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The Transcription Factor COUP-TFII Is Negatively Regulated by Insulin and Glucose via Foxo1- and ChREBP-Controlled Pathways▽

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COUP-TFII has an important role in regulating metabolism in vivo. We showed this previously by deleting COUP-TFII from pancreatic beta cells in heterozygous mutant mice, which led to abnormal insulin secretion. Here, we report that COUP-TFII expression is reduced in the pancreas and liver of mice refed with a carbohydrate-rich diet and in the pancreas and liver of hyperinsulinemic and hyperglycemic mice. In pancreatic beta cells, COUP-TFII gene expression is repressed by secreted insulin in response to glucose through Foxo1 signaling. Ex vivo COUP-TFII reduces insulin production and secretion. Our results suggest that beta cell insulin secretion is under the control of an autocrine positive feedback loop by alleviating COUP-TFII repression. In hepatocytes, both insulin, through Foxo1, and high glucose concentrations repress COUP-TFII expression. We demonstrate that this negative glucose effect involves ChREBP expression. We propose that COUP-TFII acts in a coordinate fashion to control insulin secretion and glucose metabolism.

Homeostatic mechanisms maintain blood glucose levels within a narrow range, protecting the body against hypoglycemia during periods of fasting and against excess glucose following the ingestion of a high-carbohydrate (HCHO) meal. Many genes are tightly controlled at the transcriptional level by key hormones, particularly insulin, and by glucose, which act through distinct, but synergistic, signaling pathways in the liver and pancreas. Elevated levels of glucose and insulin resistance in type 2 diabetes are the consequence of progressive defects in liver function and the insulin secretory capacity and/or beta cell mass. Only a few transcription factors are known to be controlled by insulin or glucose and to have an essential role in the pathogenesis of this disease. Among them, Foxo1, a forkhead family member, has been shown to regulate pancreatic beta cell mass and function and to control multiple metabolic pathways in liver. It can function as a transcription factor or as a coregulator, depending on the gene being regulated (9, 16). Both insulin and oxidative stress (chronic exposure to high glucose concentrations) regulate its activity through dynamic relocalization in the cell after posttranslational modification (9, 16). The carbohydrate response element binding protein ChREBP is a glucose-sensitive transcription factor regulated at the transcriptional and posttranslational levels that promotes the hepatic conversion of excess carbohydrate to fatty acids (6, 8, 14, 15). The identification of the transcription factors that mediate glucose and insulin effects on metabolic pathways is of great interest, as any modulation in these pathways will have consequences on glucose homeostasis.

Chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII) (also called NR2F2) is an orphan member of the steroid/thyroid hormone receptor superfamily that binds DNA by a Zn finger DNA binding domain on a variety of hormone response elements that contain direct or inverted imperfect AGGTCA repeats with various spacings (3). Our interest in the transcription factor COUP-TFII started when we cloned this nuclear receptor in a yeast one-hybrid screen using the glucose-responsive element (20), now called the carbohydrate response element (ChoRE). COUP-TFII acts as an inhibitor of the glucose activation of the liver pyruvate kinase (L-PK) gene by binding the ChoRE and may maintain basal L-PK gene expression in the absence of glucose (20). The presence of functional COUP-TFII response elements in promoters of numerous genes encoding metabolic enzymes suggests that COUP-TFII may have a role in glucose homeostasis (23). Using immunohistochemistry, we found that the COUP-TFII protein is present in hepatocyte nuclei and in islets (40). More recently, in mouse pancreatic islets, we observed that COUP-TFII protein expression is restricted to beta cells, and it is expressed in the beta cell line 832/13 INS-1 (28). The importance of COUP-TFII in glucose homeostasis in vivo was shown in heterozygous mutant mice with COUP-TFII deleted from pancreatic beta cells, as they have impaired glucose sensitivity and abnormal insulin secretion (2). These mice presented hyperinsulinemia in fasted and fed states and impaired glucose tolerance. Here, we set out to elucidate the molecular function of COUP-TFII in pancreas and liver and to understand how COUP-TFII expression is controlled by insulin and glucose.

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MATERIALS AND METHODS

Animals. Six-week-old male C57BL/6J mice were purchased from Elevage Janvier and allowed to adapt for 2 weeks prior to study. All mice were housed in colony cages with a 12-h light/12-h dark cycle in a temperature-controlled environment.

(i) Nutritional studies. One group of mice was fasted for 24 h and another group was fasted for 24 h and then fed with an HCHO low-fat diet (72.2% carbohydrate, 1% fat, and 26.8% protein; Safe, Chaumesnil, France) for 18 h.

(ii) Clamped mice. Mice were catheterized at least 2 days before the experiment after being anesthetized with a mixture of ketamine (100 mg/kg of body weight) and xylazine (10 mg/kg of body weight). The right jugular vein was catheterized for infusion with a silastic catheter. The free end of the catheter was tunneled under the skin to the back of the neck and passed through a piece of Tygon tubing, glued together, and secured to the skin. Lines were flushed daily with 50 μl of 0.9% NaCl containing 5 mg/ml ampicillin and 20 IU/ml heparin. Animals were housed individually after surgery and weighed daily. After a 5-h fast on the day of experiment, awake animals were placed unrestrained into their home cage during the perfusion. Three groups were studied during a 3-h infusion: saline-infused (control), glucose-infused (hyperglycemic-hyperinsulinemic [HGHI]), and insulin-infused (euglycemic-hyperinsulinemic [eGHI]) mice.

(iii) HGHI mice. Glucose (30%, wt/vol; Chaix & DuMaris, France) was infused at 15 μl/min for 3 h to produce hyperglycemia (400 to 500 mg/dl). Hyperinsulinemia is a consequence of hyperglycemia due to glucose infusion.

(iv) eGHI mice. Hyperinsulinemia was induced by a 3-h insulin infusion at a constant rate of 0.6 IU/kg/h (Actrapid Novolet; Novo-Nordisk). Euglycemia was maintained by simultaneous glucose infusion at a flow rate adjusted to the basal glycemia, around 100 mg/dl. For the three groups, blood glucose levels were determined from tail blood samples at time zero and then every 15 min (Accu-chek glucose analyzer; Roche). Steady state was attained when glucose measurements were constant for at least 20 min at a fixed glucose infusion rate and was achieved within 30 to 45 min. At the beginning (0 min) and end (180 min) of the experiment, blood samples were collected, and the plasma was separated by centrifugation and stored at −20°C. Plasma insulin concentrations were measured with a radioimmunoassay kit (Insik-5 kit; Diasorin). At the end of the infusion, mice were killed by pentobarbital injection, and the pancreas and liver were frozen in liquid nitrogen and kept at −80°C until analysis.

Other mouse models. To determine the role of glucokinase (GK) and ChREBP in the regulation of COUP-TFII, cDNA and total protein extracts were prepared from control, liver-specific hepatic GK (hGK) knockout (hGK-KO) (8), 7-day adenovirus (Ad)-green fluorescent protein (GFP)-treated *ob*/+ and *ob*/*ob*, and Ad-ChREBP short hairpin RNA (shChREBP)-treated *ob*/*ob* mice (6). All animal studies were approved by the Direction Départementale des Services Veterinaires de Paris (Paris, France).

Pancreatic islet isolation and culture. Mouse pancreatic islets were isolated using a collagenase digestion method (2). Briefly, mice were anesthetized with 3.5×10^5 Pa isoflurane–0.5×10^5 Pa oxygen (Minerve), and type V collagenase P (Roche) was injected into the common bile duct. Infused and distended pancreases were then removed and left to digest for 4 min at 37°C with gentle mixing. Islets were washed and handpicked in HEPES balanced salt solution (HBSS) (124 mM NaCl, 5 mM KCl, 0.8 mM $MgSO_4 \cdot 7H_2O$, 1 mM NaH_2PO_4, 10 mM HEPES, 1.8 mM $CaCl_2$, 14 mM $NaHCO_3$, and 0.5% defatted bovine serum albumin [BSA] [Sigma] [pH 7.4]) containing 3 mM glucose under an inverted light microscope and then separated into study groups. Islets were cultured overnight in RPMI 1640 medium (Invitrogen) containing 11 mM glucose supplemented with 10% heat-inactivated fetal bovine serum (Invitrogen), 1 mM sodium pyruvate (Invitrogen), and 100 U/ml penicillin-streptomycin (Invitrogen). The next day, islets were incubated in RPMI 1640 medium containing 3 mM glucose for about 8 h and then either kept in 3 mM glucose or stimulated overnight with the appropriate fresh medium. Total RNA was extracted from hand-picked islets using the Absolutely RNA Microprep kit according to the instructions provided by the manufacturer (Stratagene). Each RNA sample was prepared from 200 to 400 islets from about three mice. RNA (0.5 μg) was reverse transcribed for real-time quantitative PCR (RTQ-PCR) (described below).

Primary culture of hepatocytes. Hepatocytes were isolated and cultured as described previously (8) for RNA or protein extraction.

