Glucose Regulates the Expression of the Farnesoid X Receptor in Liver

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An increased prevalence of hypertriglyceridemia and gallbladder disease occurs in patients with diabetes or insulin resistance. Hypertriglyceridemia is positively associated to gall bladder disease risk. The farnesoid X receptor (FXR) is a bile acid–activated nuclear receptor that plays a key role in bile acid and triglyceride homeostasis. The mechanisms controlling FXR gene expression are poorly understood. This study evaluated whether FXR gene expression is regulated by alterations in glucose homeostasis. FXR expression was decreased in livers of streptozotocin-induced diabetic rats and normalized upon insulin supplementation. Concomitantly with diabetes progression, FXR expression also decreased in aging diabetic Zucker rats. In primary rat hepatocytes, D-glucose increased FXR mRNA in a dose- and time-dependent manner, whereas insulin counteracted this effect. Addition of xylitol, a precursor of xylulose-5-phosphate, to primary rat hepatocytes increased FXR expression to a comparable level as D-glucose. Finally, expression of the FXR target genes, SHP and apolipoprotein C-III, were additively regulated by D-glucose and FXR ligands. This study demonstrates that FXR is decreased in animal models of diabetes. In addition, FXR is regulated by glucose likely via the pentose phosphate pathway. Dysregulation of FXR expression may contribute to alterations in lipid and bile acid metabolism in patients with diabetes or insulin resistance. *Diabetes* 53:890–898, 2004

More than 300 million people worldwide are at risk of developing diabetes. Type 1 diabetes is usually of relatively early onset, resulting from the destruction of insulin-producing pancreatic β-cells. Type 2 diabetes, by contrast, develops in middle-aged adults as a result of a western lifestyle that leads to insulin resistance. Insulin resistance is initially characterized by glucose intolerance and develops subsequently in overt type 2 diabetes when pancreas failure occurs. High serum triglyceride levels, often present in type 2 diabetes, have been positively associated with gall bladder disease risk (1), a highly prevalent pathology in diabetic patients (2,3). In addition, alterations in bile composition have been described in diabetic patients (4,5). However, the molecular link between bile acid metabolism and triglyceride homeostasis in diabetes or insulin resistance remains to be determined.

Glucose is an important modulator of gene expression. Recent studies have identified transcription factors, e.g., upstream stimulatory factors, sterol response element-binding protein-1c, chicken ovoalbumin upstream promoter-transcription factor 2, and carbohydrate response element-binding protein, as mediators of glucose-induced gene regulation (6–9). However, the mechanism by which glucose regulates the transcriptional activity of these factors is still unclear. Glucose effects on gene transcription may be mediated via metabolites such as glucose-6-phosphate, a glucose-6-phosphatase complex substrate, or xylulose-5-phosphate, an intermediate metabolite in the nonoxidative branch of the pentose phosphate shunt (10,11). In addition, the hexosamine pathway is also involved in the regulation of genes by glucose (12).

The farnesoid X receptor (FXR) (NR1H4) is a member of the nuclear receptor superfamily that is expressed in adrenals, intestine, kidney, and liver (13,14). FXR binds to FXR response elements (FXREs) after heterodimerization with the retinoid X receptor (RXR). The optimal DNA binding sequence for the FXR/RXR heterodimer is an inverted repeat of two AGGTCA half-sites spaced by one nucleotide (inverted repeat-1) (15). However, FXR also binds as a monomer to extended half-sites (16,17). Bile acids are natural FXR ligands (18–20), whereas GW4064 is a synthetic nonsteroidal FXR agonist (21). FXR negatively regulates bile acid synthesis by decreasing the expression of cholesterol-7α-hydroxylase (Cyp7a1), the rate-limiting enzyme of bile acid synthesis, in part through an indirect mechanism involving the induction of the small heterodimer partner (SHP) (22,23). In addition, FXR controls the enterohepatic circulation of bile acids by increasing the transcription of the intestinal ileal-bile acid binding protein (24), inhibiting the hepatic expression of bile acid transporters Na^+-taurocholate cotransporting polypeptide (25) and inducing the bile salt export pump (26) and multidrug resistance-associated protein 2 (27). Overall, bile acid–activated FXR controls bile acid homeostasis.

FXR also regulates lipoprotein metabolism. FXR-deficient mice display elevated serum cholesterol and triglyc-

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apo, apolipoprotein; Cyp7a1, cholesterol-7α-hydroxylase; EMSA, electrophoretic mobility shift assay; FXR, farnesoid X receptor; FXRE, FXR response element; LPK, liver pyruvate kinase; RXR, retinoid X receptor; SHP, small heterodimer partner; STZ, streptozotocin.

eride levels (28,29). Moreover, FXR regulates genes controlling lipid metabolism, such as apolipoprotein (apo) C-II, apo C-III, apo A-I, apo E, and phospholipid transfer protein (16,30–33). In addition, FXR induces peroxisome proliferator–activated receptor-α expression (34), another nuclear receptor controlling triglyceride metabolism, corroborating a potential role for FXR in triglyceride metabolism.

Considering the clinical link between hypertriglyceridemia and altered bile acid metabolism in diabetes and the key role of FXR in triglyceride and bile acid homeostasis, we set out to evaluate whether FXR gene expression is regulated by conditions of altered glucose homeostasis. Our results show that streptozotocin (STZ)-induced diabetes results in a downregulation of FXR gene expression. In addition, liver FXR expression decreases in aging Zucker diabetic fatty (ZDF) rat concomitant with diabetes progression. Furthermore, using primary rat hepatocytes from nondiabetic rats, we demonstrate that D-glucose positively regulates FXR gene expression in a dose- and time-dependent manner, probably by acting at the transcriptional level. In addition, expression of the FXR target genes SHP and apo C-III is additively regulated by glucose and FXR ligands. Finally, we show that the effects of glucose are mediated via metabolites of the pentose phosphate shunt.

