

THE FARNESOID X RECEPTOR INHIBITS THE TRANSCRIPTIONAL ACTIVITY OF THE CARBOHYDRATE RESPONSE ELEMENT BINDING PROTEIN IN HUMAN HEPATOCYTES – R2

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Abbreviations :

ACC1 : Acetyl-CoA Carboxylase 1 ; ChIP : Chromatin immunoprecipitation ; ChORE : Carbohydrate-Response Element ; ChREBP : Carbohydrate Response Element Binding Protein ; DMSO : Dimethyl Sulfoxide ; FAS : Fatty Acid Synthase ; FXR : Farnesoid X Receptor ; HDAC : Histone deacetylase ; HNF4 α : Hepatocyte Nuclear Factor-4 α ; IHH : Immortalized Human Hepatocytes ; LBD : Ligand Binding Domain ; L-PK : Liver-type Pyruvate Kinase ; Mix : Max-like protein ; LXR : Liver X Receptor ; PP2A : Protein Phosphatase 2.

ABSTRACT

The glucose-activated transcription factor Carbohydrate Response Element Binding Protein (ChREBP) induces the expression of hepatic glycolytic and lipogenic genes. The Farnesoid X Receptor (FXR) is a nuclear bile acid-receptor controlling bile acid, lipid and glucose homeostasis. FXR negatively regulates hepatic glycolysis and lipogenesis in mouse liver. The aim of this study is to determine whether FXR regulates the transcriptional activity of ChREBP in human hepatocytes and to unravel the underlying molecular mechanisms. Agonist-activated FXR inhibits glucose-induced transcription of several glycolytic genes, including Liver-type Pyruvate Kinase (*L-PK*), in the human hepatocyte IHH and HepaRG cell lines. This inhibition requires the L4L3 region of the *L-PK* promoter known to bind the transcription factors ChREBP and Hepatocyte Nuclear Factor-4 α (HNF4 α). FXR interacts directly with ChREBP and HNF4 α proteins. Analysis of the protein complex bound to the L4L3 region reveals the presence of ChREBP, HNF4 α , FXR and the transcriptional co-activators p300 and CBP at high glucose concentrations. FXR activation does not affect FXR neither HNF4 α binding to the L4L3 region, but results in the concomitant release of ChREBP, p300 and CBP and in the recruitment of the transcriptional co-repressor SMRT. Thus, FXR transrepresses the expression of genes involved in glycolysis in human hepatocytes.

INTRODUCTION

The liver plays a critical role in maintaining glucose homeostasis, by controlling both glucose production and utilization (1). In the fasting state, the liver produces glucose from glycogen through the glycogenolysis pathway and from lactate, glycerol and amino acids through the gluconeogenesis pathway (2). In the fed state, energy is provided by glucose oxidation through the glycolysis pathway and glucose excess is stored as glycogen through the glycogen synthesis pathway or converted into fatty acids via the de novo lipogenesis pathway (1). These pathways are regulated not only by glucagon and insulin, but also by glucose itself.

Glucose regulates its own metabolism by controlling the transcription of glucose-handling enzymes (3). Also known as WBSCR14 or MondoB and member of the basic helix–loop–helix leucine zipper (bHLH-ZIP) family of transcription factors (4), the transcription factor Carbohydrate Response Element Binding Protein (ChREBP) is necessary for glucose-induced gene expression (5). It associates with the Max-like protein (Mlx) (6) to bind to Carbohydrate-Response Elements (ChORE) composed of two E-box-like motifs present in the promoters of its target genes, such as the glycolytic Liver-type Pyruvate Kinase (*L-PK*) and lipogenic Fatty Acid Synthase (*FAS*) and Acetyl-CoA Carboxylase 1 (*ACC1*) genes (5; 7; 8). The activation of ChREBP transcriptional activity by glucose involves its dephosphorylation by Protein Phosphatase 2 A (PP2A), allowing its nuclear translocation and binding to ChOREs (9). Generation of xylulose 5-phosphate by the pentose phosphate pathway activates PP2A, hence coupling glucose metabolism to ChREBP activation (10). However, recent reports challenged this view showing that glucose 6-phosphate mediates ChREBP activation (11; 12). A recent study showed that both xylulose 5-phosphate and glucose 6-phosphate activate ChREBP

(13). Moreover, ChREBP activity is also regulated by other post-translational modifications, such as acetylation (14) and O-GlcNAcylation (15; 16). Finally, other transcription factors, including the nuclear receptors Hepatocyte Nuclear Factor-4 α (HNF-4 α) (17) and Liver X Receptors (LXR) (18), are involved directly or indirectly in the transcriptional response to glucose, offering multiple entry points for the fine tuning of cellular responses to this nutrient.

The nuclear bile acid-receptor Farnesoid X Receptor (FXR) maintains bile acid homeostasis by regulating the expression of key enzymes involved in bile acid synthesis and transport in the liver and intestine (19-22). FXR also controls lipid metabolism, as illustrated by the phenotype of the FXR-deficient (FXR^{-/-}) mouse which displays elevated serum triglyceride and cholesterol levels (23; 24). More recently, we and others have shown that FXR modulates glucose homeostasis and insulin sensitivity (25-28). Hepatic FXR expression is decreased in rodent models of diabetes and regulated *in vitro* by insulin and glucose in rodent hepatocytes (29). Moreover, FXR^{-/-} mice display an accelerated hepatic response to high carbohydrate refeeding (30). FXR negatively regulates the expression of several glucose-regulated genes, such as *L-PK*, *FAS* and *ACC1*, in rodent primary hepatocytes, which led us to hypothesize that FXR could interfere with the transcriptional activity of ChREBP.

In the present study, we show that ligand-activated FXR represses the induction of *L-PK* gene expression during the fasting-high carbohydrate transition period in livers of wild type mice. Further, this regulation is conserved in Immortalized Human Hepatocytes (IHH) which display glucose and insulin-responsiveness (31). Investigation of the molecular mechanisms by which FXR inhibits the response to glucose shows that FXR inhibits glucose-induced *L-PK* expression by a transrepressive mechanism involving the

release of the transcription factor ChREBP and the recruitment of the transcriptional co-repressor SMRT to the ChORE of the *L-PK* promoter. The expression of several other previously identified ChREBP or glucose target genes is similarly regulated. These results identify a novel regulatory mechanism of glucose-induced genes by the nuclear bile acid receptor FXR, involving the transrepression of ChREBP-controlled pathways.