Cell culture. The rat insulinoma 832/13 INS-1 cell line (generously provided by C. Newgard) was used between passages 19 and 29 (13). Cells were cultured at 5% CO_2–95% air at 37°C in RPMI 1640 medium containing 11 mM D-glucose supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum, 100 U/ml penicillin-streptomycin, 10 mM HEPES, 1 mM sodium pyruvate (Invitrogen), and 50 μM beta-mercaptoethanol (Invitrogen) (INS-1 medium).

For insulin and glucose stimulation, cells were seeded in 12-well plates at a density of 0.9×10^6 cells/well in INS-1 medium. The next day, cells were incubated in medium containing 5 mM glucose for 24 h and then incubated in fresh stimulating medium, which contained either 20 mM glucose (Merck), 100 nM insulin (Actrapid; Novo-Nordisk), 15 mM KCl (Merck), 100 μM HNMPA-$(AM)_3$ (hydroxy-2-naphthalenylmethyl phosphonic acid tris acetoxy methyl ester) (Biomol), 20 μM verapamil (Sigma), or 2.5 μM actinomycin D (Sigma) prior to RNA isolation or protein extraction.

Gene silencing in insulinoma cells and hepatocytes with siRNA. A 21-nucleotide RNA against COUP-TFII was designed and synthesized by Qiagen SA (2-For-Silencing small interfering RNA [siRNA]). The siRNA sequence targeting mouse and rat COUP-TFII (GenBank accession numbers 009697 and 080778, respectively), but not human COUP-TFII (GenBank accession number 021005) or mouse COUP-TFI (GenBank accession number 010151), was from position 1023 relative to the mouse gene start codon. The COUP-TFII siRNA sequence is r(AGUGUGCUUUGGAAGAGUA)dTdT (sense) and r(UACUC UUCCAAAGCACACU)dGdG (antisense). Nonspecific control siRNA from Qiagen SA was used. 832/13 INS-1 cells were grown to 75 to 80% confluence in 10-cm dishes. The cells were then trypsinized and transiently transfected by electroporation using an Amaxa Nucleofector II device (solution T, program T20; Amaxa Biosystems) and 68 pmol of siRNA duplex per 1.2×10^6 cells. Nucleofection was performed on cells in INS-1 medium in 12-well plates for subsequent RNA or protein extraction. Culture medium was changed 24 h after electroporation. Each reaction was performed 48 h postelectroporation. Rat Foxo1 siRNA was obtained from Invitrogen (using Foxo1-MSS226200 and control 12935200) (29). In these experiments, nucleofection was performed as described above except that cells were cultured in medium containing 5 mM glucose, and RNA extraction was performed 24 h postelectroporation.

Primary cultures of C57BL/6J mouse hepatocytes were transiently transfected with either ChREBP or control siRNAs using adenovirus/polyethyleneimine as described previously (8). The same method was used for mouse Foxo1 siRNA silencing (using FKHR siRNA, catalog number sc-35382, and control siRNA, catalog number sc-37007; Santa Cruz).

TG content in 832/13 INS-1 cells. 832/13 INS-1 cells plated in 12-well plates were nucleofected with COUP-TFII siRNA or nonspecific control siRNA and cultured for 48 h in INS-1 medium. Cellular lipids were then extracted in chloroform-methanol (2:1, vol/vol) with vigorous shaking for 10 min. After centrifugation for 25 min at $1{,}000 \times g$, the lower organic phase was collected, dried, and solubilized in chloroform-methanol. Lipid classes were separated by thin-layer chromatography on silica gel plates by using petroleum ether-diethyl ether-acetic acid (85:15:0.5, vol/vol/vol) as the mobile phase. Lipids were visualized with I_2 vapor. Bands were scraped from the plate, and triglycerides (TGs) were extracted from silica in acetone. After centrifugation for 10 min at $1{,}500 \times g$ to remove silica and evaporation, TGs were measured with a PAP 150 TG kit (Biomerieux, Marcy l'Etoile, France).

Measurement of fatty acid esterification flux. After 48 h of nucleofection with COUP-TFII siRNA or control siRNA, cells were preincubated at 37°C for 30 min in HBSS medium containing 2.5 mM glucose. Cells were then incubated for 2 h at 37°C in 1 ml of fresh HBSS containing 2.5 or 20 mM glucose in the presence of 250 μM [1-^{14}C]palmitate (0.01 Ci/mol; GE Healthcare) bound to 1% (wt/vol) defatted BSA and 1 mM carnitine. Cells were washed and scraped into cold phosphate-buffered saline, and total cellular lipids were extracted and separated by thin-layer chromatography to measure the incorporation of labeled palmitate into diacylglycerides, TGs, and phospholipids as previously described (31).

Glucose-stimulated insulin secretion and insulin content in insulinoma cells. 832/13 INS-1 cells were seeded into 24-well plates at a density of 1.2×10^6 cells/well in INS-1 culture medium. Forty-eight hours after plating, cells were washed and preincubated in HBSS containing 3 mM glucose for 1 h at 37°C, followed by static incubation for 1 h in the same buffer containing 3 or 20 mM glucose. The supernatant was collected for insulin secretion assays, with insulin release being expressed relative to that of cells in the 3 mM glucose buffer.

To measure insulin content, the cells were first lysed in 1 M acetic acid–0.1% BSA free fatty acid (Sigma) and sonicated. Insulin content was normalized to the DNA content/well. Insulin levels were determined by radioimmunoassay using the INSIK-5 kit (DiaSorin) according to the manufacturer's protocol. The kit detects both human and rat mature insulins.

Preparation of a recombinant virus expressing COUP-TFII and Ad infection. The full-length cDNA encoding human COUP-TFII was inserted into the KpnI and XhoI sites of the pAdTrack-CMV shuttle vector, which also contained a cytomegalovirus-GFP expression reporter gene to monitor the efficiency of adenoviral infection (Ad-hCOUP-TFII). Recombinant adenoviral (Ad-hCOUP-TFII) and control (pAdTrack with no exogenous gene) plasmids were produced by INSERM U649. Ad-Foxo1-ADA was provided by T. Kitamura (25).

832/13 INS-1 cells were seeded into 12-well plates at a density of 0.9×10^6 cells/well in INS-1 medium and were exposed overnight to Ad at 2 or 5 PFU/cell 24 h later. Virus-containing medium was removed the next day and replaced with fresh medium (containing 11 mM glucose for COUP-TFII experiments or 5 mM glucose ± 100 nM insulin for Foxo1 experiments) for 24 h before RNA and proteins were extracted. Isolated pancreatic islets were cultured for 2 h in 11 mM glucose before exposing them to Ad at 200 PFU/cell for 90 min. Islets were then cultured in fresh 11 mM glucose medium for 3 days prior to RNA or protein extraction.

C57BL/6J mouse hepatocytes were cultured for 24 h in the presence of 5 mM glucose, infected for 4 h with Ad-GFP or Ad-Foxo1-ADA as indicated (0.5 and 2 PFU/cell), and cultured for 24 h in the presence of 5 mM glucose or 25 mM glucose plus 100 nM insulin before RNA extraction.

Isolation of total mRNA and detection by RTQ-PCR. Total RNA was extracted and purified from tissues and cultured cells using the RNA-Plus reagent (Q-BIOgene) according to the instructions provided by the manufacturer. Reverse transcription was done with 2 μg of total RNA using Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's protocols. RTQ-PCR was performed with 6.25 ng of reverse-transcribed total RNA, 10 μM of each primer (Eurogentec), and 2 mM $MgCl_2$ in 1× LightCycler DNA Master Sybr green I mix using a LightCycler apparatus (Roche). All samples were normalized to the threshold cycle value for cyclophilin mRNA, which was chosen as an invariant control. Forward and reverse primers used for the specific amplification of cDNA fragments and designed to hybridize to rat and mouse transcripts were 5′-CGC TCC TTG CCG CTG CT-3′ and 5′-AAG AGC TTT CCG AAC CGT GTT-3′ for COUP-TFII, 5′-TTG CCA TTC CTG GAC CCA AA-3′ and 5′-ATG GCA CTG GTG GCA AGT CC-3′ for cyclophilin, 5′-CTT GCT CTA CCG TGA GCC TC-3′ and 5′-ACC ACA ATC ACC AGA TCA CC-3′ for L-PK, 5′-GCC CAG CTT AAT GCC ATC TTT-3′ and 5′-CAA AAG GGC TGC CTT CTG TAA-3′ for NeuroD1, 5′-GCG CTG AGA GTC CGT GAG-3′ and 5′-CCG GGG TAG GGA GCT ACA-3′ for pancreatic and duodenal homeobox factor 1 (Pdx1), 5′-CAG TAG TTC TCC AGC TGG TA-3′ and 5′-GGC TTC TTC TAC ACA CCC A for proinsulins I and II, 5′-AGC AGT GCT GGC TAC CTT CAA-3′ and 5′-AAT ATG TAG CCA CCC CCT TGG-3′ for PPARα, 5′-CTG GGG ACC TAA ACA GGA GC-3′ and 5′-GAA GCC ACC CTA TAG CTC CC-3′ for ChREBP, 5′-TCT TGT GGT TGG GAT ACT GG-3′ and 5′-GCA ATG CCT GAC AAG ACT C-3′ for G6Pase, and 5′-GTC CTG GGC CAA AAT GTA ATG-3′ and 5′-AGC CTG ACA CCC AGC TGT GTG-3′ for Foxo1.