RESEARCH DESIGN AND METHODS

Male Wistar rats (~150 g), purchased from IFFA CREDO (L'Arbresle, France), were housed in a temperature-controlled environment with an alternating 12-h light/dark cycle and had free access to food and water.

Male Zucker diabetic fatty (ZDF/GMI-*fa/fa*) and lean rats (ZLC/GMI-+/−), 5 weeks of age, obtained from Genetic Models (Charles River Laboratories, Wilmington, MA), were maintained with ad libitum access to a Purina 5008 diet (PMI Nutrition International, Wellingborough, Northamptonshire, U.K.). A total of 48 rats were divided into three groups of 16 animals (8 ZDF rats and 8 ZLC rats) at 6, 10, and 20 weeks of age. Body weights and food consumption were monitored throughout the experiment.

Blood samples were collected by retro-orbital puncture under anesthesia and glucose (enzymatic assay; Sigma, St. Louis, MO), and insulin levels were determined (radioimmunoassay; Cis Bio International, Shering SA, Gif/Yvette, France). All experiments were performed according to the institutional guidelines for the care and use of laboratory animals in research (*Guide for the Care and Use of Laboratory Animals*, 7th edition, National Institutes of Health, 1996).

STZ-induced diabetes. The protocol used for STZ-induced diabetes in rats was previously described (35). Diabetes was induced by a single intraperitoneal injection of STZ (6 mg/100 g body wt; $n = 14$) (Pharmacia & Upjohn, Kalamazoo, MI). Control rats ($n = 5$) received an intraperitoneal injection of the solvent (3% sodium citrate). Induction of diabetes was perceived by development of hyperphagia, polydipsia, and polyuria and confirmed by determination of the degree of hyperglycemia. Three weeks after STZ injection, one group of diabetic rats ($n = 7$) was treated subcutaneously with insulin (long-acting insulin [Humulin NPH; Eli Lilly, Nieuwegein, the Netherlands]; 1 IU in the morning and 2 IU in the evening). Experiments were performed at 4 weeks after STZ injection. None of the rats receiving STZ died during the 4 weeks of the experiment.

Primary rat hepatocytes. Rat hepatocytes were isolated by collagenase perfusion from livers of Wistar rats (36). Hepatocytes (cell viability >85% by a trypan blue exclusion test) were cultured as a monolayer in serum-free William's E medium supplemented with 2 mmol/l glutamine, 25 μg/ml gentamicine, 100 nmol/l dexamethasone, and 0.1% fatty acid–free BSA at 37°C in a humidified atmosphere of 5% CO_2, 95% air. After 4 h of incubation, medium was changed to glucose-free Dulbecco's modified Eagle's medium (Invitrogen, France) supplemented with 2 mmol/l glutamine, 25 μg/ml gentamicine, 100 nmol/l dexamethasone, 10 mmol/l lactate, and 5.5 mmol/l D-glucose. After an overnight culture, cells were incubated for the indicated time in fresh culture medium supplemented with 2 mmol/l glutamine, 25 μg/ml gentamicine, 100 nmol/l dexamethasone, and the different compounds as described in RESULTS. As indicated, cells were cultured with actinomycin D (2.5–5 μg/ml), and D-glucose was added to the culture medium as described in the figure legends.

RNA extraction and quantitative PCR analysis. Total RNA was isolated from liver or primary hepatocytes (2.5×10^6 cells/plate) using the Trizol reagent according to the manufacturer's instructions (Life Technologies, Cergy-Pontoise, France). RNA was reverse transcribed using random hexamer primers and 200 units of MMLV reverse transcriptase (Invitrogen). FXR, SHP, Cyp7a1, liver pyruvate kinase (LPK), apo C-III, and 36B4 mRNA were quantified by real-time quantitative PCR on an MX-4000 apparatus (Stratagene) using specific primers (FXR forward: 5′-CCA ACC TGG GTT TCT ACC C-3′; FXR reverse: 5′-CAC ACA GCT CAT CCC CTT T-3′; SHP forward: 5′-ACT GCC TGT GCC AGC AAC AC-3′; SHP reverse: 5′-AGC CGT CGC TGA TCC TCA TG-3′; Cyp7a1 forward: 5′-ATG ATG ACT ATT TCT TTG ATT TGG GGA-3′; Cyp7a1 reverse: 5′-CTT CTG TGT CCA AAT GCC TTC GCA GAA-3′; LPK forward: 5′-CGT GCG AGT GTG GCT CAA CTG ACC C-3′; LPK reverse: 5′-GTC GTC AAT GTA GAT GCG GCC CCC C-3′; apo C-III forward: 5′-GAA CAA GCC TCC AAG ACG GT-3′; apo C-III reverse: 5′-GGG ATT TGA AGC GAT TGT CC-3′; 36B4 forward: 5′-CAT GCT CAA CAT CTC CCC CTT CTC C-3′; and 36B4 reverse: 5′-GGG AAG GTG TAA TCC GTC TCC ACA G-3′). PCR amplifications were performed in a volume of 25 μl containing 100 nmol/l of each primer, 4 mmol/l $MgCl_2$, the Brilliant Quantitative PCR Core Reagent Kit mix (Stratagene), and SYBR Green 0.33X (Sigma-Aldrich, Saint-Quentin, France). The conditions were 95°C for 10 min, followed by 40 cycles of 30 s at 95°C, 30 s at 55°C, and 30 s at 72°C. mRNA levels were normalized to 36B4 mRNA.