MATERIALS AND METHODS

In vivo study and blood and tissue sampling

All studies were approved by the ethical committee. Twelve wildtype mice were purchased from Charles River (Wilmington, USA), housed in a pathogen-free barrier facility with a 12-h light/12-h dark cycle and maintained on a standard laboratory chow diet (UAR AO3, Villemoisson/Orge, France). The fasting-refeeding experiments were performed as described in (30). Before feeding the high carbohydrate diet, the mice were pre-treated (30min) by gavage with vehicle alone (0.5% carboxymethyl cellulose (CMC)–0.1% Tween80) or 30mg/kg INT-747 (6-CDCA). Plasma glucose concentrations were determined using Glucotrend 2 (Roche Diagnostics). Total RNA was isolated from livers by guanidinium thiocyanate/phenol/chloroform extraction (32).

IHH and HepaRG cell culture, stimulations and transfections

IHH and HepaRG cells were cultured and stimulated by respectively 11mM and 25mM of glucose as described previously (31; 33; 34). When indicated, IHH cells were pre-treated for 1h by Trichostatin A (TSA)(300µg/ml) before incubation with glucose.

IHH cells were transfected with plasmids using Jet PEI (QBiogene, Illkirch, France) in Williams E (Invitrogen, Cergy-Pontoise, France) medium for 16h and then stimulated with glucose. IHH and HepaRG cells were transfected with small interfering RNA (siRNA) (OnTargetPlus SmartPools: scramble (control) (D-001810, Dharmacon, Lafayette, CO), FXR (L-003414; Dharmacon), ChREBP (L-009253; Dharmacon), CBP (L-003477; Dharmacon) and p300 (L-003486; Dharmacon); siGenome SmartPool:

scramble (control) (D-001206, Dharmacon), SMRT (M-020145; Dharmacon)), as previously described (31; 35).

Total RNA extraction and quantitative PCR

Total RNA was extracted from cells with Trizol reagent (Invitrogen) according to the manufacturer's protocol. Reverse transcription was performed using the High Capacity cDNA reverse transcription kit (Applied Biosystems, Life Technologies, Cergy Pontoise, France) and 1 µg of total RNA. Quantitative PCR were performed on an Mx3500p apparatus (Agilent Technologies, Santa Clara, USA) by using the SYBR Green Brilliant II Fast kit (Agilent Technologies). mRNA levels were normalized to the 36B4 gene mRNA levels and the fold induction was calculated using the ddCt method (36).

GST-pull down

Glutathione-S-Transferase (GST) fusion proteins were adsorbed to glutathione Sepharose 4B beads (GE Healthcare, Chalfont St. Giles, UK) for 1h at 4°C in lysis buffer (1X PBS, 1mM DTT, 0.1X complete Protease Inhibitor Cocktail (PIC) (Roche Diagnostics, Meylan, France), 0.5mM benzamidine). Proteins of interest were produced using the T7 or SP6 TNTsystem (TNT Quick Coupled Transfection/Translation kit; Promega, Madison, USA) containing [³⁵S]-methionine according to the manufacturer's protocol. GST or GST-fusion protein were pre-incubated with vehicle (Dimethyl Sulfoxide (DMSO)) or synthetic FXR ligand (GW4064, 5µM) for 30min at 20°C in binding buffer (20mM Tris-HCl pH8, 100mM KCl, 20% Glycerol, 0.2mM EDTA, 0.05% NP40, 1mM DTT, 1mM Benzamidine, 2.5mg/mL BSA, 0.1X PIC), then with 15µL of *in vitro* translated-proteins for 2h at 20°C in the same buffer. Beads were then washed

three times with wash buffer (20mM Tris-HCl pH8, 100mM KCl, 20% Glycerol, 0.2mM EDTA, 0.05% NP40, 1mM DTT, 1mM Benzamidine, 0.1X complete PIC). Bound proteins were separated by 10% SDS-PAGE. Gels were dried and radiolabeled proteins were detected after a 2 days exposure by phosphorimaging.

Total and nuclear protein extraction, immunoprecipitation and western blot analysis

For analysis of protein expression after gene silencing, total proteins were extracted by lysing cells in a buffer containing 20 mM Tris-HCl pH 7.5, 150mM NaCl, 1mM EDTA, 1mM EGTA, 1% Triton and supplemented with 2.5mM sodium pyrophosphate, 1mM β -glycerophosphate, 1mM Na_3VO_4 and 1 $\mu\text{g}/\text{ml}$ leupeptin.

For immunoprecipitation experiments, cytoplasmic proteins were extracted in a buffer containing 20mM Hepes pH7.9, 10mM KCl, 1mM EDTA, 0.2% NP40 and 0.1X complete PIC for 5min at 4°C. Nuclear proteins were then extracted in the same buffer without NP40 and supplemented with 0.35M NaCl for 30min at 4°C. Protein concentrations were determined by the Peterson method and equal protein amounts were analysed by western blot analysis. Nuclear extracts were immunoprecipitated without antibody (control) or with the antibodies against FXR (AB1, PP-A9033A-00, R&D Systems, Minneapolis, USA ; AB2, sc-13063, SantaCruz) for 16h at 4°C and then 2h at 4°C with protein A-conjugated agarose beads (Millipore, Molsheim, France). Bound proteins were eluted in Laemmli buffer by heating (90°C, 5min) and then analysed by western blotting. Protein samples were resolved in 10% SDS-PAGE. After protein transfer, nitrocellulose membranes were incubated with primary antibodies against ChREBP (sc-21189; Santa Cruz Biotechnology, Santa Cruz, USA), FXR (PP-A9033A-00, R&D Systems), SMRT

(06-891, Millipore), LaminA/C (sc-6215, Santa Cruz) or beta-actin (sc-1616, Santa Cruz) at 4°C for 16h and then with secondary antibodies for 1h at room temperature. Proteins were detected using the enhanced chemiluminescence Western Blot detection kit (Amersham ECL plus western blotting Detection system; GE Healthcare).