Western blot analysis and ECL detection. 832/13 INS-1 and pancreatic islets were washed in cold phosphate-buffered saline and centrifuged. To extract total proteins, cell pellets were lysed in 100 μl of lysis buffer (50 mM Tris-HCl [pH 7.5], 150 mM NaCl, 5 mM EDTA, 30 mM $Na_4P_2O_7$, 50 mM NaF, 1% Triton, 10 mg/ml leupeptin, 10 mg/ml pepstatin, 10 mg/ml aprotinin, and 1 mM phenylmethylsulfonyl fluoride). For nuclear protein extraction, the NE-PER Nuclear and Cytoplasmic Extraction Reagents kit was used as recommended by the manufacturer (Pierce Perbio). Immunoblotting was done as described previously (2). Blots were incubated with ECL SuperSignal West Pico chemiluminescent reagents (Pierce) and visualized using the high-end CDD LAS-3000 imaging system (Fujifilm). The images were quantified by densitometry using the Multi-gauge 3.0 image processor program (Fuji Film), normalizing each band intensity to those of loading controls. The antibodies and dilutions used were as follows: antihemagglutinin (anti-HA) tag at a 1:1,000 dilution (catalog number 16B12; Covance-Babco), anti-COUP-TFII at a 1:500 dilution (NR2F2, catalog number PP-H7147-00; R&D Systems), anti-α-tubulin at a 1:3,000 dilution (catalog number T9026; Sigma), anticyclophilin at a 1:3,000 dilution (catalog number 07-313; Upstate), and anti-glyceraldehyde-3-phosphate dehydrogenase (anti-GAPDH) at a 1:200 dilution (catalog number sc-25778; Santa Cruz).

Statistical analysis. Quantitative results are expressed as means ± standard errors of the means (SEM). Statistical analyses were carried out using the Mann-Whitney test, a nonparametric statistical program appropriate when the sample number is less than 10. Null hypotheses were rejected at P values of >0.05. All experiments were performed at least three times.

RESULTS

Levels of COUP-TFII expression are decreased in mouse pancreas and liver by insulin and glucose. We analyzed COUP-TFII mRNA expression by RTQ-PCR in the pancreas and liver of C57BL/6J mice subjected to a 24-h fast and refed with an HCHO diet. This regimen caused a major switch in the use of energy-providing molecules from fatty acid to glucose, leading to elevated plasma glucose and insulin levels in the refed mice. The nutritional status of the mice was evaluated by measuring the level of expression of genes known to be modulated by glucose and insulin in liver, as previously reported (8, 10, 36, 39). As expected, L-PK and GK mRNA levels increased markedly upon HCHO refeeding compared to that in the fasted state. The inverse was true for liver carnitine palmitoyltransferase I (L-CPT I) and hepatocyte nuclear factor 4α (HNF4α) mRNA (data not shown). In the refed mice, there was less COUP-TFII mRNA in the pancreas (Fig. 1A) and less COUP-TFII mRNA and protein in the liver (Fig. 1B) than in fasted controls. These results suggest that COUP-TFII expression is regulated by changes in blood glucose and/or insulin levels.

Because insulinemia and glycemia are so tightly linked, we dissected their respective impacts on COUP-TFII mRNA expression in pancreas and in liver using clamped mouse models. COUP-TFII mRNA was downregulated in the pancreas (Fig. 1C, left) and liver (Fig. 1C, right) of HGHI animals compared to those of control mice as well as eGHI mice, animals which were infused with insulin in order to induce high hyperinsulinemia with euglycemia maintained by glucose infusion. These mouse models show that insulin decreases COUP-TFII expression levels in liver and in pancreas. We cannot exclude the possibility that glucose per se could also have an effect on COUP-TFII mRNA expression.

Glucose and exogenous insulin reduce levels of COUP-TFII mRNA and protein expression in the 832/13 INS-1 beta cell line and pancreatic islet beta cells. In order to investigate the insulin and glucose signaling pathways leading to the inhibition of COUP-TFII expression in these two tissues, we used ex vivo pancreatic and hepatic cell culture models. First, we investigated COUP-TFII repression by insulin and glucose in cultured mouse pancreatic islets and rat 832/13 INS-1 beta cells. Figure 2A shows that the expression of COUP-TFII mRNA is downregulated by elevated glucose concentrations in primary cultures of mouse islets. Glucose also reduces the levels of COUP-TFII transcripts in 832/13 INS-1 cells in a dose-dependent manner, with a maximal effect at 20 mM glucose (Fig. 2B). It is now well documented that in beta cells, glucose effects on gene and protein expression can occur indirectly via the autocrine/paracrine action of insulin (1, 4, 18, 22, 27, 38). To evaluate whether exogenous insulin affects COUP-TFII mRNA expression, we added insulin to the cells after an overnight starvation. Similarly to the response to glucose, the addition of insulin (10 nM and 100 nM) resulted in a dose-dependent downregulation of COUP-TFII mRNA levels (Fig. 2C). In order to determine if this decrease in COUP-TFII mRNA expression levels could be linked to changes in its stability, we added actinomycin D, an inhibitor of RNA synthesis, to samples treated with 5 mM glucose with or without 100 nM insulin. We observed that insulin does not lead to a further reduction in COUP-TFII mRNA levels (Fig. 2D) when actinomycin is present. Therefore, insulin does not affect COUP-TFII mRNA stability.

The glucose- and insulin-regulated changes in COUP-TFII mRNA levels were reflected at the protein level, as elevated glucose and/or exogenous insulin concentrations reduce COUP-TFII protein levels by 40 to 50% (Fig. 2E).

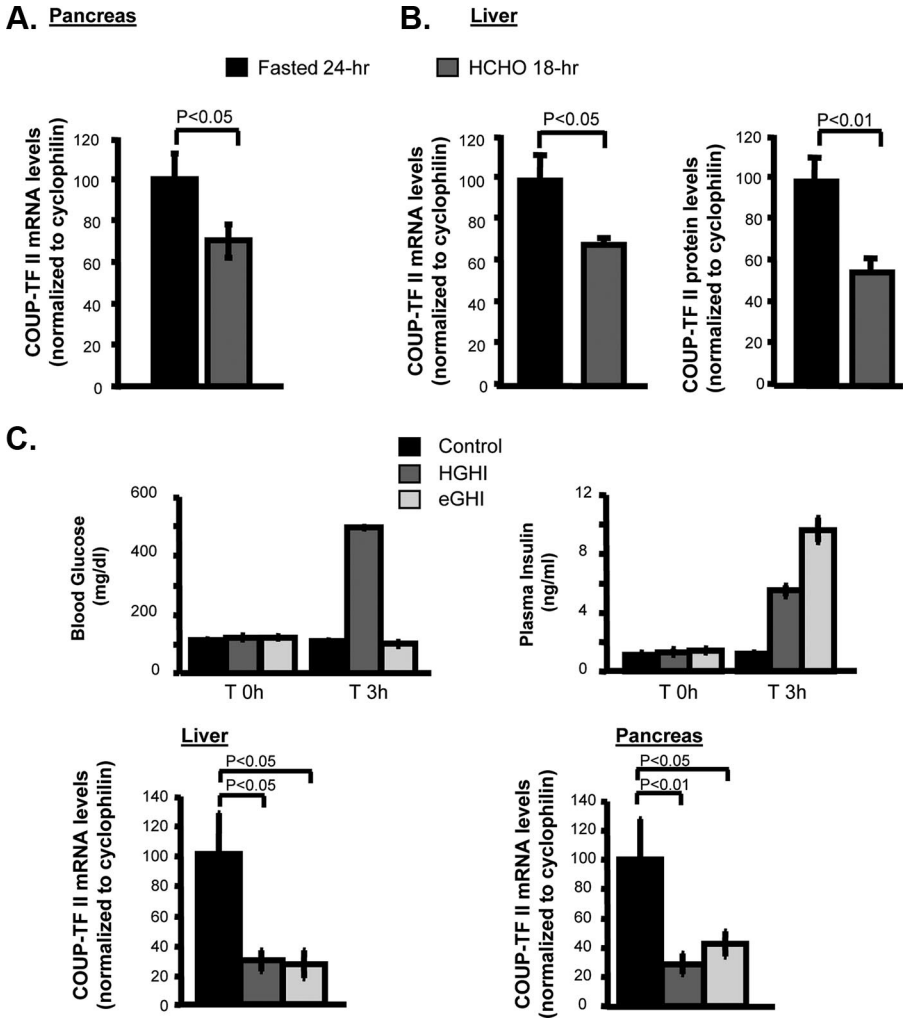

FIG. 1. In vivo COUP-TFII expression in pancreas and liver. C57BL/6J mice were either fasted for 24 h or fed an HCHO diet for 18 h. (A and B) RTQ-PCR analysis of COUP-TFII in pancreas (A) and liver (B, left) and quantification from triplicate Western blots of COUP-TFII protein from liver (B, right). (C) Blood glucose, plasma insulin, and regulation of COUP-TFII in liver and pancreas of HGHI and eGHI clamped mice. Results are means ± SEM (where $n = 6$ mice/group).