Electrophoretic mobility shift assays. Electrophoretic mobility shift assays (EMSAs) were performed as described (16) using the radiolabeled FXRE consensus probe (IR-1: 5′-GATCTCAAGAGGTCATTGACCTTTTTG-3′). For experiments using nuclear extracts, proteins were purified as described (37) and dialyzed for 1 h at 4°C to remove salt excess. Nuclear extracts (3 μg) were incubated for 30 min at 4°C in EMSA buffer (16) and for a further 30 min at 4°C after addition of the probe, before electrophoresis at 4°C. For supershift experiments, 0.2 μg anti-FXR antibody (C-20; Santa Cruz) was preincubated for 20 min in the binding buffer before addition of the probe.

Statistical analysis. Statistical significance was analyzed by using the Student's t test or ANOVA followed by a Mann-Whitney U test. All values are reported as means ± SD. Values of $P < 0.05$ were considered significant.

RESULTS

Hepatic FXR gene expression is reduced in diabetic rats. To determine whether FXR gene expression is regulated in vivo under conditions of insulinopenic hyperglycemia, liver FXR mRNA levels were measured in rats in which diabetes was induced by STZ injection (Fig. 1*A*). Blood glucose values were higher in STZ-induced diabetic (25.5 ± 3.6 mmol/l) compared with control-treated (5.2 ± 0.5 mmol/l) or insulin-treated (5.3 ± 2.7 mmol/l) animals (Fig. 1*B*). A significant reduction of liver FXR mRNA expression was observed in STZ-induced diabetic rats compared with control animals (Fig. 1*A*). Interestingly, administration of insulin to the diabetic rats restored FXR mRNA to similar levels as control animals (Fig. 1*A*).

To evaluate whether liver FXR expression is also altered in a model of type 2 diabetes, the ZDF rat model that develops type 2 diabetes progressively with age was studied (Fig. 1*C*). Analysis of age-matched ZDF and ZLC control rats showed progressively increasing blood glucose levels in ZDF rats (7.9 ± 0.8 mmol/l at 6 weeks; 28.1 ± 2.9 mmol/l at 20 weeks). By contrast, plasma insulin levels were very high at 6 weeks of age, indicative of existing insulin resistance, and decreased progressively thereafter, as diabetes developed (222 ± 44 μIU/ml; 53 ± 8 μIU/ml) (Fig. 1*D*). Liver FXR mRNA levels in ZDF rats were significantly increased compared with age-matched ZLC rats at 6 weeks. Interestingly, liver FXR mRNA levels decreased progressively with advancing age in ZDF rats (Fig. 1*C*). In ZDF rats at 20 weeks, liver FXR mRNA levels were similar to liver FXR mRNA levels in ZLC rats at 6 weeks. In contrast to ZDF rats, lean ZLC rats displayed an

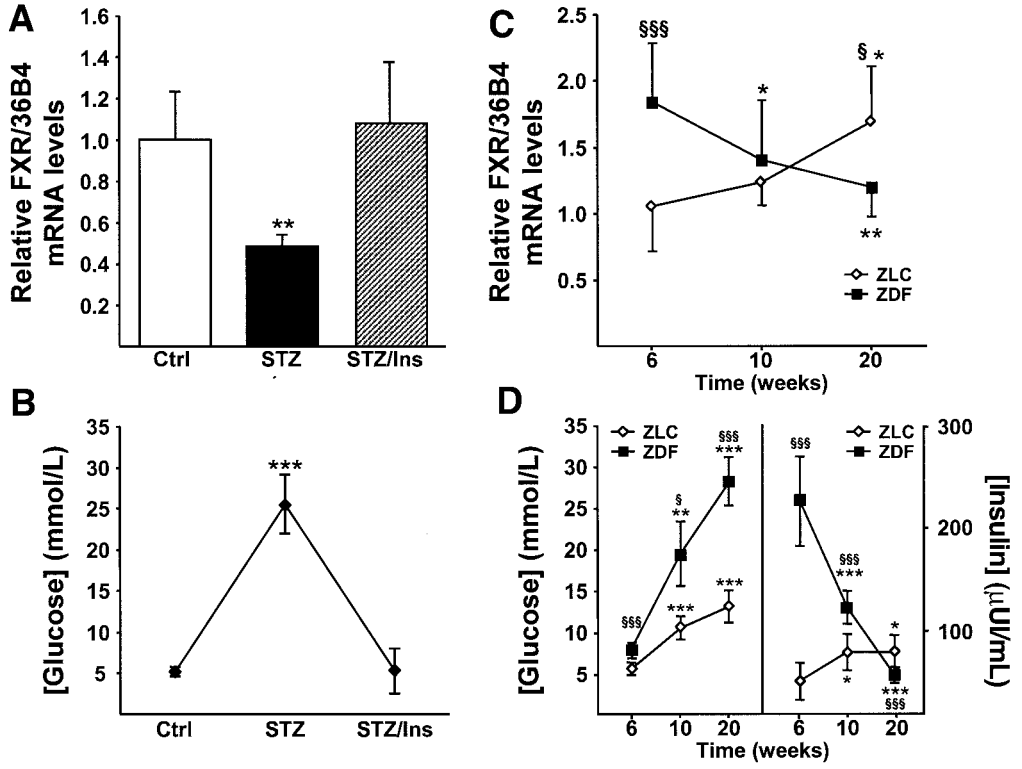

FIG. 1. FXR expression is decreased in diabetic rat models. FXR mRNA levels were measured by quantitative PCR in livers of vehicle-treated (Ctrl; $n = 5$) or STZ-induced diabetic rats treated without insulin (STZ; $n = 7$) or with insulin (STZ/Ins; $n = 7$) as described in RESEARCH DESIGN AND METHODS (*A*) and livers of ZDF and ZLC rats ($n = 8$/group) (*C*). FXR mRNA levels are normalized relative to 36B4 mRNA. *B*: Plasma glucose levels from vehicle-treated STZ-induced diabetic or insulin-treated STZ-induced diabetic rats. *D*: Plasma glucose and insulin levels of ZDF (■) and ZLC (◇) rats at different ages. Data are expressed as means ± SD. Statistically significant differences from Ctrl and from ZDF/ZLC at 6 weeks (*$P < 0.05$; **$P < 0.005$; ***$P < 0.001$) or between ZDF and ZLC at each age (§$P < 0.05$; §§§$P < 0.001$) are indicated.