Chromatin immunoprecipitation (ChIP) experiments

Cells were fixed using 0.07% ethylene glycol bis-succinimidylsuccinate (EGS) (Pierce, Rockford, USA) for 30min and 1% formaldehyde (Sigma-Aldrich, St. Quentin Fallavier, France) for 10min at 20°C. Chromatin fragmentation was performed using the Bioruptor® system (Diagenode, Liège, Belgium). 200µg of cross-linked DNA-protein complexes were immunoprecipitated with 4µg of ChREBP (sc-21189, SantaCruz and NB400-135, Novus Biologicals, Littleton, USA), HNF4α (sc-8987, SantaCruz), FXR (equal mix of sc-13063, SantaCruz and ab28676, Abcam, Paris, France), CBP (sc-369, SantaCruz), p300 (sc-584, SantaCruz), SMRT (06-891, Millipore), H3K9-Ac (07-473, Millipore) antibodies or no antibody as a negative control. Complexes were captured with 50µL of a 50% protein A agarose bead slurry (Millipore). DNA-protein complexes were eluted with elution buffer (1% SDS, 0.1M NaHCO₃, 200mM NaCl). DNA protein cross-linking was reversed by overnight incubation at 65°C. DNA was then purified using a QIAQuick PCR purification Kit (QIAGEN, Courtaboeuf, France). *L-PK* or *TxNIP* promoter regions containing the ChoRE or myoglobin promoter (negative control) were targeted for amplification by quantitative PCR by using specific primers [*L-PK*, 5'-AGAGCCTCCCGTGTGTTAAA-3' (forward), 5'-GTGTCACCACTGTCTCCTGTTC -3' ; *TxNIP*, 5'-GCCGCTCCAGAGCGCAACAAC -3' (Forward), 5'-GCCCTCGTGACATCCCTCCC -3' (reverse) ; *myoglobin*, 5'-AGC ATG GTG CCA

CTG TGC T-3' and 5'- GGC TTA ATC TCT GCC TCA TGA TG-3']. Promoter occupancy was calculated on the basis of the difference between amplification obtained after immunoprecipitation and input and using the myoglobin gene promoter region as a negative control.

Microarray analysis

Total RNA was prepared from IHH cells stimulated by low and high glucose concentrations as previously described (31) in presence of DMSO or GW4064 (5 μ M) for 24h. mRNA quality was analyzed on a Bioanalyzer 2100 (Agilent Technologies) and only RNA preparations with high integrity (RNA-integrity number (RIN)>9) were used. RNA was amplified and labeled using the Quick Amp Labeling kit (Agilent Technologies) and hybridized to Agilent whole human genome GE 4x44K microarrays (Agilent Technologies). Analyses were performed using Genespring v11.0 (Agilent) and DAVID Bioinformatics Resources 6.7 (<http://david.abcc.ncifcrf.gov/>).

Statistics

Statistical significance was analysed using the unpaired Student's test. All values are reported as means \pm SD. Values with $p < 0.05$ were considered significant (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$).

RESULTS

FXR activation inhibits glucose-mediated induction of *L-PK* expression in mouse liver and in human IHH and HepaRG hepatocytes

We have previously shown that the glucose-mediated induction of the *L-PK* gene expression depends on FXR in mouse liver (30). To assess the effect of FXR activation on *L-PK* expression *in vivo*, wildtype mice were treated, after a 24h-fasting period, by oral administration with INT-747 (6-ECDA, 30 mg/kg), a synthetic FXR agonist (37) and then subjected 30min later to high carbohydrate diet during 8h. Plasma glucose levels were significantly induced by high carbohydrate refeeding (**Fig1A, left panel**). Hepatic *L-PK* mRNA levels increased significantly upon high carbohydrate refeeding, whereas INT-747 treatment significantly repressed this induction (**Fig1A, right panel**).

To test whether FXR activation affects also the induction of glucose-regulated gene expression in human hepatocytes, IHH and HepaRG cells, which respond to glucose and insulin via induction of the glycolytic and lipogenic pathways (31), were incubated at low glucose concentration (IHH, 1mM ; HepaRG, 0.5mM) for 16h mimicking the *in vivo* 'fasting state', and then switched to a medium containing high glucose concentrations (IHH, 11mM ; HepaRG, 25mM) for 24h in the presence of insulin mimicking the *in vivo* high carbohydrate 'refed state'. IHH cells were treated with the synthetic FXR agonists GW4064 (5 μ M), WAY362450 (5 μ M), INT-747 (10 μ M), the natural FXR agonist Chenodeoxycholic Acid (CDCA, 100 μ M) or DMSO (vehicle) at the time of high glucose incubation. HepaRG cells were treated with the synthetic FXR agonist GW4064 (5 μ M) or DMSO (vehicle). High glucose concentrations induced *L-PK* gene expression in IHH (**Fig1B**) and HepaRG (**SupFig1**) cells. This induction was strongly blunted upon FXR

activation in both cell models. FXR gene knockdown by specific siRNAs in IHH cells, leading to a clear decrease of FXR protein levels (**Fig1C, bottom panel**), abolished the inhibitory effect of GW4064 (**Fig1C, top panel**).

IHH cells were then transfected with an expression vector containing the highly conserved L4L3 region of the rat *L-PK* promoter, containing the ChREBP (ChORE) and HNF4 α (DR-1) binding sites involved in glucose induction of *L-PK* expression (**Fig1D**) (38). L4L3 region-driven promoter activity increased at high glucose concentrations, while FXR activation with GW4064 prevented the glucose-induced activation of the L4L3-driven promoter (**Fig1E**). These results show that the L4L3 region of the *L-PK* promoter mediates the FXR-dependent inhibition of the glucose-induced response.

FXR does not alter ChREBP nuclear translocation in IHH cells, but interacts physically with the ChREBP and HNF4 α proteins

Since the L4L3 region is sufficient to drive FXR inhibition of transcription, we hypothesized that FXR could interfere with the transcriptional activity of ChREBP and/or HNF4 α . Indeed, these transcription factors bind to the L4L3 region (39) and are necessary for the induction of *L-PK* gene expression in IHH cells (35). Moreover, it has been shown that both ChREBP and HNF4 α interact directly (17). Our first hypothesis was that FXR could interfere with the nuclear translocation of ChREBP. The nuclear expression of the ChREBP protein was examined by western blot analysis in IHH cells exposed to either low or high glucose concentrations in the presence of GW4064 or not (**Fig2A**). Nuclear ChREBP protein concentrations were increased at high compared to low glucose concentrations. The nuclear enrichment of ChREBP protein was not affected by FXR activation.

We then investigated whether FXR interacts with ChREBP and/or HNF4 α . *In vitro* GST pull-down experiments showed that FXR interacts directly with both ChREBP, in a FXR-ligand stimulated manner (signal intensity: 1.3(with GW4064) *versus* 1(without GW4064), $p < 0.01$, $n = 12$) (**Fig2B**), and HNF4 α proteins, in a FXR ligand-independent manner (signal intensity: 1.3(with GW4064) *versus* 1(without GW4064), $p = 0.3$, $n = 4$) (**Fig2B**), but not with Mlx (**Fig2B**). Analysis of FXR deletion mutants revealed that FXR interacts with ChREBP via its Activation Function-1 (AF-1) domain (F1-105 and F106-196 FXR fragments) and via its Ligand Binding Domain (LBD) (F215-300 FXR fragment) (**Fig2C, lanes3&4**). Finally, FXR coimmunoprecipitated with ChREBP in nuclear extracts from IHH cells transfected with ChREBP and FXR and incubated at high glucose concentrations (**Fig2D**). This interaction was observed with two distinct FXR antibodies, AB1 and AB2.