Glucose represses COUP-TFII mRNA expression via insulin secretion. We determined which mechanisms of glucose-stimulated insulin secretion are required for the downregulation of COUP-TFII expression. The incubation of pancreatic beta cells with stimulatory glucose concentrations leads to the activation of a cascade of reactions that ends in the exocytosis of stored insulin. Briefly, the elevation in the ATP/ADP ratio induced by glucose metabolism leads to closure of ATP-sensitive K^+ channels, which in turn results in the depolarization of the plasma membrane. The subsequent opening of voltage-gated L-type Ca^{2+} channels leads to an increase in the cytoplasmic free Ca^{2+} concentration, which promotes insulin secretion.

As shown in Fig. 2B and C, COUP-TFII mRNA is downregulated by 20 mM glucose and to a similar extent by insulin at low glucose concentrations (Fig. 3A).

First, in the presence of 20 mM glucose, verapamil was used to block voltage-gated L-type Ca^{2+} channels, and this abolished the decrease in COUP-TFII mRNA levels (Fig. 3A). Second, the effects of 20 mM glucose were mimicked by KCl, which is known to stimulate insulin secretion by depolarizing the beta cell plasma membrane, leading to the influx of extracellular Ca^{2+} through voltage-gated L-type Ca^{2+} channels. As shown in Fig. 3A, the stimulation of 832/13 INS-1 cells with 15 mM KCl in the presence of 5 mM glucose led to a decrease in levels of COUP-TFII mRNA expression.

As pancreatic beta cells express the insulin receptor, we next investigated the potential involvement of paracrine insulin secretion in the observed glucose COUP-TFII inhibition. A specific insulin receptor tyrosine kinase inhibitor, HNMPA-$(AM)_3$ (18, 34), added to 832/13 INS-1 cells significantly blocked the decrease in levels of COUP-TFII mRNA induced by exogenous insulin or by glucose-induced insulin secretion (Fig. 3A). The efficacy of these agents was verified by measuring the mRNA levels of the PPARα gene, which is downregulated by glucose (Fig. 3B); of the L-PK gene, which is upregulated by glucose (Fig. 3C); and of the insulin I and II genes, which are upregulated by insulin (Fig. 3D) (4, 19, 32, 33, 37).

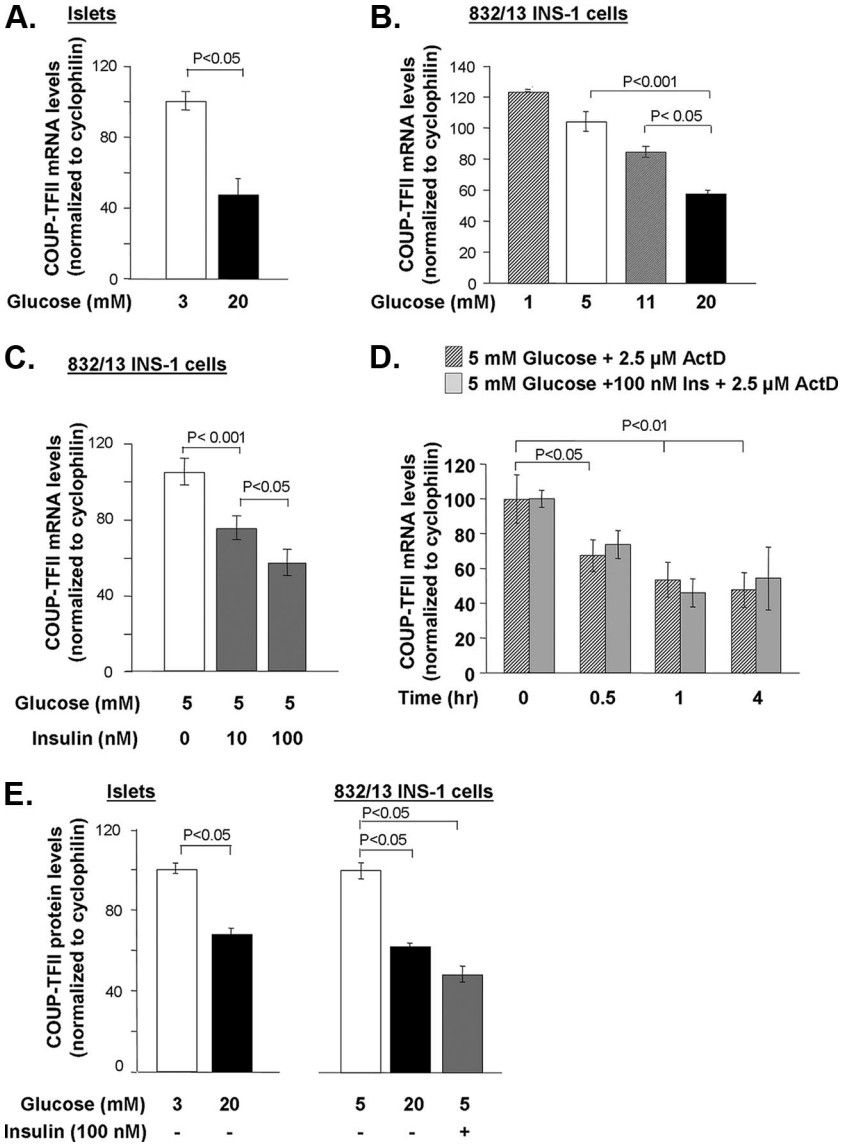

FIG. 2. Ex vivo COUP-TFII expression in pancreatic beta cells. After a 24-h preincubation at low glucose concentrations, 832/13 INS-1 cells were incubated for 4 h or C57BL/6J mouse islets were incubated for 12 h in the presence of glucose or insulin. (A) RTQ-PCR analysis of islet COUP-TFII mRNA after glucose stimulation. (B and C) RTQ-PCR analysis of the effects of different glucose (B) and insulin (C) concentrations on COUP-TFII mRNA levels in 832/13 INS-1 cells. (D) Insulin does not affect the stability of COUP-TFII transcripts in 832/13 INS-1 cells. After a 24-h preincubation in 5 mM glucose, cells were incubated for different periods in the presence of 5 mM glucose with 2.5 μM actinomycin D (Act D) (banded bars) or 5 mM glucose plus 100 nM insulin with 2.5 μM actinomycin D (gray bars). (E) Quantification from triplicate Western blots of COUP-TFII protein from islets and 832/13 INS-1 cells cultured as described above (A to C). Data are the means ± SEM for at least three independent experiments.

All these data are consistent with the hypothesis that the major effect of glucose leading to the repression of COUP-TFII expression in pancreatic beta cells is mediated by secreted insulin acting on its own receptor.

Foxo1 induces COUP-TFII mRNA expression in 832/13 INS-1 cells. As a major downstream target of the insulin-signaling pathway, the Foxo1 transcription factor mediates adaptive gene expression programs in many insulin-sensitive tissues like the liver and pancreatic beta cells (9, 16). Insulin induces the phosphorylation of Foxo1 through the phosphatidylinositol 3-kinase–Akt signaling pathway. Phosphorylated Foxo1 is excluded from the nucleus, thereby attenuating its transcriptional activity. To investigate whether Foxo1 mediates the action of insulin on COUP-TFII expression, we measured endogenous COUP-TFII mRNA expression levels in cultured 832/13 INS-1 beta cells with a gain or loss of function of Foxo1. Firstly, we infected cells with an Ad encoding a constitutively nuclear Foxo1 mutant (Foxo1-ADA) (17). Foxo1-ADA is always located in the nucleus, as it cannot be phosphorylated by Akt and thus is constitutively active. After infection of beta cells with this adenoviral vector, the Foxo1-ADA protein was readily detectable by Western blotting (Fig. 4A). Increasing concentrations of Foxo1-ADA Ad prevented the inhibition of COUP-TFII mRNA expression caused by the addition of in-