increase of liver FXR mRNA levels at 20 weeks (Fig. 1*C*), which paralleled the moderate increase of blood glucose concentrations with advancing age (13.0 ± 1.8 mmol/l) (Fig. 1*D*).

To evaluate whether decreased expression of liver FXR mRNA in diabetes models is associated with altered FXR target gene expression, Cyp7a1 expression was analyzed. Liver Cyp7a1 expression tended to increase in STZ-induced diabetic rats and normalized after insulin administration (Fig. 2*A*). Hepatic expression of Cyp7a1 in ZDF rats increased progressively with age (Fig. 2*B*). These results indicate that the regulation of hepatic FXR expression is influenced by states of altered glucose homeostasis.

D-Glucose induces FXR expression in a dose- and time-dependent manner in rat hepatocytes. To start determining the factors mediating the regulation of FXR expression under conditions of altered glucose and insulin plasma levels, the influence of glucose and insulin on FXR mRNA was analyzed in primary cultures of rat hepatocytes from nondiabetic Wistar rats, a model commonly used to study insulin and glucose regulation of gene expression (9,38). Incubation of primary rat hepatocytes in high

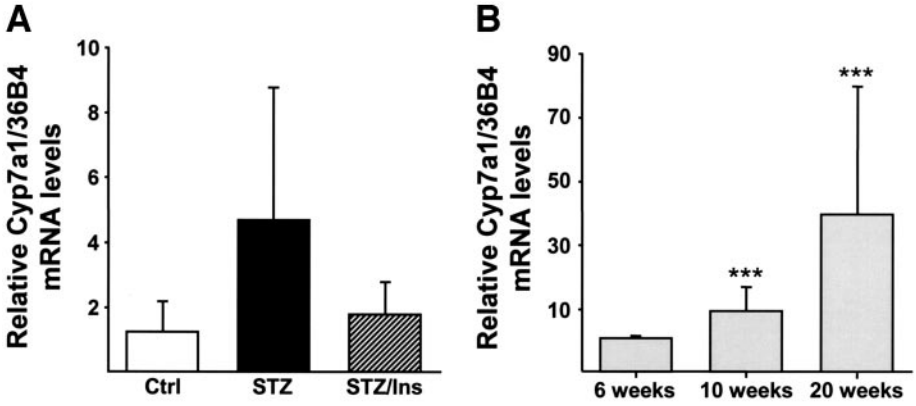

FIG. 2. Liver Cyp7a1 expression levels in diabetic rats. Cyp7a1 mRNA was measured by quantitative PCR in liver samples (described in Fig. 1) and normalized relative to 36B4 mRNA. Data are expressed as means ± SD. Statistically significant differences from ZDF rats at 6 weeks are indicated (*$P < 0.001$).**

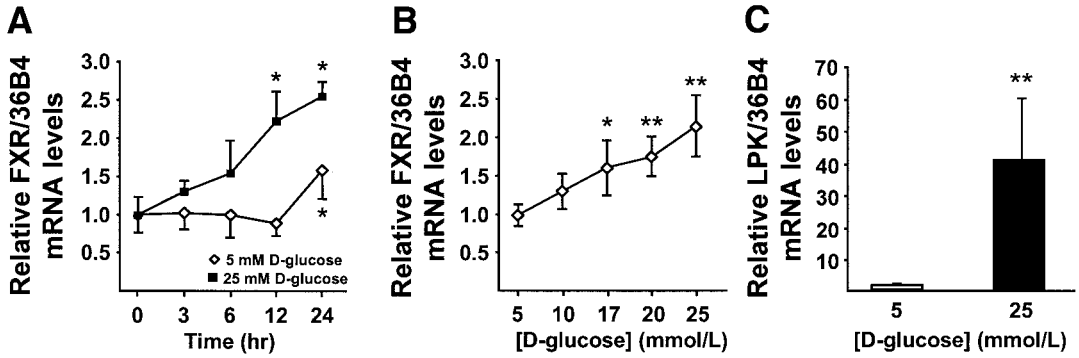

FIG. 3. Glucose induces FXR expression in a dose- and time-dependent manner. FXR mRNA levels in primary rat hepatocytes were measured as described in RESEARCH DESIGN AND METHODS. *A*: Hepatocytes were cultured for different times in 5 mmol/l (◇) or 25 mmol/l (■) D-glucose and 1 nmol/l insulin. Statistically significant differences from 5 mmol/l D-glucose at each time point are indicated (P* < 0.05). *B*: Hepatocytes were cultured for 12 h in the presence of increasing D-glucose concentrations and 1 nmol/l insulin. *C*: LPK expression in primary rat hepatocytes cultured with high (25 mmol/l) or low (5 mmol/l) D-glucose concentrations. Data are expressed as means ± SD (*n* = 6). Statistically significant differences from a 5 mmol/l D-glucose control are indicated (**P* < 0.05; ***P* < 0.005). Results are representative of three different experiments.**

concentrations of D-glucose (25 mmol/l) in the presence of 1 nmol/l insulin resulted in a time-dependent increase of FXR mRNA levels, which reached a maximum of more than twofold after 12 h (Fig. 3*A*). Moreover, a dose-dependent increase of FXR mRNA levels was observed in rat hepatocytes incubated for 12 h with D-glucose (Fig. 3*B*). As a control, LPK expression was strongly induced in hepatocytes cultured in a high D-glucose concentration (Fig. 3*C*), thus confirming previous observations (9,38).