FXR activation decreases ChREBP, but not HNF4 α or FXR, binding on the L4L3 region of the *L-PK* promoter in IHH cells

To study the complex bound to the L4L3 region of the *L-PK* promoter, Chromatin immunoprecipitation (ChIP) experiments were performed using cross-linked chromatin from IHH incubated at either low or high glucose concentrations and treated with GW4064 or not for 5h. The relative *L-PK* promoter occupancy by ChREBP, HNF4 α and FXR was measured (**Fig3**). Increasing glucose concentration significantly enhanced ChREBP and FXR recruitment to the L4L3 region, whereas the occupancy for HNF4 α did not change. FXR activation strongly decreased ChREBP binding to the L4L3 region, while HNF4 α and, to a lesser extent, FXR proteins were retained on the L4L3 region (**Fig3**).

FXR activation decreases CBP and p300 transcription co-activator binding to the L4L3 region of the *L-PK* promoter in IHH cells

p300 and CBP are transcriptional co-activators associated with ChREBP and/or HNF4 α at the regulatory regions of glucose-induced genes in the liver and pancreas (14; 40; 41). Furthermore, p300 and CBP are necessary for the glucose-mediated induction of *L-PK* gene expression, as shown by the inhibition of the glucose response after p300 and/or CBP silencing (**SupFig2**). ChIP experiments showed a significant enrichment of p300 and a trend to increased occupation of CBP on the *L-PK* promoter at high glucose concentrations in IHH cells (**Fig4A**). Strikingly, FXR activation resulted in the release of both co-activators from the *L-PK* promoter (**Fig4A**).

FXR activation induces the recruitment of the transcriptional co-repressor SMRT and leads to the deacetylation of Histone H3 Lysine 9

SiRNA-mediated silencing of the expression of the transcriptional co-repressor SMRT (**Fig4B, right panel**) in IHH cells did not interfere with glucose-induced *L-PK* expression, but totally prevented the FXR-dependent repression of *L-PK* expression at high glucose concentrations (**Fig4B, left panel**). *In vitro* GST pull-down experiments showed that FXR interacts directly with SMRT protein (**Fig4C, lanes 3&4**). Furthermore, SMRT occupancy of the L4L3 region of the *L-PK* promoter was not modulated by increasing glucose concentrations, but was significantly enhanced upon FXR activation (**Fig4D**). Of note, the co-repressor NCoR was not recruited on the L4L3 region of the *L-PK* promoter and silencing of its gene expression did not interfere with the FXR-dependent repression of *L-PK* gene expression (*Data not shown*).

SMRT recruits and activates Histone Deacetylase (HDAC) 3 (42) which removes acetyl groups from histones. IHH cells were thus pre-treated for 1h with TSA, a selective HDAC inhibitor, before incubation of 24h in medium containing low and high glucose concentrations supplemented with GW4064 or not. Whereas the glucose-induced *L-PK* expression was diminished by TSA treatment, the FXR ligand-dependent inhibition was totally prevented (**Fig4E**). Moreover, ChIP experiments showed a trend for increased acetylation of Histone H3 Lysine 9 (H3K9-Ac) on the *L-PK* promoter in high glucose concentrations, which was significantly inhibited upon FXR activation (**Fig4F**). Taken together, these data suggest that FXR recruits SMRT to the *L-PK* promoter, leading to histone deacetylation and repression of promoter activity.

FXR inhibits glucose induction of several glucose-induced genes

To investigate whether FXR activation generally inhibits the expression of other glucose-induced genes, the expression of known ChREBP-target genes, such as *FAS* (7), Apolipoprotein (Apo) CIII (35), patatin-like phospholipase domain-containing 3 (*PNPLA3*) (43) and Thioredoxin-interacting protein (*TxNIP*) (44), was measured. The expression of these genes which, albeit to differing extents, was induced by high glucose concentrations, was consistently inhibited upon FXR activation (**Fig5A**). To investigate if the same type of molecular mechanism could be involved in these FXR-mediated regulations, the occupancy of the region around the ChORE binding site of the *TxNIP* promoter was analysed using ChIP experiments (**Fig5B**). ChREBP, FXR, p300 and CBP were recruited to the *TxNIP* promoter at high glucose concentrations. FXR activation resulted in the release of ChREBP, p300 and CBP, with little change in FXR. Moreover, FXR activation led to the recruitment of SMRT, while the acetylation of H3K9

decreased. These results suggest that the activation of FXR inhibits the expression of several ChREBP-target genes, probably by a mechanism similar to that observed for the *L-PK* gene.

To map the other glucose-induced genes whose expression is inhibited by FXR, a microarray experiment was performed on mRNA isolated from IHH cells cultured in low glucose concentrations for 16h followed by another 24h in either low or high glucose concentrations in the presence of GW4064 or not. Gene array analysis by DAVID Bioinformatic Database software identified a gene cluster that contained six genes coding for enzymes of metabolic pathways involved in glucose utilization and whose expression is regulated both by glucose and FXR (**SupFig3**). The expression of these genes was induced at high glucose concentrations in IHH cells (**Fig6**), in a ChREBP- (**SupFig4**) and HNF4 α - (**SupFig5**) dependent manner. Furthermore, the glucose-mediated induction was inhibited upon FXR activation (**Fig6**). Thus, FXR-mediated inhibition seems to be a general phenomenon affecting several ChREBP-regulated genes.

DISCUSSION

We report here a novel molecular mechanism of transrepression of glucose-induced gene expression by the nuclear receptor FXR. This mechanism was illustrated using two glucose-responsive human hepatocyte cell lines, IHH and HepaRG. This FXR-dependent regulation is mediated by the highly conserved L4L3 region of the *L-PK* promoter, a region that binds the transcription factors ChREBP and HNF4 α and which is necessary for glucose-induction of *L-PK* gene expression. FXR does not interfere with the nuclear translocation of ChREBP, but interacts with *in vitro* the ChREBP and HNF4 α proteins. High glucose concentrations enhanced the recruitment of ChREBP, p300, CBP and, surprisingly, FXR, but not to the L4L3 region, whereas FXR activation led to the release of ChREBP. In parallel, the transcription co-activators p300 and CBP were released, while the transcription co-repressor SMRT was recruited. Similar results, with the exception of HNF4 α which does not regulate this gene, were observed for *TxNIP*, another ChREBP target gene (44). Finally, microarray experiments performed using IHH cells allowed the identification of six genes involved in glucose utilization pathways whose expression is regulated in a similar manner by ChREBP and FXR.

