxr−/− mice were pretreated with vehicle (Me2SO) or GW4064 (5
µmol/liter) for 12 h, and then cultured for 6 additional hours in the
presence of low (5 mmol/liter) or high (25 mmol/liter) glucose and
insulin (1 nmol/liter). Lpk (A) and Shp (B) mRNA levels were measured
by quantitative real-time PCR. Values were normalized relative to
36B4 mRNA and are expressed relative to those of vehicle-treated
hepatocytes cultured in low glucose medium, which was arbitrarily set
at 1. The experiment shown is representative of two independent
experiments.

**FXR Inhibits the Activation of Glucose-responsive Promoters at
the Transcriptional Level—**To determine the mechanism by
which Fxr regulates glucose-induced gene expression, primary
rat hepatocytes were co-transfected with the Lpk and Acc-1
promoters and subsequently incubated in either high or low

glucose concentrations and treated with GW4064. High glucose
strongly increased Lpk promoter activity, an effect that was
prevented by GW4064 treatment (Fig. 7A, left panel). A similar
effect, albeit in a lesser extent than for Lpk, was observed on the
Acc-1 promoter (Fig. 7A, right panel). Moreover, a hetero-
logous promoter driven by the L3L4-Lpk glucose response ele-
ment was also repressed by activated Fxr and this effect was
enhanced by cotransfected Fxr (Fig. 7B). In a similar way,
glucose induction of an heterologous promoter containing the
ChoRE-Spot14 region was also repressed by activated Fxr (Fig.
7C). Under similar transfection conditions, GW4064 and Fxr
induced the expression of a heterologous promoter driven by a
consensus FXRE site (Fig. 7D). These results suggest that
activated Fxr modulates the glucose response via negative in-
terference with the ChoRE.

In light of the strong effect of activated Fxr on Lpk promoter
activity, it was determined whether FXR binds physically to
the L3L4 site. As previously described (25), FXR can bind to
consensus IR-1 FXRE either as a monomer or as a heterodimer
in the presence of RXR (Fig. 7E, lanes 2 and 4). FXR bound to
the L3-Lpk site as monomer (Fig. 7E, lane 7), because the
presence of FXR did not modify FXR complex mobility (Fig. 7E,
lane 8). The complex was specific, because the addition of an
antibody against FXR was able to eliminate FXR binding (Fig.
7E, lanes 11 and 12). These results suggest that FXR also bind
to the ChoRE region of the Lpk promoter, an effect that may
contribute to the modulation of its glucose response.
could explain the phenotype of FXR<sup>−/−</sup> mice. Primary hepatocytes were used to differentiate between systemic and hepatocyte-specific effects on glycolytic and lipogenic gene transcription. Interestingly, activation of Fxr with its specific ligand GW4064 significantly reduced glucose-induced expression of Lpk, Acc-1, and S14. This effect was glucose-dependent, because insulin concentrations were kept constant throughout the experiments. The genes encoding these enzymes all contain ChoREs in their promoters. In the case of the Lpk promoter, four nuclear protein-binding regions (L1 to L4) have been described (8). The L4 site contains the Lpk ChoRE (9) and binds the transcription factor Chrebp, which is thought to translate glucose signaling in transcriptional responses (6). The contiguous L3 site binds Hnf4α and several studies suggest that the L3/L4 site is required for full promoter activity (9, 37). In addition, the Acc-1 and S14 promoters also contain ChoRE and are regulated by ChREBP (7, 32). Results from transfection assays demonstrated that FXR inhibits glucose-induced Lpk and Acc-1 promoter transcription. Moreover, our results indicate that FXR negatively interferes with the isolated ChoRE from the Lpk and S14 genes and decreases the glucose response. In addition, gel shift assays indicated that FXR physically interacts with the L3 region of the Lpk promoter, an effect that may contribute to the strong effect of Fxr on its transcriptional activity induced by glucose.

Our in vivo results indicate that Fxr regulates glucose-induced Lpk gene expression. Moreover, in vitro in primary mouse hepatocytes, Fxr deficiency results in a derepression of basal Lpk expression. Carbohydrate responsiveness is mainly mediated by the L4 region to which, among other transcription factors, Chrebp binds (6, 7). However, Chrebp expression was not different in the FXR<sup>−/−</sup> mice. Thus, additional molecular mechanisms for Fxr-mediated inhibition of glucose-regulated genes may exist. Considering that Srebp-1c may be negatively controlled by BAs (26, 38), its expression levels were also measured to determine its potential involvement in the regulation of glycolytic and lipogenic gene expression in FXR<sup>−/−</sup> mice. However, Fxr deficiency was not associated with an increased Srebp-1c expression<sup>in vivo</sup>. Therefore, it is unlikely that the observed effects in FXR<sup>−/−</sup> mice occur via an indirect pathway implicating alterations in expression of Srebp-1c or Chrebp. Because both Chrebp and Srebp-1c are mainly regulated at the post-translational level, it cannot be excluded that Fxr interferes with the activity of any or more of these transcription factors. Taken together, our observations suggest that at least one of the molecular mechanisms of Fxr action on carbohydrate metabolism implicate a direct effect on the expression of glucose-regulated genes, such as Lpk.

FXR modulates the kinetics rather than the amplitude of the response to dietary carbohydrate intake, because a strong in-
Fxr inhibits BA synthesis by inhibition of ACC-1 mRNA levels occurred as early as 6 h after refeeding in FXR−/− mice. Thus, Fxr appears to act as a molecular modulator of carbohydrate metabolism during the fasting-refeeding transition. The nutritional regulation of Fxr expression correlates well with the changes in hepatic glucose metabolism in wild-type mice. During fasting, Fxr is induced by Pgc-1α (26). However, the increased expression and activity of Cyp7a1, a negative regulated Fxr target gene (15, 16), during fasting (20) suggests that the transcriptional activity of Fxr is decreased. From a physiological point of view, this would prepare BAs for the digestion and absorption of fats after a subsequent meal. Upon meal ingestion, the increased enterohepatic circulation of BA negatively feedbacks BA synthesis. In a subsequent meal. Upon meal ingestion, the increased enterohepatic cycling of BAs, our findings suggest a novel physiological role of bile acids in the control of post-prandial carbohydrate metabolism via Fxr activation.

For Foxo1 was not different in livers of refeed FXR+/− and FXR−/− mice, suggesting that the observed differences in Foxo1 mRNA levels likely have a minor physiological impact. Another hypothesis may be that increased hepatic carbohydrate utilization in FXR−/− mice may interfere with PEPCk expression. Indeed, the glucose-dependent inhibition of Pepek gene expression (29) was decreased in response to Fxr activation in primary hepatocytes. Alternatively, an increased glycolytic flux per se may be involved because it decreases gluconeogenic substrates and directly pyruvate to the lipogenic pathway (41). Additionally, our findings suggest that unaltered hepatic insulin signaling in FXR−/− mice, the phosphorylation level of |

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