Insulin negatively regulates FXR gene expression in rat hepatocytes. Because the insulin-signaling pathway plays a key role in glucose metabolism, the effect of insulin on the regulation of FXR expression was assessed in primary rat hepatocytes cultured in a high and low concentration of D-glucose. FXR mRNA levels were reduced by increasing insulin concentrations (Fig. 4). These results indicate that high concentrations of insulin negatively regulate FXR expression. However, insulin does not prevent the upregulation of FXR expression by D-glucose.

Glucose induction of FXR requires ongoing transcription. To examine whether increased FXR mRNA levels in response to D-glucose are a result of changes in transcription or mRNA stability, experiments were performed in primary rat hepatocytes using the RNA polymerase inhibitor actinomycin D (Fig. 5). Because basal FXR expression is low, cells were preincubated for 12 h in a high concentration of D-glucose to increase FXR mRNA. Subsequently, the medium was changed, and cells were incubated with actinomycin D in the presence of either 5 or 25 mmol/l D-glucose, and FXR mRNA levels were measured at different times thereafter. Comparison of mRNA decay curves (Fig. 5*A*) showed that the half-life of FXR mRNA was identical in high and low D-glucose concentrations. Thus, FXR mRNA stability did not appear to be altered by glucose.

To determine if the effect of glucose requires ongoing transcription, primary rat hepatocytes were pretreated for 90 min with actinomycin D before incubation in high and low concentrations of D-glucose. As expected (Fig. 3), an increase of FXR mRNA levels was observed when hepatocytes were incubated for 12 h in high D-glucose (Fig. 5*B*). However, this effect was completely abolished in the presence of actinomycin D. These results suggest that D-glucose regulates FXR expression at the transcriptional level.

D-Glucose induces nuclear FXR DNA-binding activity. To examine whether increased FXR mRNA levels result in an increased accumulation of nuclear FXR protein, primary rat hepatocytes were incubated with low and high concentrations of D-glucose for 24 h. Nuclear protein extracts were isolated and analyzed for DNA binding by EMSA using a consensus inverted repeat-1 FXRE as a probe (Fig. 6). A complex with similar migration properties to the FXR/RXR heterodimer was observed in nuclear extracts of hepatocytes, and its intensity was increased in high D-glucose concentrations (Fig. 6, *lane 4* vs. *lane 6*). Addition of an FXR antibody prevented formation of this complex (Fig. 6, *lanes 5* and *7*). Thus, enhanced FXR DNA-binding activity occurs in nuclei from cells incubated in high concentrations of D-glucose.

Glucose induction of FXR enhances the response of FXR target genes to the FXR agonist GW4064. To evaluate whether high D-glucose induction of FXR results in an increased responsiveness of FXR target genes to FXR agonists, mRNA levels of the direct FXR target genes SHP and apo C-III were analyzed in cells incubated with high and low D-glucose concentrations for 12 h and treated with

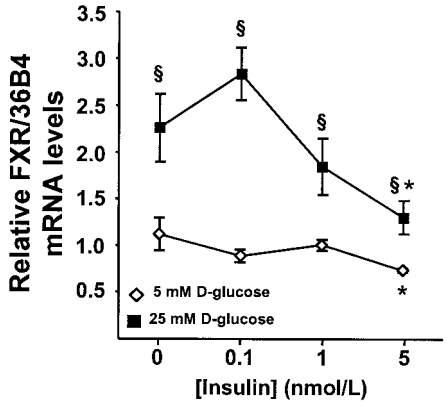

FIG. 4. Insulin negatively regulates FXR expression. FXR mRNA levels were measured in primary rat hepatocytes cultured for 12 h in 5 or 25 mmol/l D-glucose and increasing insulin concentrations. Statistically significant differences with 0 nmol/l (P* < 0.05) or between 5 and 25 mmol/l D-glucose at each insulin concentration (§*P* < 0.05) are indicated. Data are expressed as means ± SD (*n* = 6). Results are representative of three different experiments.**