The glucose response is mainly mediated by the transcription factor ChREBP (5) in association with Mlx (6) and in cooperation with other transcription factors, in which can be, HNF4 α in the case of *L-PK* (39), *FAS* (17) and *apoCIII* (35), but not *TxNIP*. The transcription factor HNF4 α is a master regulator of liver-specific genes (45), regulating the expression of genes involved in glucose utilization (glycolysis) and production (gluconeogenesis) (46). Our results allow us to propose a model of transrepression by the nuclear receptor FXR involving ChREBP, and HNF4 α in the case of *L-PK* (**Fig7**). At high glucose concentrations, ChREBP and HNF4 α are bound to their binding sites in the

L4L3 region of the *L-PK* promoter and activate *L-PK* expression through the recruitment of the transcriptional co-activators p300 and CBP (**Fig7A**). Surprisingly, FXR presence is induced by high glucose concentration in this protein complex likely via ligand-independent mechanism, possibly involving post-translational modifications (47). Upon FXR ligand-activation, at high glucose concentrations, ChREBP is released from the promoter, although HNF4 α is still bound, as well as FXR probably through its interaction with HNF4 α (**Fig7B**). Moreover, p300 and CBP are released, whereas the transcriptional co-repressor SMRT and HDACs are recruited, thus establishing a repressed transcriptional state. The active recruitment of SMRT by a liganded nuclear receptor contrasts to its known preferential interaction with unliganded nuclear receptors (42), but in line with the proposed mechanism of transrepression by PPAR γ (48). Interestingly, FXR appears to participate both in the glucose-induction and the inhibition of *L-PK* gene expression, which suggests a dual role for this nuclear receptors in *L-PK* regulation. Similar regulation by transrepression was described for other nuclear receptors, such as the glucocorticoid receptor (GR) and peroxisome proliferator-activated receptor (PPAR) α and γ , which transrepress inflammation pathways (49). To our knowledge, this is the first transrepression mechanism acting on metabolic pathways.

Interestingly, the expression of other ChREBP-target genes is regulated in a similar mechanism by FXR and likely through similar mechanisms as the *L-PK* gene, at least for *FAS*, *apoCIII* and *PNPLA3*, whose regulation by glucose are both ChREBP- and HNF4 α -dependent ((17; 35; 43) and **SupFig5**). On the other hand, the glucose-mediated regulation of TxNIP is ChREBP-dependent (44), but not HNF4 α -dependent (*Data not shown*). Moreover, using cDNA microarray analysis, we have identified six

genes involved in metabolic pathways of glucose utilization, of which glycolysis, and whose expression was both glucose-induced and FXR-inhibited in IHH cells. Except for Lactate Dehydrogenase (50), these genes have not been previously shown to be ChREBP-target genes. We show that the glucose-mediated induction of their expression is ChREBP-dependent for all (**SupFig4**) and HNF4 α -dependent for at least five of them (**SupFig5**). Finally, we have used data from ChIP-sequencing (ChIP-seq) experiments performed on mouse liver (available on the UCSC Genome Bioinformatics group website) to localize the peaks of FXR enrichment on the promoter of these genes and the DNA sequences 30kb before and 30kb after every FXR enrichment peak were analyzed to identify potential ChORE and FXRE (**SupTable1**). The DNA regions around approximately 50% of the FXR enrichment peaks do not contain classical FXRE sequences, which suggests that FXR could be recruited to the promoter without direct binding on DNA. Amongst these regions, one third contained a ChORE, suggesting a same molecular mechanism of regulation as for the *L-PK* and *TxNIP* genes. This percentage is likely an underestimation, since the ChIP-seq data available were obtained on livers of fasting mice. The other regions contained no ChORE, which suggests that FXR could also transregulate gene expression through interaction with a non-identified transcription factor bound to the DNA. These analyses open new perspectives about the identity of FXR-regulated genes, the molecular mechanisms involved and the functions of FXR.

In summary, our results propose an original model of regulation of FXR-target gene expression by transrepression and interference with ChREBP transcriptional activity. This new molecular mechanism enhances our understanding on the regulation of glucose-handling and lipogenesis by ChREBP and its modulation by FXR.

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FIGURE LEGENDS

Figure 1. FXR activation inhibits the glucose-induced expression of the *L-PK* gene in mouse liver and in IHH cells.

A, Glycemia (*Left panel*) and hepatic *L-PK* mRNA levels (*Right panel*) in wildtype mice subjected to 24h fasting (F) and then refed (RF) for 8h with a high carbohydrate diet after being pre-treated (30min) by gavage with vehicle alone (0.5% CMC–0.1% Tween80) or INT-747 (30mg/kg). Liver mRNA levels were measured by real-time quantitative PCR. Values were expressed relative to those in the fasting state, arbitrarily set to 1. **B**, *L-PK* mRNA expression in IHH incubated in a medium containing low (1mM) or high (11mM) glucose concentrations and vehicle (DMSO) or GW4064 (5 μ M), WAY362450 (5 μ M), INT-747 (10 μ M) or Chenodeoxycholic Acid (CDCA, 100 μ M) for 24h. **C**, Effect of FXR silencing in IHH at low (1mM) or high (11mM) glucose concentrations and vehicle (DMSO) or GW4064 (5 μ M) for 24h on *L-PK* mRNA (*Top panel*) and FXR protein (*Bottom panel*) levels. *L-PK* and control *36B4* mRNA levels were measured by real-time quantitative PCR and values were expressed relative to those at low glucose concentration with vehicle, arbitrarily set to 1. Total protein was extracted and analyzed as indicated in the ‘Materials and Methods’. FXR protein level was quantified by densitometry and normalized to actin protein level. **D**, Schematic representation of the L4L3 (-169/-125) region of the human *L-PK* promoter that contains the ChREBP (ChORE) and HNF4 α (DR1) binding sites. **E**, Activity of the L4L3 region of the *L-PK* promoter in IHH cells transfected with pGL3-TK-(L4L3)-LPK and incubated in medium containing low (1mM) or high (11mM) glucose concentrations and vehicle (DMSO) or GW4064 (5 μ M) for 24h. Values were normalized to β -galactosidase activity and were expressed relative to those of pGL3-TK-(L4L3)-LPK and pCMV-Sport6 (B) or pcDNA3

(C) or to those of pGL3-TK-(L4L3)-LPK with vehicle at low glucose concentration (D), which were arbitrarily set to 1.