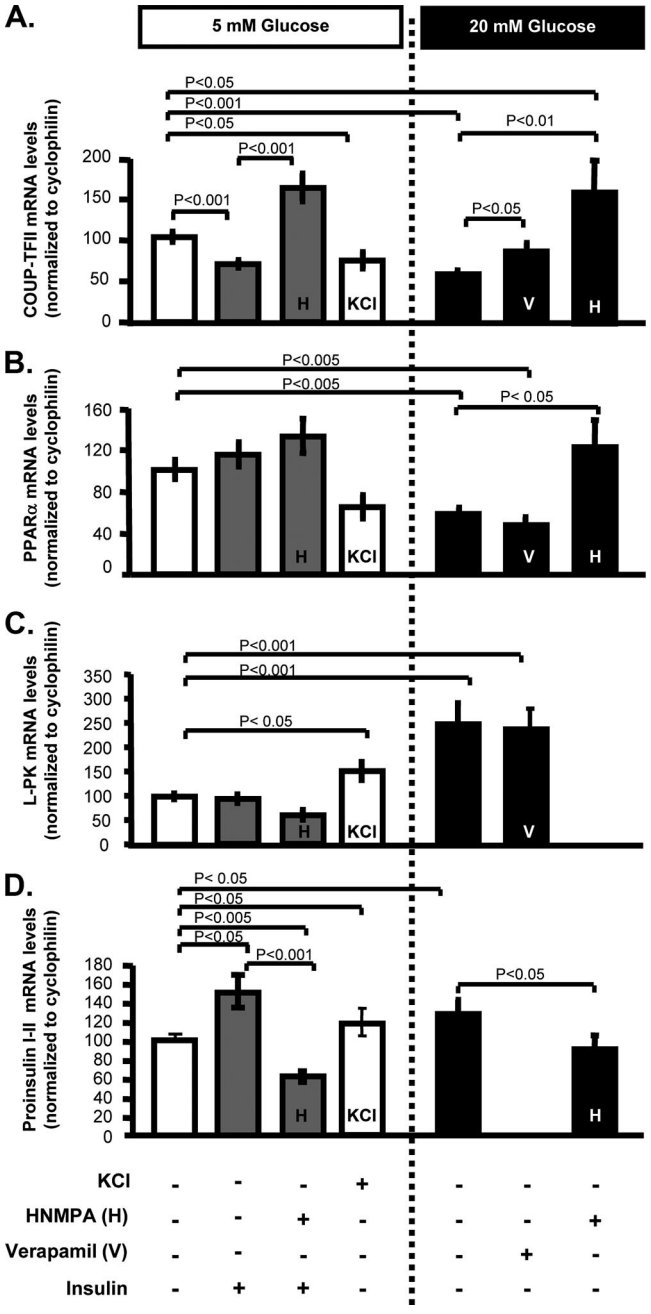

FIG. 3. Glucose-stimulated insulin secretion decreases COUP-TFII mRNA expression levels in 832/13 INS-1 cells. Following a 24-h preincubation in 5 mM glucose, cells were incubated for 4 h with 5 mM glucose, 5 mM glucose plus 100 nM insulin, 5 mM glucose plus 100 μM HNMPA-$(AM)_3$, 5 mM glucose plus 15 mM KCl, 20 mM glucose, 20 mM glucose plus 20 μM verapamil, or 20 mM glucose plus 100 μM HNMPA-$(AM)_3$. The resulting COUP-TFII (A), PPARα (B), L-PK (C), and proinsulin I and II (D) mRNA levels were measured by RTQ-PCR. Results are means ± SEM for six independent experiments.

sulin. The expression of the Foxo1 protein strongly increased COUP-TFII expression, suggesting that there is a positive transcriptional effect of Foxo1 on the COUP-TFII gene (Fig. 4B); there was no change in control PPARα mRNA expression (Fig. 4B). Secondly, we tested the effects of Foxo1 suppression using a Foxo1 siRNA and measuring gene expression in 832/13 INS-1 cells cultured in 5 mM glucose, a steady state where the Foxo1 protein is transcriptionally active. The specific knockdown of Foxo1 was confirmed by a 38% reduction in Foxo1 mRNA levels and a 20% reduction in Pdx1 mRNA expression, a known Foxo1 target (Fig. 4C). Under these conditions, endogenous COUP-TFII mRNA expression was inhibited, whereas there was no change in control PPARα mRNA expression (Fig. 4C).

Insulin genes are COUP-TFII targets. Knockout mice with a heterozygous deletion of the COUP-TFII gene in pancreatic beta cells have a defect in insulin secretion (2). In this study, we developed an ex vivo system in which to investigate the in vivo observations. 832/13 INS-1 cells were treated with COUP-TFII siRNA, which abrogated COUP-TFII expression in a pancreatic beta cell line, allowing us to analyze the consequences on insulin expression first.

Endogenous proinsulin I and II mRNA expression in response to insulin inversely correlates with COUP-TFII mRNA expression (Fig. 3A and D). We tested whether insulin genes are COUP-TFII-dependent targets in two ways. First, we downregulated endogenous COUP-TFII expression (by 80%) by electroporating 832/13 INS-1 cells with a specific COUP-TFII siRNA (Fig. 5A and B). The insulin I and II gene transcript levels were markedly increased (Fig. 5C), whereas there was no change in control Pdx1 or NeuroD mRNA levels (Fig. 5C). Second, we overexpressed the COUP-TFII protein using a recombinant Ad encoding a human COUP-TFII protein in 832/13 INS-1 cells and in mouse pancreatic islets. Based on GFP reporter expression, 80% of 832/13 INS-1 cells and 50% of islet beta cells were infected (data not shown). Immunoblotting showed that COUP-TFII was strongly expressed in infected cells (Fig. 5D and E, left). Ad-mediated COUP-TFII expression resulted in a 60% inhibition of proinsulin I and II mRNA expression in 832/13 INS-1 cells and in pancreatic islets (Fig. 5D and E, right).

COUP-TFII negatively regulates insulin content and insulin secretion in 832/13 INS-1 cells. As we observed a modulation in insulin gene expression linked to COUP-TFII expression, we then determined the effects of COUP-TFII expression on insulin secretion and total cellular insulin content in 832/13 INS-1 cells treated with COUP-TFII siRNA. In both 3 mM and 20 mM glucose, insulin secretion was significantly enhanced (Fig. 5F), and there was a loss of glucose-stimulated insulin secretion. The total insulin content was also higher, independent of the glucose concentration, and is consistent with the observed increase in the levels of expression of insulin genes (Fig. 5G).

COUP-TFII inhibition results in an increase in TG content in 832/13 INS-1 cells. As insulin is known to stimulate TG production, we first examined whether the elevated insulin release induced by COUP-TFII suppression in 832/13 INS-1 cells was associated with an increase in cellular TG content. Compared with control electroporated cells, COUP-TFII siRNA-treated cells have significantly more intracellular TGs 48 h postnucleofection (control, 16.9 ± 0.4 nmol/mg of protein; COUP-TFII siRNA, 20.3 ± 0.1 nmol/mg of protein [$P < 0.02$; $n = 4$]). To determine if this increased TG content resulted from an increase in the fatty acid esterification capacity,