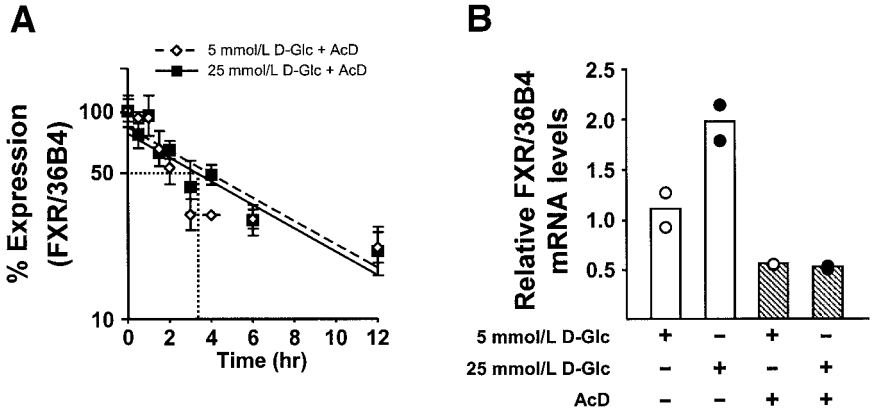

FIG. 5. Glucose induction of FXR requires transcription but does not implicate changes in FXR mRNA stability. *A*: Primary rat hepatocytes were pretreated for 12 h in medium containing high (25 mmol/l) D-glucose concentrations and subsequently shifted to a medium containing actinomycin D (AcD; 2.5 μg/ml) and either high (25 mmol/l) or low (5 mmol/l) D-glucose (D-Glc) concentrations ($t = 0$). FXR mRNA levels were measured at different times thereafter. To compare mRNA decay curves, FXR mRNA levels were expressed relative to the corresponding $t = 0$ point set at 100%. *B*: FXR mRNA levels were measured in primary rat hepatocytes pretreated for 90 min with actinomycin D (5 μg/ml; ▨) or vehicle (□) and subsequently incubated for 12 h in a medium containing 5 mmol/l (○) or 25 mmol/l (●) D-glucose and 1 nmol/l insulin as indicated. Bars represent the average of two samples. Results are representative of two independent experiments.

the FXR agonist GW4064 for a further 24 h. As expected, GW4064 strongly induced SHP expression. Interestingly, this effect was significantly enhanced in cells incubated in the high concentration of D-glucose (Fig. 7*A*). In addition, mRNA of apo C-III, a negatively regulated FXR target gene, was slightly decreased by GW4064 at low D-glucose levels, an effect that was significantly enhanced by high D-glucose levels (Fig. 7*B*).

D-Glucose regulates FXR mRNA expression via the pentose phosphate pathway in primary rat hepatocytes. To determine the metabolic pathway by which D-glucose regulates FXR expression, rat hepatocytes were incubated with different glucose metabolites. To examine whether the metabolites of the glycolytic pathway stimulate FXR expression, lactate (10 or 20 mmol/l), glycerol (2 or 10 mmol/l), and pyruvate (1 or 5 mmol/l) were tested.

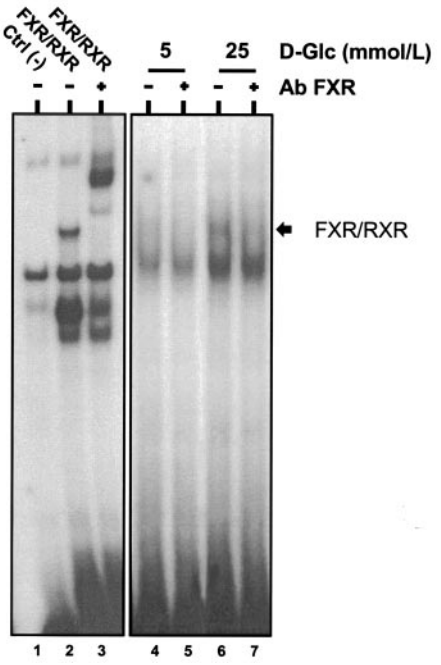

FIG. 6. D-Glucose (D-Glc) increases FXR DNA-binding activity in nuclear extracts from primary rat hepatocytes. Primary rat hepatocytes were exposed for 24 h to 5 or 25 mmol/l D-glucose. Nuclear proteins were isolated as described in RESEARCH DESIGN AND METHODS, and gel shift assays were performed with labeled double-stranded consensus FXRE oligonucleotides in the presence or absence of FXR antibody (Ab FXR). In vitro translated FXR and RXRα (*lanes 2* and *3*) or unprogrammed reticulocyte lysates [Ctrl(-), *lane 1*] were analyzed in parallel.

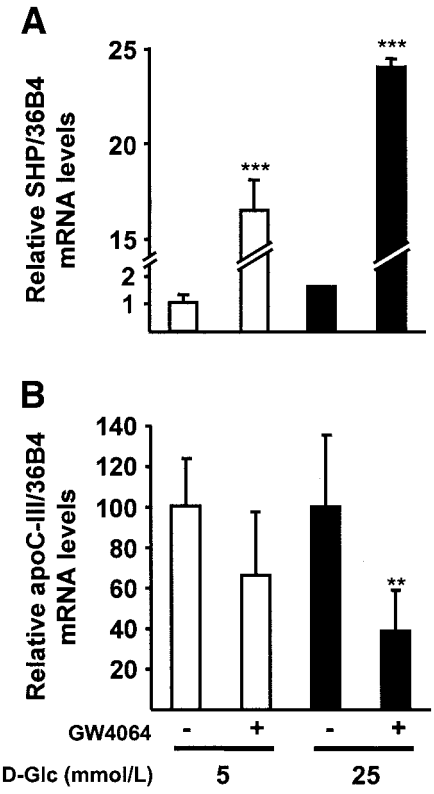

FIG. 7. Glucose enhances the effect of ligand-activated FXR on the expression of FXR target genes. SHP and apo C-III mRNA levels were measured in primary rat hepatocytes treated with the indicated concentrations of D-glucose (D-Glc) in the presence of GW4064 (5 μmol/l) or vehicle (DMSO). Values represent means ± SD ($n = 6$). Statistically significant differences between GW4064- or vehicle-treated hepatocytes at each D-glucose concentration are indicated (Student's *t* test: $^{**}P < 0.005$; $^{***}P < 0.001$).