Figure 2. FXR physically interacts with ChREBP and HNF4 α *in vitro*.

A, ChREBP protein levels in nuclear extracts from IHH incubated in a medium containing low (1mM) or high (11mM) glucose concentrations and vehicle (DMSO) or GW4064 (5 μ M) for 24 hours. The expression of ChREBP protein was analyzed by Western blot using a specific antibody. **B**, *In vitro* GST pull-down experiments using full length GST-FXR and *in vitro* transcribed/translated (TNT) ChREBP, Mlx or HNF4 α in presence of [³⁵S]-methionine. **C**, *In vitro* GST pull-down experiments using GST (lane1) and the indicated FXR deletion mutants (lanes3&4) FXR protein and TNT ChREBP in presence of [³⁵S]-methionine and vehicle (DMSO) or FXR ligand (GW4064, 5 μ M). AF-1 : Activation Function-1 ; DBD : DNA Binding Domain ; LBD : Ligand Binding Domain. **D**, Co-immunoprecipitation of ChREBP using two distinct anti-FXR antibodies in nuclear extracts from IHH cells transfected with pSG5-FXR, pCMV-Sport6-ChREBP and pcDNA3-Mlx and incubated in a medium containing high (11mM) glucose concentration. The expression of FXR and ChREBP proteins was detected 24h after transfection by Western blot using specific antibodies.

Figure 3. FXR activation at high glucose concentrations releases ChREBP, but not FXR and HNF4 α from the L4L3 region of the *L-PK* promoter.

Relative *L-PK* promoter occupancy by ChREBP, HNF4 α and FXR on the L4L3 region. The occupancy was evaluated by quantitative PCR in CHIP experiments performed using total extracts from IHH incubated in a medium containing low (1mM) or high

(11mM) glucose concentrations and vehicle (DMSO) or GW4064 (5 μ M) for 5h. Occupancies were expressed relative to those at low glucose concentration with vehicle, arbitrarily set to 1. Each experiment was performed at least 3 times and results are the average of these experiments.

Figure 4. FXR activation leads to the release of co-activators p300 and CBP and the recruitment of the co-inhibitor SMRT on the L4L3 region at high glucose concentration.

A, Relative *L-PK* promoter occupancy by p300 and CBP on the L4L3 region. **B**, Effect of SMRT gene silencing in IHH transfected with specific siRNAs and incubated at low (1mM) or high (11mM) glucose concentration and vehicle (DMSO) or GW4064 (5 μ M) for 24h on *L-PK* mRNA (*left panel*) and SMRT protein (*right panel*) levels. Proteins were extracted and analyzed as indicated in 'Materials and Methods'. SMRT protein level was quantified by densitometry and normalized to actin protein level. **C**, *In vitro* GST pull-down experiments using full length GST-SMRT and TNT FXR in presence of ³⁵S methionin. **D**, Relative *L-PK* promoter occupancy by SMRT. **E**, Effect of TSA treatment on *L-PK* gene expression in IHH at low (1mM) or high (11mM) glucose concentration and vehicle (DMSO) or GW4064 (5 μ M) for 24h. **F**, Relative H3K9 acetylation of the *L-PK*. For A, D&F, the occupancy was evaluated by quantitative PCR in ChIP experiments performed using total extracts from IHH incubated in a medium containing low (1mM) or high (11mM) glucose concentrations and vehicle (DMSO) or GW4064 (5 μ M) for 5h. Occupancies were then expressed relative to those at low glucose concentration with vehicle, arbitrarily set to 1. Each experiment was performed at least 3 times and results are the average of these experiments. For B(*left panel*)&E, *L-PK* and control *36B4*

mRNA levels were measured by real-time quantitative PCR. The values were expressed relative to those at low glucose concentration with vehicle, which were arbitrarily set to 1.

Figure 5. The expression of other ChREBP-regulated genes is also inhibited by FXR by a similar mechanism as for *L-PK*.

A, mRNA expression of ChREBP-target genes in IHH incubated in a medium containing low (1mM) or high (11mM) glucose concentrations and vehicle (DMSO) or GW4064 (5 μ M) for 24h. Gene and control *36B4* mRNA levels were measured by real-time quantitative PCR. Values were expressed relative to those measured at low glucose concentration with vehicle, arbitrarily set to 1. **B**, Relative *TxNIP* promoter occupancy by ChREBP, HNF4 α and FXR (top panels), p300 and CBP (middle panel) and SMRT and H3K9 (bottom panels). The occupancy was evaluated by quantitative PCR amplification of the region of the *TxNIP* promoter that contains the ChORE in ChIP experiments performed using total extracts from IHH at low (1mM) or high (11mM) glucose concentrations and vehicle (DMSO) or GW4064 (5 μ M) for 5h. Occupancies were expressed relative to those at low glucose concentration with vehicle, arbitrarily set to 1. Each experiment was performed at least three times and results from a representative experiment were shown.

Figure 6. FXR inhibits the expression of glucose-induced genes.

mRNA expression of genes identified as being regulated by both glucose and FXR using microarray analysis in IHH incubated in a medium containing low (1mM) or high (11mM) glucose concentrations and vehicle (DMSO) or GW4064 (5 μ M) for 24h. HK3 :

hexokinase 3 ; PGM1 : phosphoglucomutase 1 ; TPI1 : triosephosphate isomerise 1 ; PGAM1 : phosphoglycerate mutase 1 ; ACSS1 : acyl-CoA synthetase short-chain family member 1 ; LDHA : lactate deshydrogenase A. Gene and control 36B4 mRNA levels were measured by real-time quantitative PCR. The values were expressed relative to those at low glucose concentration with vehicle, which were arbitrarily set to 1.

Figure 7. Model of transrepression of glucose-induced *L-PK* gene expression by FXR.

A, At high glucose concentrations without FXR activation, ChREBP and HNF4 α are bound on the L4L3 region of the *L-PK* promoter and trans-activate gene expression, in part due to the recruitment of the transcriptional co-activators p300 and CBP. FXR is integrated into this protein complex, probably through its direct interaction with ChREBP and HNF4 α . **B**, At high glucose concentration after FXR activation, ChREBP, as well as p300 and CBP, are released from the *L-PK* promoter. FXR and HNF4 α are still bound on the *L-PK* promoter. Tethered to the promoter through its interaction with HNF4 α , FXR recruits transcriptional co-inhibitor SMRT and represses the transcription through the recruitment of HDACs and deacetylation of H3 histones.