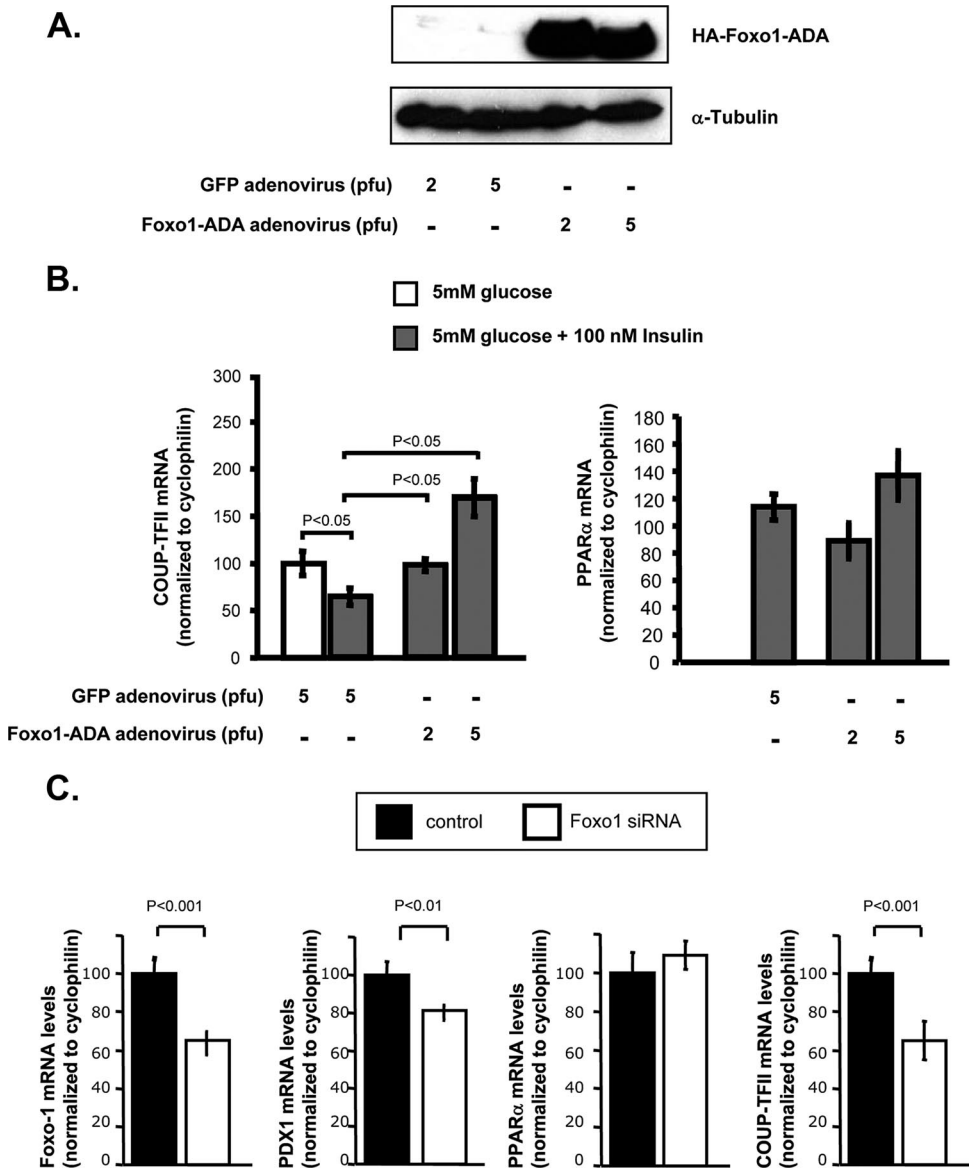

FIG. 4. COUP-TFII mRNA expression is negatively controlled by insulin via the Foxo1 pathway in 832/13 INS-1 cells. (A and B) Cells were cultured in 5 mM glucose and infected overnight with Ad-GFP or Ad-Foxo1-ADA as indicated (2 to 5 PFU/cell) in the presence of 5 mM glucose plus 100 nM insulin. (A) Representative Western blot of total protein (25 μg/lane) showing HA-Foxo1-ADA (top) as detected with antibodies against the HA tag and α-tubulin as a loading control (bottom). (B) RTQ-PCR of steady-state COUP-TFII mRNA and control PPARα mRNA levels. (C) 832/13 INS-1 beta cells were electroporated with control or with rat Foxo1 siRNA and incubated with 5 mM glucose for 24 h. Shown are data for RTQ-PCR measuring Foxo1, Pdx1, PPARα, and COUP-TFII mRNA levels. Results are means ± SEM for three independent experiments.

we then measured [1-^{14}C]palmitate esterification flux over a 2-h incubation period in both low (2.5 mM) and high (20 mM) glucose. The high glucose concentration induced sixfold and fivefold increases in [1-^{14}C]palmitate esterification into TGs in control and COUP-TFII siRNA-treated cells, respectively (Fig. 5H). At the low glucose concentration, we observed that the downregulation of COUP-TFII expression led to a significant 45% increase in labeled TGs (Fig. 5H) that was accompanied by a tendency in the increase of intermediate in ^{14}C nonesterified fatty acid, [^{14}C]diacylglyceride, and [^{14}C]phospholipid levels (data not shown).

Insulin and glucose decrease COUP-TFII mRNA expression levels in hepatocytes. Our in vivo data also showed that insulin and possibly glucose were involved in reducing the expression of COUP-TFII in the liver of fed mice. Using hepatocytes in primary cultures, we investigated the pathways by which insulin and/or glucose controls COUP-TFII expression.

The effect of insulin and glucose on COUP-TFII mRNA levels in primary cultures of C57BL/6J mouse hepatocytes was measured. When cells were cultured in low glucose, the COUP-TFII mRNA levels were maximal. On the other hand, insulin (100 nM) and glucose (25 mM), both individually and

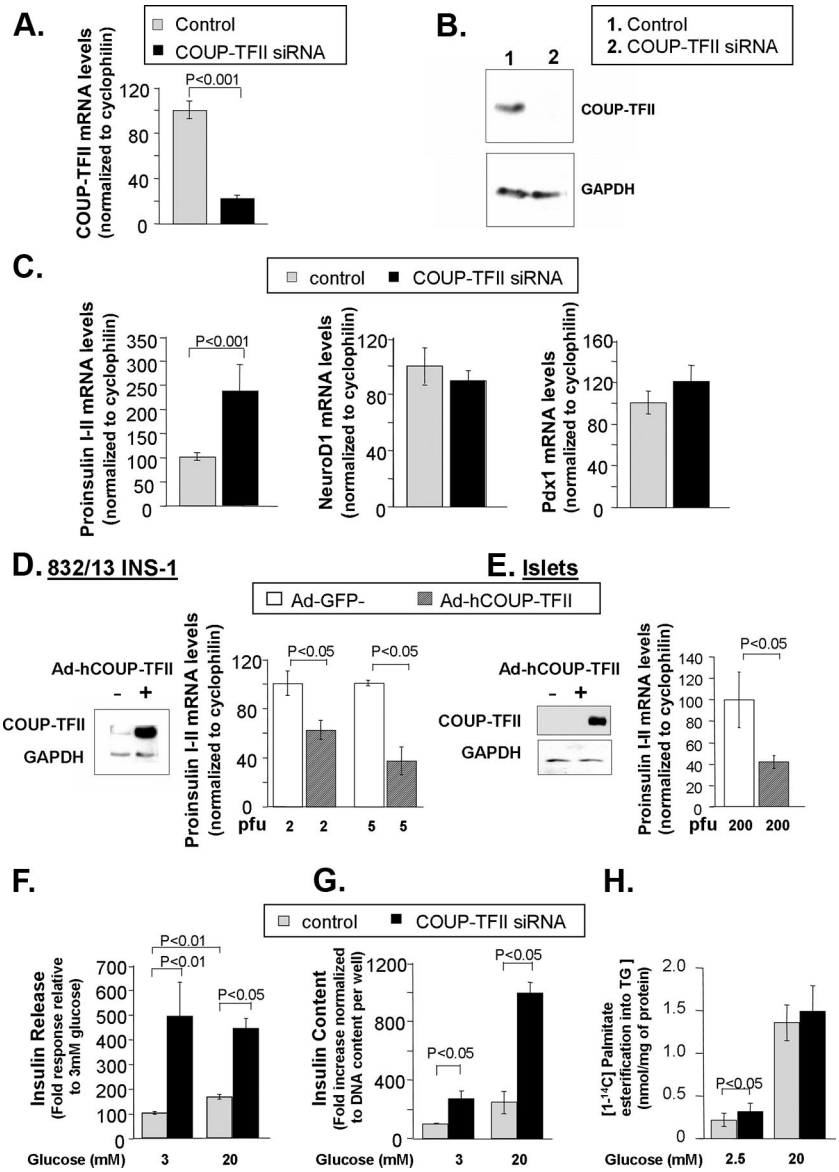

FIG. 5. COUP-TFII represses insulin mRNA expression and insulin release and lowers insulin content and intracellular TG content. 832/13 INS-1 cells were electroporated with control or specific COUP-TFII siRNA and cultured for 48 h. (A) COUP-TFII mRNA levels measured by RTQ-PCR. Results are means ± SEM for eight independent experiments. (B) Representative Western blot with 20 μg/lane of nuclear extracts showing COUP-TFII protein (top) and the GAPDH loading control (bottom). (C) RTQ-PCR analysis of proinsulin I and II and control NeuroD1 and Pdx1 mRNA levels in control and COUP-TFII knockdown 832/13 INS-1 cells. Results are means ± SEM for eight independent experiments. (D and E) 832/13 INS-1 cells (D) and islets (E) were cultured with 11 mM glucose and infected with Ad-GFP or Ad-hCOUP-TFII as indicated (2 to 5 and 200 PFU/cell, respectively). Shown are representative Western blots of 20 μg/lane of nuclear extracts showing COUP-TFII (top) and GAPDH (bottom) as the loading control and RTQ-PCR analysis of proinsulin I and II mRNA levels. (F and G) Insulin release and insulin content in control (white bars) and COUP-TFII siRNA-electroporated (black bars) 832/13 INS-1 cells. (F) Induction of glucose-stimulated insulin secretion in 832/13 INS-1 cells. (G) Increase in insulin content normalized to the DNA content/well. Data are the means ± SEM for three independent experiments. (H) [1-^{14}C]palmitate esterification into cellular TGs was measured in control and COUP-TFII-suppressed 832/13 INS-1 cells at the end of a 2-h incubation period in the presence of either 2.5 mM or 20 mM glucose. Results are means ± SEM of data from four independent experiments.

combined, significantly decreased COUP-TFII expression levels. In the range of concentrations used, combining both effectors did not have a synergistic effect (Fig. 6A).