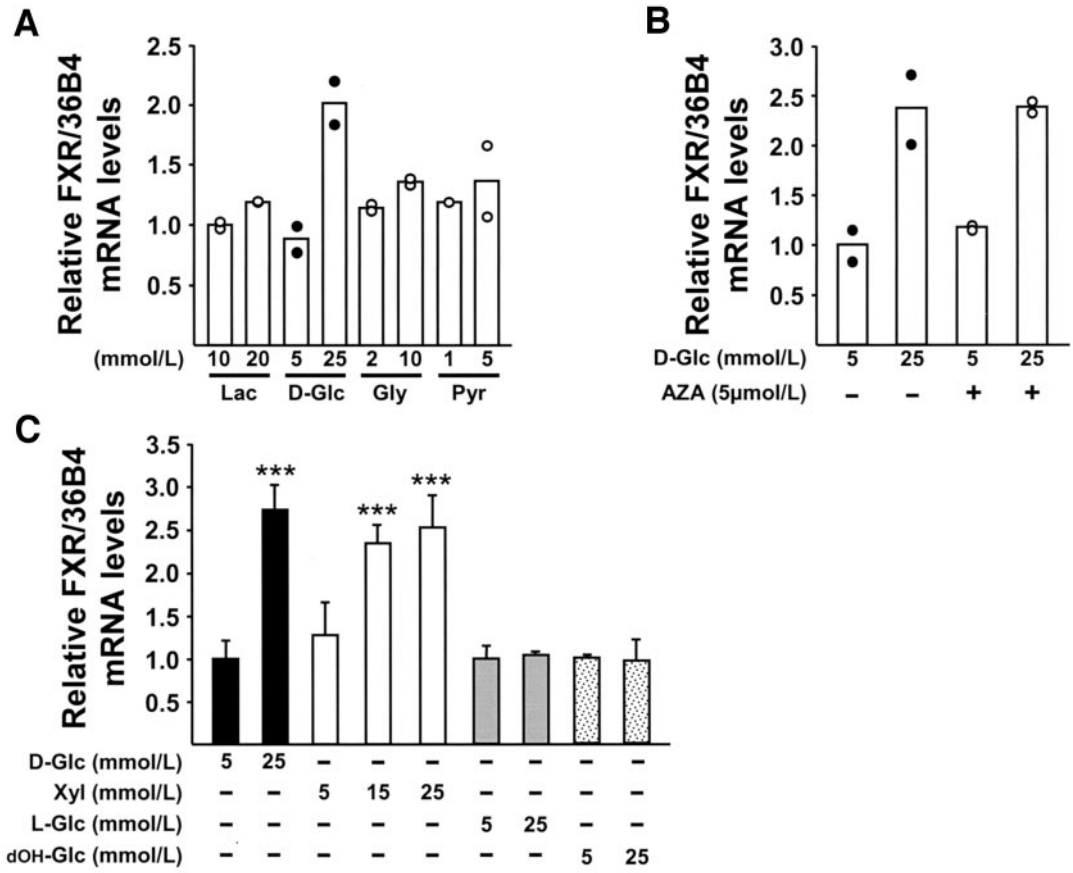

FIG. 8. Effect of glucose metabolites on FXR expression. FXR mRNA levels were measured in primary rat hepatocytes cultured for 12 h in medium containing insulin (1 nmol/l) and different chemicals as indicated. *A*: Lac, 10–20 mmol/l lactate; D-Glc, 5–25 mmol/l D-glucose; Gly, 2–10 mmol/l glycerol; Pyr, 1–5 mmol/l pyruvate. *B*: D-Glc, 5–25 mmol/l D-glucose; AZA, 5 μmol/l azaserine. Bars represent the average of two samples. *C*: D-Glc, 5–25 mmol/l D-glucose; Xyl, 5–25 mmol/l xylitol; L-Glc, 5–25 mmol/l L-glucose; dOH-Glc, 5–25 mmol/l deoxyglucose. Statistically significant differences from a 5-mmol/l glucose control are indicated (*$P < 0.001$). Data are expressed as means ± SD ($n = 6$).**

None of these molecules influenced FXR mRNA levels (Fig. 8*A*), thus indicating that the D-glucose–dependent induction of FXR is not mediated by glycolysis metabolites.

To evaluate whether the hexosamine pathway could mediate the induction of FXR expression by D-glucose, rat hepatocytes were cultured with azaserine (5 μmol/l), an inhibitor of glutamine/fructose-6-phosphate amidotransferase, the rate-limiting enzyme in the conversion of glucose to glucosamine. The induction of FXR expression in 25 mmol/l D-glucose was not inhibited by azaserine, suggesting that the hexosamine pathway is not implicated either (Fig. 8*B*).

Finally, it was analyzed whether the pentose pathway is implicated in the glucose-dependent induction of FXR. To this end, hepatocytes were incubated with xylitol, a precursor of xylulose-5-phosphate and an intermediary molecule of the pentose phosphate pathway. Increasing concentrations of xylitol induced FXR expression in a dose-dependent manner (Fig. 8*C*) and at a similar extent as D-glucose, thus suggesting that the pentose pathway could mediate the D-glucose–dependent induction of FXR in rat hepatocytes. In addition, high concentrations of L-glucose and deoxyglucose, nonmetabolizable sugars, did not influence the expression of FXR, indicating that intracellular processing of glucose is needed to promote FXR induction by D-glucose.

DISCUSSION

Data from clinical and experimental studies show an altered synthesis and transport of bile acids in diabetes (4,5). Moreover, hypertriglyceridemia is one of the most frequent lipid abnormalities that is also positively associated with gall bladder disease (1). FXR is a key factor controlling the synthesis and transport of bile acids as well as triglyceride metabolism. We therefore decided to investigate whether FXR gene expression is regulated by conditions of altered glucose homeostasis. In the present study, we demonstrate that hepatic FXR expression is decreased in two different rat models of diabetes. Moreover, using primary rat hepatocytes as a model, we show that FXR is a glucose- and insulin-regulated gene. Whereas insulin represses FXR gene expression, D-glucose increases FXR mRNA and nuclear protein levels in a dose- and time-dependent manner. Moreover, this regulation is mimicked by glucose metabolites of the pentose phosphate pathway.