REFERENCES

1. **Bouché C, Serdy S, Kahn CR, Goldfine AB.** 2004. The cellular fate of glucose and its relevance in type 2 diabetes. *Endocr. Rev.* **25**:807-830.
2. **Nordlie RC, Foster JD, Lange AJ.** 1999. Regulation of glucose production by the liver. *Annu. Rev. Nutr.* **19**:379-406.
3. **Girard J, Ferré P, Foufelle F.** 1997. Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. *Annu. Rev. Nutr.* **17**:325-352.
4. **de Luis O, Valero MC, Jurado LA.** 2000. WBSCR14, a putative transcription factor gene deleted in Williams-Beuren syndrome: complete characterisation of the human gene and the mouse ortholog. *Eur. J. Hum. Genet.* **8**:215-222.
5. **Yamashita H, Takenoshita M, Sakurai M, Bruick RK, Henzel WJ, Shillinglaw W, Arnot D, Uyeda K.** 2001. A glucose-responsive transcription factor that regulates carbohydrate metabolism in the liver. *Proc. Natl. Acad. Sci. U.S.A.* **98**:9116-9121.
6. **Ma L, Tsatsos NG, Towle HC.** 2005. Direct role of ChREBP.Mlx in regulating hepatic glucose-responsive genes. *J. Biol. Chem.* **280**:12019-12027.
7. **Iizuka K, Bruick RK, Liang G, Horton JD, Uyeda K.** 2004. Deficiency of carbohydrate response element-binding protein (ChREBP) reduces lipogenesis as well as glycolysis. *Proc. Natl. Acad. Sci. U.S.A.* **101**:7281-7286.
8. **Ishii S, Iizuka K, Miller BC, Uyeda K.** 2004. Carbohydrate response element binding protein directly promotes lipogenic enzyme gene transcription. *Proc. Natl. Acad. Sci. U.S.A.* **101**:15597-15602.
9. **Kawaguchi T, Takenoshita M, Kabashima T, Uyeda K.** 2001. Glucose and cAMP regulate the L-type pyruvate kinase gene by phosphorylation/dephosphorylation of the

carbohydrate response element binding protein. *Proc. Natl. Acad. Sci. U.S.A.* **98**:13710-13715.

10. **Kabashima T, Kawaguchi T, Wadzinski BE, Uyeda K.** 2003. Xylulose 5-phosphate mediates glucose-induced lipogenesis by xylulose 5-phosphate-activated protein phosphatase in rat liver. *Proc. Natl. Acad. Sci. U.S.A.* **100**:5107-5112.

11. **Dentin R, Tomas-Cobos L, Foufelle F, Leopold J, Girard J, Postic C, Ferré P.** 2012. Glucose 6-phosphate, rather than xylulose 5-phosphate, is required for the activation of ChREBP in response to glucose in the liver. *J. Hepatol.* **56**:199-209.

12. **Li MV, Chen W, Harmancey RN, Nuotio-Antar AM, Imamura M, Saha P, Taegtmeyer H, Chan L.** 2010. Glucose-6-phosphate mediates activation of the carbohydrate responsive binding protein (ChREBP). *Biochem. Biophys. Res. Commun.* **395**:395-400.

13. **Diaz-Moralli S, Ramos-Montoya A, Marin S, Fernandez-Alvarez A, Casado M, Cascante M.** 2012. Target metabolomics revealed complementary roles of hexose- and pentose-phosphates in the regulation of carbohydrate-dependent gene expression. *Am. J. Physiol. Endocrinol. Metab.* **303**:E234-42.

14. **Bricambert J, Miranda J, Benhamed F, Girard J, Postic C, Dentin R.** 2010. Salt-inducible kinase 2 links transcriptional coactivator p300 phosphorylation to the prevention of ChREBP-dependent hepatic steatosis in mice. *J. Clin. Invest.* **120**:4316-4331.

15. **Guinez C, Filhoulaud G, Rayah-Benhamed F, Marmier S, Dubuquoy C, Dentin R, Moldes M, Burnol A, Yang X, Lefebvre T, Girard J, Postic C.** 2011. O-GlcNAcylation increases ChREBP protein content and transcriptional activity in the liver. *Diabetes* **60**:1399-1413.

16. **Sakiyama H, Fujiwara N, Noguchi T, Eguchi H, Yoshihara D, Uyeda K, Suzuki K.** 2010. The role of O-linked GlcNAc modification on the glucose response of ChREBP. *Biochem. Biophys. Res. Commun.* **402**:784-789.
17. **Adamson AW, Suchankova G, Rufo C, Nakamura MT, Teran-Garcia M, Clarke SD, Gettys TW.** 2006. Hepatocyte nuclear factor-4alpha contributes to carbohydrate-induced transcriptional activation of hepatic fatty acid synthase. *Biochem. J.* **399**:285-295.
18. **Cha J, Repa JJ.** 2007. The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. *J. Biol. Chem.* **282**:743-751.
19. **Lefebvre P, Cariou B, Lien F, Kuipers F, Staels B.** 2009. Role of bile acids and bile acid receptors in metabolic regulation. *Physiol. Rev.* **89**:147-191.
20. **Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B.** 1999. Identification of a nuclear receptor for bile acids. *Science* **284**:1362-1365.
21. **Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, Lehmann JM.** 1999. Bile acids: natural ligands for an orphan nuclear receptor. *Science* **284**:1365-1368.
22. **Wang H, Chen J, Hollister K, Sowers LC, Forman BM.** 1999. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol. Cell* **3**:543-553.
23. **Lambert G, Amar MJA, Guo G, Brewer HBJ, Gonzalez FJ, Sinal CJ.** 2003. The farnesoid X-receptor is an essential regulator of cholesterol homeostasis. *J. Biol. Chem.* **278**:2563-2570.