Foxo1 induces COUP-TFII mRNA expression in hepatocytes. To investigate whether Foxo1 mediates the action of insulin in primary mouse hepatocytes, as observed in pancreatic beta cells, we examined Foxo1 effects on endogenous COUP-TFII gene expression in two ways. Firstly, we examined the effects of constitutively active Foxo1 on gene expression in hepatocytes infected with adenoviral vectors expressing the constitutively nuclear Foxo1 mutant plus GFP (Foxo1-ADA) or GFP alone. Hepatocytes were treated with 100 nM insulin and 25 mM glucose for 24 h, conditions comparable to those of the fed state, and RNA was harvested for gene expression

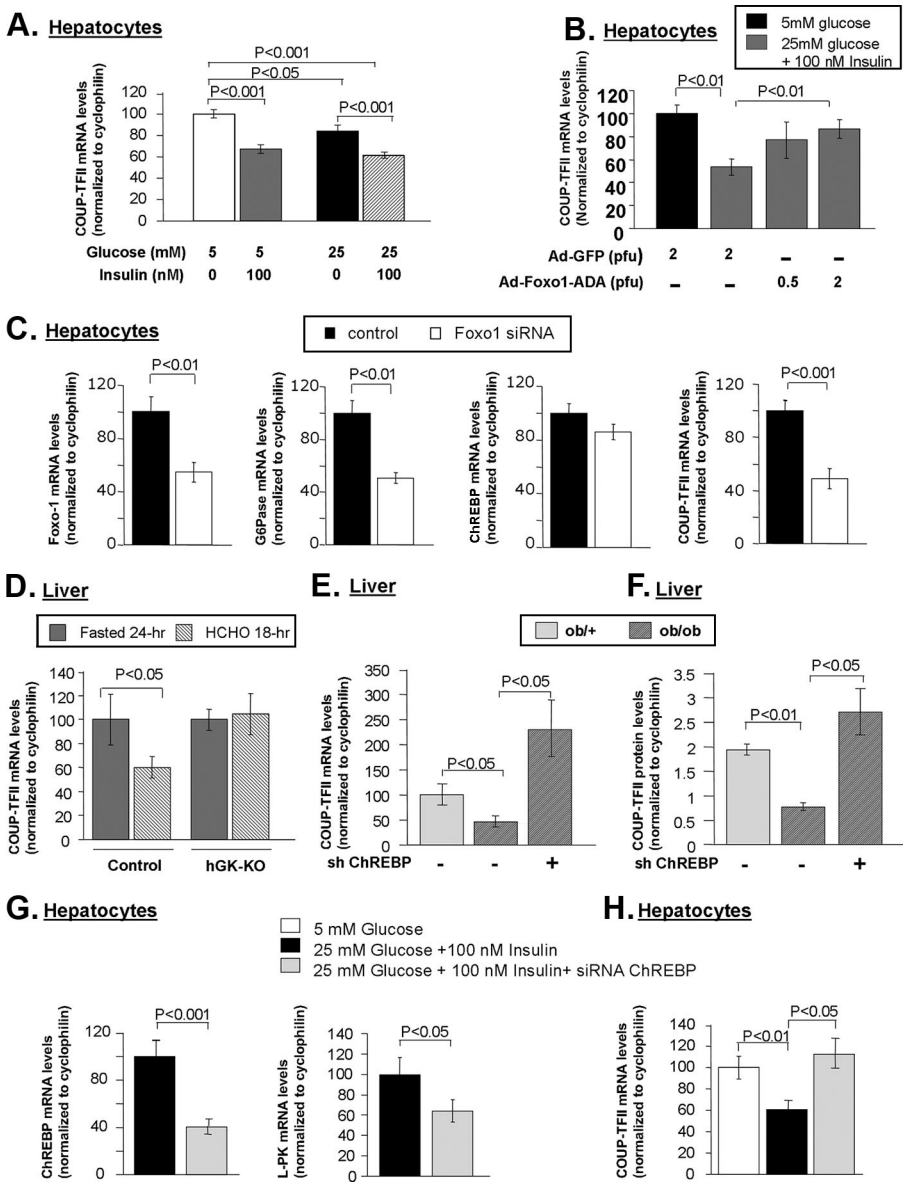

FIG. 6. In hepatocytes, COUP-TFII expression is negatively controlled by insulin via the Foxo1 pathway and by glucose via the ChREBP pathway. (A) C57BL/6J mouse hepatocytes were cultured for 24 h in the presence of 5 mM glucose. Cells were then incubated with either 5 mM or 25 mM glucose with or without 100 nM insulin. After 24 h, steady-state COUP-TFII mRNA levels were determined by RTQ-PCR. Data are the means ± SEM for five independent experiments. (B) C57BL/6J mouse hepatocytes were cultured for 24 h in the presence of 5 mM glucose and infected for 4 h with Ad-GFP or Ad-Foxo1-ADA as indicated (0.5 and 2 PFU/cell) and then cultured for 24 h in the presence of 25 mM glucose plus 100 nM insulin. Shown are data for RTQ-PCR of steady-state COUP-TFII mRNA levels. Results are means ± SEM for three independent experiments. (C) C57BL/6J mouse hepatocytes were cultured for 12 h in the presence of 5 mM glucose. Hepatocytes were then transfected with control or Foxo1 siRNA and incubated with 5 mM glucose for 48 h. RTQ-PCR measured Foxo1, G6Pase, ChREBP, and COUP-TFII mRNA expression levels. (D) Effect of fasting and HCHO refeeding in control and hGK-KO mice. Total RNA was extracted from liver, and COUP-TFII mRNA levels were analyzed by RTQ-PCR. Results are means ± SEM from six mice/group. (E and F) *ob*+/− and *ob*/*ob* mice treated for 7 days with Ad-GFP (−) or Ad-shChREBP (+). (E) RTQ-PCR measuring COUP-TFII mRNA levels in liver. (F) Quantification from triplicate Western blots of COUP-TFII protein from total liver extracts. Cyclophilin expression was used as a loading control. Results are means ± SEM from six mice/group. (G and H) C57BL/6J mouse hepatocytes were cultured for 24 h in the presence of 5 mM glucose and transfected with control or ChREBP siRNA in the presence of 5 mM glucose and incubated with 25 mM glucose plus 100 nM insulin for 24 h the next day. RTQ-PCR measured ChREBP and L-PK (G) and COUP-TFII (H) mRNA expression levels.

analysis by real-time PCR. As shown in Fig. 6B, Foxo1-ADA Ad prevented the inhibition of COUP-TFII mRNA expression caused by insulin and glucose. At the same time, Foxo1 stimulated the expression of IGFBP-1 and suppressed the expression of the key lipogenic enzyme acetyl-coenzyme A carboxylase, consistent with data from previous reports (data not shown) (41). Secondly, we tested the effects of Foxo1 suppression using a Foxo1 siRNA, and we measured gene expression in hepatocytes cultured in 5 mM glucose, a steady state where the Foxo1 protein is transcriptionally active. The specific

knockdown of Foxo1 was confirmed, with a 50% reduction in Foxo1 mRNA levels and a 55% reduction in G6Pase mRNA expression, a known Foxo1 target (Fig. 6C). Under these conditions, endogenous COUP-TFII mRNA expression was inhibited, whereas there was no change in control ChREBP mRNA expression (Fig. 6C).

Loss of glucose inhibition of COUP-TFII mRNA levels in the liver of hGK-KO mice. hGK catalyzes the phosphorylation of glucose to glucose-6-phosphate, and therefore, glucose metabolism in hepatocytes is strongly dependent on its expression. We previously showed that hGK-KO mice have perturbed glucose metabolism and display basal hyperglycemia (30). To determine whether COUP-TFII expression was dependent on active glucose metabolism, we measured the relative COUP-TFII mRNA levels in livers of control and hGK-KO mice in fasted or refed states. In the liver of control mice, COUP-TFII mRNA levels were maximal after a 24-h fast, when insulin and glucose concentrations are at their lowest, but decreased upon HCHO refeeding, as previously observed (Fig. 6D). In contrast, in the liver of hGK-KO mice, HCHO refeeding did not reduce COUP-TFII gene expression (Fig. 6D). Thus, active glucose metabolism in the liver is a key element in the inhibition of COUP-TFII gene expression.

COUP-TFII expression is lower in *ob*/*ob* mouse liver but can be rescued by a long-term knockdown of ChREBP. Mice homozygous for the leptin-deficient (*ob*) gene are obese, hyperglycemic, and hyperinsulinemic and are widely used as a model for type 2 diabetes. Recently, it was reported that *ob*/*ob* mice have increased levels of ChREBP expression in liver, and its liver-specific inhibition improved hepatic steatosis and insulin resistance. In *ob*/*ob* mice, ChREBP inhibition, by the expression of a ChREBP short hairpin RNA, improved plasma insulin and blood glucose concentrations (6). *ob*/*ob* mice treated for 7 days with Ad-shChREBP are therefore a useful model for testing COUP-TFII expression with respect to metabolic status. Expression levels of COUP-TFII mRNA (Fig. 6D) and protein (Fig. 6E) are lower in the livers of *ob*/*ob* mice than in their lean *ob*/+ littermates, as we predicted. Interestingly, ChREBP knockdown in the liver of *ob*/*ob* mice, which improved insulin resistance, resulted in a clear increase in levels of COUP-TFII expression (Fig. 6D and E). Taken together, these results led us to hypothesize that ChREBP has a direct effect in the glucose inhibition of COUP-TFII expression.