Different studies demonstrated that STZ, a molecule used to destroy the pancreatic β-cell and to induce type 1 diabetes, increases the bile acid pool independently of any hepatotoxicity (35). Furthermore, physiological levels of insulin downregulate Cyp7a1 gene transcription, with a consequent suppression of bile acid synthesis (39). These data suggest that the increased bile acid synthesis ob-

served in STZ-induced diabetes is a consequence of a lack of negative insulin-mediated regulation of genes encoding bile acid synthesis enzymes. However, Cyp7a1 is also negatively regulated by FXR (22,23). In the present study, we show that FXR expression is reduced in STZ-induced diabetes, which seems to be paralleled by an increased expression of liver Cyp7a1 mRNA. Thus, the increased bile acid synthesis observed in STZ-induced diabetes could also be due, at least in part, to an increased activity of Cyp7a1 due to the decreased expression of its negative regulator FXR.

The ZDF rat is a model of insulin resistance and obesity. At a young age, ZDF rats are hyperinsulinemic but normoglycemic, and, with advancing age, they develop overt diabetes characterized by hypoinsulinemia and hyperglycemia (40). Thus, ZDF rats recapitulate the phases of type 2 diabetes development similar to what is observed in human type 2 diabetes. Our results show that hepatic FXR expression decreases in ZDF rats in parallel with diabetes progression. By contrast, a progressive increase of liver Cyp7a1 expression was observed. These data suggest a role for FXR in the dysregulation of Cyp7a1 expression and consequently of bile acid metabolism during diabetes development. Livers of ZDF rats display reduced glucose uptake and elevated glucose production as a consequence of hepatic insulin resistance (41), factors that could contribute to the dysregulation of FXR expression. Interestingly, FXR expression increased with advancing age in lean ZLC rats, an effect that was associated with an increase in blood glucose levels. Although the mechanisms behind the changes in FXR expression in these in vivo models are undoubtedly complex, our observations indicate that FXR expression is (dys)regulated in animal models of altered glucose homeostasis.

The mechanisms controlling FXR gene expression are poorly understood. A recent study demonstrated that a total removal of the bile acid pool/flux decreased liver FXR mRNA and nuclear protein levels, suggesting that a minimum level of bile acids in the enterohepatic circulation is required for maintaining FXR expression (42). Furthermore, hepatocyte nuclear factor-1α as well as the cytokines tumor necrosis factor-α and interleukin-1β have been identified to regulate FXR expression (43,44). Using primary rat hepatocytes as a model, we demonstrated that FXR gene expression is also controlled by changes in glucose and insulin concentrations.

Several lines of evidence indicate that induction of FXR by glucose translates in a more functional protein activity. First, gel shift data showed that high concentrations of glucose enhanced FXR DNA-binding activity to a consensus FXRE. Second, the regulation of the nuclear receptor SHP and apo C-III, two FXR target genes (22,23,33), was more pronounced when primary rat hepatocytes were cultured with a high concentration of glucose. These results suggest that increasing glucose concentration induces the expression of functionally active FXR.

Glucose is an important modulator of gene expression by controlling gene transcription through glucose/carbohydrate response elements present in the promoter region of its target genes (45). Several nuclear factors, including upstream stimulatory factors (6), sterol response element-binding protein-1c (7), chicken ovoalbumin upstream promoter-transcription factor 2 (8), and carbohydrate response element-binding protein (9), have been proposed to mediate glucose-regulated gene expression. The regulation mechanisms are not totally known, but may implicate a high activity of the hexosamine pathway (12) or of glucose metabolites such as glucose-6-phosphate or xylulose-5-phosphate (10,11). Our results show that xylitol induces FXR expression in a dose-dependent manner to a similar extent as glucose, suggesting that the pentose phosphate pathway could mediate FXR induction by glucose. In fact, xylitol is metabolized to xylulose-5-phosphate (46). Different studies demonstrated that xylulose-5-phosphate levels increase strongly in response to increased extracellular glucose concentrations (47,48). Xylulose-5-phosphate is an intermediate in the nonoxidative branch of the pentose phosphate shunt, which has been proposed to function as a sensor for hepatic glucose fluxes, and it is able to regulate gene transcription in the liver (10). Our data suggest that glucose induces the expression of FXR through the nonoxidative branch of the pentose phosphate pathway via an increase in the concentration of xylulose-5-phosphate.

At first sight, it may appear that the data from the chronic and highly complex in vivo models of diabetes are not completely consistent with the more acute effects observed in cultured hepatocytes. However, differences in hepatocellular glucose metabolism exist between both models. For instance, high physiological concentrations of glucose cause only slight increases in glycogen synthesis in isolated hepatocytes, whereas oral glucose administration readily induces hepatic glycogen synthesis in vivo in rats (49). Thus, it can be envisioned that differences in intracellular glucose metabolism, e.g., the partitioning of glucose-6-phosphate into the pentose phosphate pathway, partly underlie the divergent results. In addition, several indirect effects, due to alterations in metabolic and hormonal status or levels of inflammatory cytokines in these pathophysiological models of diabetes, may contribute to the modulation of FXR expression in vivo.

In summary, our results demonstrate that hepatic FXR expression is decreased in diabetic animal models. Studies using normal insulin-sensitive hepatocytes identify FXR as a positive glucose-regulated gene through a mechanism that implicates the pentose phosphate pathway. Thus, dysregulation of FXR expression in diabetic and insulin-resistant patients may, at least partially, contribute to the observed increased incidence of gall stone disease and hypertriglyceridemia in these populations.

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