24. **Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, Gonzalez FJ.** 2000. Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* **102**:731-744.
25. **Cariou B, van Harmelen K, Duran-Sandoval D, van Dijk T, Grefhorst A, Bouchaert E, Fruchart J, Gonzalez FJ, Kuipers F, Staels B.** 2005. Transient impairment of the adaptive response to fasting in FXR-deficient mice. *FEBS Lett.* **579**:4076-4080.
26. **Cariou B, van Harmelen K, Duran-Sandoval D, van Dijk TH, Grefhorst A, Abdelkarim M, Caron S, Torpier G, Fruchart J, Gonzalez FJ, Kuipers F, Staels B.** 2006. The farnesoid X receptor modulates adiposity and peripheral insulin sensitivity in mice. *J. Biol. Chem.* **281**:11039-11049.
27. **Ma K, Saha PK, Chan L, Moore DD.** 2006. Farnesoid X receptor is essential for normal glucose homeostasis. *J. Clin. Invest.* **116**:1102-1109.
28. **Prawitt J, Abdelkarim M, Stroeve JHM, Popescu I, Duez H, Velagapudi VR, Dumont J, Bouchaert E, van Dijk TH, Lucas A, Dorchies E, Daoudi M, Lestavel S, Gonzalez FJ, Oresic M, Cariou B, Kuipers F, Caron S, Staels B.** 2011. Farnesoid X receptor deficiency improves glucose homeostasis in mouse models of obesity. *Diabetes* **60**:1861-1871.
29. **Duran-Sandoval D, Mautino G, Martin G, Percevault F, Barbier O, Fruchart J, Kuipers F, Staels B.** 2004. Glucose regulates the expression of the farnesoid X receptor in liver. *Diabetes* **53**:890-898.
30. **Duran-Sandoval D, Cariou B, Percevault F, Hennuyer N, Grefhorst A, van Dijk TH, Gonzalez FJ, Fruchart J, Kuipers F, Staels B.** 2005. The farnesoid X receptor

modulates hepatic carbohydrate metabolism during the fasting-refeeding transition. *J. Biol. Chem.* **280**:29971-29979.

31. **Samanez CH, Caron S, Briand O, Dehondt H, Duplan I, Kuipers F, Hennuyer N, Clavey V, Staels B.** 2012. The human hepatocyte cell lines IHH and HepaRG: models to study glucose, lipid and lipoprotein metabolism. *Arch. Physiol. Biochem.* **118**:102-111.

32. **Chomczynski P, Sacchi N.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156-159.

33. **Gripon P, Rumin S, Urban S, Le Seyec J, Glaise D, Cannie I, Guyomard C, Lucas J, Trepo C, Guguen-Guillouzo C.** 2002. Infection of a human hepatoma cell line by hepatitis B virus. *Proc. Natl. Acad. Sci. U.S.A.* **99**:15655-15660.

34. **Schippers IJ, Moshage H, Roelofsen H, Müller M, Heymans HS, Ruiters M, Kuipers F.** 1997. Immortalized human hepatocytes as a tool for the study of hepatocytic (de-)differentiation. *Cell Biol. Toxicol.* **13**:375-386.

35. **Caron S, Verrijken A, Mertens I, Samanez CH, Mautino G, Haas JT, Duran-Sandoval D, Prawitt J, Francque S, Vallez E, Muhr-Tailleux A, Berard I, Kuipers F, Kuivenhoven JA, Biddinger SB, Taskinen M, Van Gaal L, Staels B.** 2011. Transcriptional activation of apolipoprotein CIII expression by glucose may contribute to diabetic dyslipidemia. *Arterioscler. Thromb. Vasc. Biol.* **31**:513-519.

36. **Livak KJ, Schmittgen TD.** 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{(-Delta Delta C(T))} Method. *Methods* **25**:402-408.

37. **Pellicciari R, Fiorucci S, Camaioni E, Clerici C, Costantino G, Maloney PR, Morelli A, Parks DJ, Willson TM.** 2002. 6alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J. Med. Chem.* **45**:3569-3572.

38. **Yamada K, Noguchi T.** 1999. Nutrient and hormonal regulation of pyruvate kinase gene expression. *Biochem. J.* **337 (Pt 1)**:1-11.
39. **Bergot MO, Diaz-Guerra MJ, Puzenat N, Raymondjean M, Kahn A.** 1992. Cis-regulation of the L-type pyruvate kinase gene promoter by glucose, insulin and cyclic AMP. *Nucleic Acids Res.* **20**:1871-1877.
40. **Burke SJ, Collier JJ, Scott DK.** 2009. cAMP prevents glucose-mediated modifications of histone H3 and recruitment of the RNA polymerase II holoenzyme to the L-PK gene promoter. *J. Mol. Biol.* **392**:578-588.
41. **Cha-Molstad H, Saxena G, Chen J, Shalev A.** 2009. Glucose-stimulated expression of Txnip is mediated by carbohydrate response element-binding protein, p300, and histone H4 acetylation in pancreatic beta cells. *J. Biol. Chem.* **284**:16898-16905.
42. **Watson PJ, Fairall L, Schwabe JWR.** 2012. Nuclear hormone receptor co-repressors: structure and function. *Mol. Cell. Endocrinol.* **348**:440-449.
43. **Perttilä J, Huaman-Samanez C, Caron S, Tanhuanpää K, Staels B, Yki-Järvinen H, Oikkonen VM.** 2012. PNPLA3 is regulated by glucose in human hepatocytes, and its I148M mutant slows down triglyceride hydrolysis. *Am. J. Physiol. Endocrinol. Metab.* **302**:E1063-9.
44. **Pang S, Hsieh W, Chuang C, Chao C, Weng W, Juang H.** 2009. Thioredoxin-interacting protein: an oxidative stress-related gene is upregulated by glucose in human prostate carcinoma cells. *J. Mol. Endocrinol.* **42**:205-214.
45. **Parviz F, Matullo C, Garrison WD, Savatski L, Adamson JW, Ning G, Kaestner KH, Rossi JM, Zaret KS, Duncan SA.** 2003. Hepatocyte nuclear factor 4alpha controls

the development of a hepatic epithelium and liver morphogenesis. *Nat. Genet.* **34**:292-296.

46. **Gonzalez FJ.** 2008. Regulation of hepatocyte nuclear factor 4 alpha-mediated transcription. *Drug Metab. Pharmacokinet.* **23**:2-7.

47. **Gineste R, Sirvent A, Paumelle R, Helleboid S, Aquilina A, Darteil R, Hum DW, Fruchart J, Staels B.** 2008. Phosphorylation of farnesoid X receptor by protein kinase C promotes its transcriptional activity. *Mol. Endocrinol.* **22**:2433-2447.

48. **Pascual G, Fong AL, Ogawa S, Gamliel A, Li AC, Perissi V, Rose DW, Willson TM, Rosenfeld MG, Glass CK.** 2005. A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-gamma. *Nature* **437**:759-763.

49. **Li M, Yang X.** 2011. A Retrospective on Nuclear Receptor Regulation of Inflammation: Lessons from GR and PPARs. *PPAR Res* **2011**:742785.

50. **Jeong Y, Kim D, Lee YS, Kim H, Han J, Im S, Chong HK, Kwon J, Cho Y, Kim WK, Osborne TF, Horton JD, Jun H, Ahn Y, Ahn S, Cha J.** 2011. Integrated expression profiling and genome-wide analysis of ChREBP targets reveals the dual role for ChREBP in glucose-regulated gene expression. *PLoS One* **6**:e22544.