ChREBP inhibits COUP-TFII expression. When hepatocytes are cultured in the presence of 5 mM glucose, the ChREBP protein is absent from nuclei (7). High glucose and insulin concentrations stimulate its translocation from the cytosol to the nucleus, where transcriptional activity is enabled (7). We therefore investigated the role of ChREBP in the control of COUP-TFII gene expression using a ChREBP siRNA to specifically silence CHREBP (8) in primary cultures of normal C57BL/6J mouse hepatocytes cultured in the presence of 25 mM glucose and 100 nM insulin. The specific knockdown of ChREBP was confirmed with a 60% reduction in ChREBP mRNA levels (Fig. 6G) and a 45% reduction in its target L-PK gene expression (Fig. 6G). Under these conditions, levels of COUP-TFII mRNA expression were equivalent to those in 5 mM glucose (Fig. 6H). Overall, these results demonstrate that ChREBP expression is essential for COUP-TFII gene inhibition under fed conditions.

DISCUSSION

The major role played by transcription factors such as ChREBP and Foxo1 in the control of glucose homeostasis has been demonstrated in vivo by rescuing a diabetic phenotype in insulin-resistant mice (6, 24). Here, using mouse models, pancreatic beta cells, and hepatocytes in primary cultures, we reveal that the COUP-TFII transcription factor gene is a novel negative target of the insulin and glucose signaling pathways. We show that Foxo1 and ChREBP are involved in this regulation, and we confirm that COUP-TFII has an essential role in insulin secretion.

Beta cell insulin secretion is under the control of an autocrine positive feedback loop by alleviating COUP-TFII and Foxo1 repression. We previously showed that even with 50% of the normal COUP-TFII expression in pancreatic beta cells, mutant mice present hyperinsulinemia in both fasted and fed states and impaired glucose tolerance (2). To further understand the molecular mechanisms regulating COUP-TFII, we aimed to clarify its role in cultured beta cells using both 832/13 INS-1 cells and isolated mouse islets. Here, we showed that insulin secreted by pancreatic beta cells in response to elevated glucose concentrations is a signal for the activation of insulin gene expression through insulin receptors, as has generally been reported (1, 4, 18, 21, 22, 27, 38). Interestingly, we found that COUP-TFII mRNA expression is conversely controlled; i.e., it is repressed when insulin gene mRNAs are activated. This observation led us to address how the modulation of COUP-TFII expression could affect the expression of insulin genes and the physiological consequences either by specific COUP-TFII silencing or by COUP-TFII overexpression in 832/13 INS-1 and pancreatic islets. We observed that the COUP-TFII knockdown strongly increased insulin gene transcript levels, thereby significantly increasing insulin content. Accordingly, the overexpression of COUP-TFII led to a pronounced reduction in insulin gene transcript levels.

In our COUP-TFII knockdown experiments, there was more insulin secretion under both basal and glucose-stimulated conditions resulting from the loss of glucose responsiveness. The functional consequences of lowered levels of COUP-TFII expression on insulin secretion might be partly explained by our observations that levels of fatty acid esterification and cellular TG content were increased in COUP-TFII knockdown cells when glucose levels were low. Indeed, lipid metabolism in pancreatic beta cells is known to be critical for the regulation of insulin secretion with, but not only, lipid intermediates of the esterification pathway acting as coupling factors in insulin secretion (26). Further investigations of the role of COUP-TFII in beta cell lipid signaling will therefore be of particular interest.

To understand the nature of the signal that inhibits COUP-TFII expression, we studied the regulation of COUP-TFII by insulin and glucose. We showed in vivo that insulin decreases COUP-TFII expression levels in the pancreas. In cultured pancreatic beta cells, we showed that glucose inhibits COUP-TFII gene expression by stimulating the release of insulin. Moreover, insulin downregulates COUP-TFII expression through an autocrine loop. Our data suggest that insulin suppresses COUP-TFII gene expression by inhibiting Foxo1, the effector of insulin signaling.

COUP-TFII expression is negatively controlled by insulin and by glucose in hepatocytes: demonstration that the negative glucose effect is dependent on ChREBP expression. The control of COUP-TFII expression in the liver was studied in mouse models treated with insulin and glucose. Insulin and possibly glucose decrease COUP-TFII expression. Primary culture of hepatocytes is a suitable experimental model that allows us to dissect hormone and nutrient effects. As in beta cells, we found that insulin inhibits the expression of COUP-TFII and that Foxo1 ablation results in a decrease in levels of COUP-TFII expression. Moreover, Foxo1 overexpression prevents the insulin-mediated suppression of COUP-TFII expression. Our results also indicate that elevated concentrations of glucose significantly downregulate the expression of COUP-TFII in hepatocytes. The repressive effects of insulin and glucose were not additive in our experiments. It was previously shown that the repressive effects of insulin and glucose on hormone-stimulated PEPCK gene promoter activity are additive only when concentrations of both agents are suboptimal (35). To date, the PEPCK gene is the only example of a gene whose expression is downregulated by glucose per se. It is possible that a similar metabolic intermediate, the amount of which is dependent on GK activity, could mediate the action of glucose by up- and downregulating the expression of some genes (35). In the HCHO refeeding studies, we observed that the lack of hepatic GK impairs the downregulation of COUP-TFII mRNA by an HCHO diet. Furthermore, in the genetically obese *ob*/*ob* mouse model, COUP-TFII expression is inhibited in the liver. Reestablishing the normal balance of these parameters in liver-specific Ad-shChREBP-treated *ob*/*ob* mice leads to a total rescue of COUP-TFII mRNA and protein expression. ChREBP silencing in normal hepatocytes in primary culture in the presence of high glucose concentrations and insulin led to a maximal expression of COUP-TFII. Our data suggest that ChREBP might be the transcription factor that controls the glucose inhibition of COUP-TFII.

However, the total rescue of COUP-TFII expression (Fig. 6G) in the ChREBP silencing experiment highlights the lack of a blunted negative insulin effect under these conditions. Is it therefore possible that Foxo1 controls ChREBP expression in liver? We did not detect any effect of Foxo1 on ChREBP mRNA expression. Evidently, other aspects of the network, such as a potential effect of Foxo1 on ChREBP, still need to be investigated.

Finally, our findings about COUP-TFII regulation may have wider implications since COUP-TFII interacts with several other nuclear receptor family members involved in metabolic regulation.

In conclusion, these results reinforce the concept that pancreatic beta cells are targets for positive insulin action on insulin genes or insulin secretion and implicate a novel partner, the transcription factor COUP-TFII, as being a negative regulator. It is also suggested that high levels of expression of COUP-TFII during fasting inhibit insulin secretion. In the coordinated adaptation of the liver to fasting, COUP-TFII could participate in hepatic glucose production through the activation of the gluconeogenic PEPCK gene (2, 5, 11, 12, 23; M. Vasseur-Cognet, unpublished data). In this regulation, as illustrated in Fig. 7, Foxo1 could activate COUP-TFII expression directly or indirectly. In the fed state, COUP-TFII expression is doubly blocked by glucose via the "glucose sensor" ChREBP and by insulin.

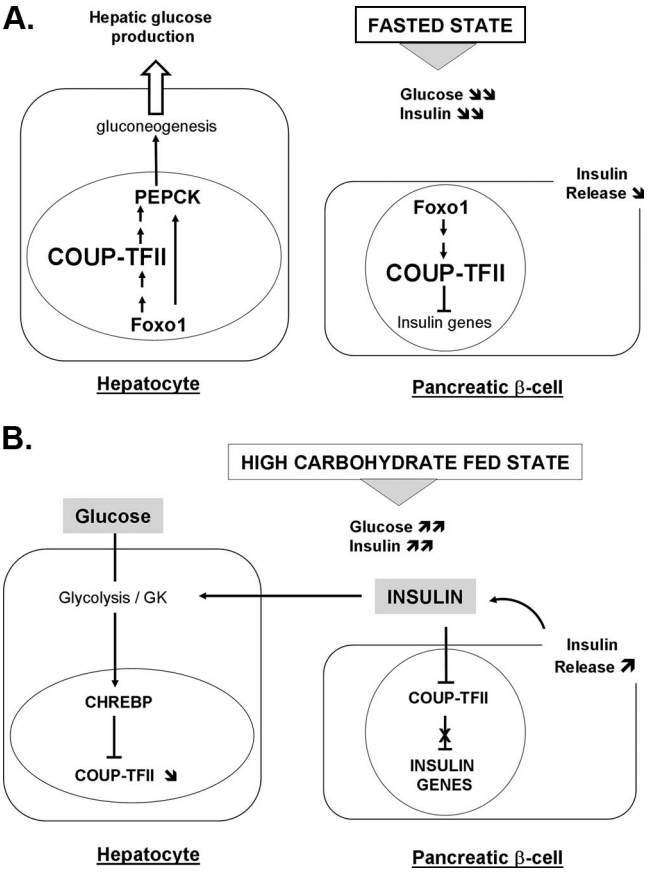

FIG. 7. Proposed scheme of events underlying the regulation of insulin secretion in pancreatic beta cells and glucose metabolism in hepatocytes by COUP-TFII. Shown are positive (pointed arrows) and negative (flat-headed arrows) effects of COUP-TFII in hepatocytes and pancreatic beta cells in fasted (A) and HCHO-fed (B) states.

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