Coordinated control of cholesterol catabolism to bile acids and of gluconeogenesis via a novel mechanism of transcription regulation linked to the fasted-to-fed cycle

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Running title:
Bile acids affect HNF-4α/coactivator complex

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Summary

Bile acid metabolism plays an essential role in cholesterol homeostasis and is critical for the initiation of atherosclerotic disease. However, in spite of the recent advances, the molecular mechanisms whereby bile acids regulate gene transcription and cholesterol homeostasis in mammals still need further investigations. Here, we show that bile acids suppress transcription of the gene (CYP7A1) encoding cholesterol 7α-hydroxylase, the rate-limiting enzyme in bile acid biosynthesis, also through an unusual mechanism not involving the bile acid nuclear receptor, farnesoid X receptor (FXR). By performing cell-based reporter assays, protein-protein interaction and chromatin immunoprecipitation (ChIP) assays, we demonstrate that bile acids impair the recruitment of PPARγ-coactivator-1α (PGC-1α) and CREB-binding protein (CBP) by hepatocyte nuclear factor-4α (HNF-4α), a master regulator of CYP7A1. We also show for the first time that bile acids inhibit transcription of the gene (PEPCK) encoding phosphoenolpyruvate carboxykinase, the rate-limiting enzyme in gluconeogenesis, through the same FXR-independent mechanism. ChIP assay reveals that bile acid-induced dissociation of coactivators from HNF-4α decreases the recruitment of RNA polymerase II on the core promoter and downstream in the 3′-untranslated regions of these two genes, reflecting the reduction of gene transcription. Finally, we found that Cyp7a1 expression is stimulated in fasted mice in parallel to Pepck whereas the same genes are repressed by bile acids. Collectively, these results reveal a novel regulatory mechanism that controls gene transcription in response to extracellular stimuli and argue that the transcriptional regulation by bile acids of genes central to cholesterol and glucose metabolism should be viewed dynamically in the context of the fasted-to-fed cycle.
Introduction

A fundamental problem in biology is understanding the mechanisms by which extracellular stimuli transduce their signal to the nucleus and affect gene transcription. Genes are expressed in temporal and spatial pattern during development and can be regulated by hormonal and dietary stimuli or by pathogenic factors, thus contributing to disease initiation and progression. The appreciation of such mechanisms can help to unravel disease pathogenesis or even disclose targets for novel therapeutic interventions. A typical example is the mechanism whereby bile acids regulate their own synthesis by acting on transcription of the gene encoding cholesterol 7α-hydroxylase (CYP7A1), the rate-limiting enzyme of the so-called “classic” pathway (1,2). CYP7A1 has been implicated in genetic susceptibility to atherosclerosis (3,4), since it controls the main route whereby cholesterol is removed from the body in mammals (2,5). The most relevant type of regulation of CYP7A1 is the feedback that bile acids exert on the expression of this gene (2). The complete understanding of such mechanisms represents an opportunity to describe unusual regulatory circuits controlling gene transcription and is of great value for potential biomedical applications in the design of new generations of drugs affecting cholesterol metabolism. Stroup et al. (6) first identified a Bile Acid Responsive Element (BARE) located at nt –149/-128 of CYP7A1, which contains hormone response element-like sequences recognized by several members of the nuclear receptor superfamily, such as hepatocyte nuclear factor 4α (HNF-4α, NR2A1), chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII, NR2F2) and α-fetoprotein transcription factor (FTF), also known as CPF or LRH-1 (FTF, NR5A2) (7-10). The recent discovery of the bile acid receptor, farnesoid X receptor (FXR/BAR, NR1H4) (8,11-14), allowed an elegant model to be developed to explain the mechanisms of feedback regulation of CYP7A1 by bile acids (9,10,15). However, recent data from independent
laboratories have shown that FXR, SHP (NR0B2) and FTF are not the only nuclear receptors that are responsible. At this regard, for instance, we showed that bile acids inhibit the transcription of CYP7A1 by repressing the transactivation potential of HNF-4α, a strong activator of CYP7A1, via a mitogen-activated protein kinase (MAPK)-dependent signaling cascade (16). Furthermore, by using Shp+/− mice models Wang et al. (17) and Kerr et. al (18) have recently demonstrated that bile acids can repress CYP7A1 transcription in a SHP-independent fashion, thus corroborating the observations made in in vitro models. These results raise the question on the molecular events underlying the additional regulatory mechanisms of CYP7A1 transcription by bile acids through the nuclear receptor HNF-4α. A possible scenario could lie in the bile acid-induced dissociation of the HNF-4α/coactivator complex. Nuclear receptors and transcription factors recruit coactivators to assemble the preinitiation complex efficiently on the core promoter of specific genes (19). Among the candidate coactivators possibly affected by bile acid signaling, we considered PPARγ Coactivator-1α (PGC-1α) and cAMP Response Element Binding Protein (CREB)-Binding Protein (CBP). PGC-1α and CBP interact tightly with and co-activate HNF-4α. The association of PGC-1α with HNF-4α is also a key event in the regulation of the gene encoding the gluconeogenic enzyme PEPCK in response to the glucagon/cAMP cascade (20,21).

Since HNF-4α is a key sensor of bile acid concentration in liver cells (16), we tested the hypothesis that bile acids could affect the association of HNF-4α with these transcription coactivators.

In what follows we show for the first time that bile acids suppress the transcription of CYP7A1 by blocking the association of HNF-4α with the coactivators PGC-1α and CBP. Furthermore, we found that PEPCK, a target of the HNF-4α/PGC-1α complex that mediates the response to starvation (21), is subject to the same regulation by bile acids in vitro and in vivo. We also
provide strong evidence that Cyp7a1 is stimulated in parallel with Pepck in fasted mice. This work reveals bile acids as key regulators of glucose and lipid metabolism.
**Experimental Procedures**

*Plasmid Constructs*

The expression vector pcDNA3 containing the human FXR and the reporter plasmid containing two copies of the FXRE in front of the SV40 minimal promoter (pGL3-Promoter, Promega, USA) were kindly donated by Dr. Krister Bamberg (AstraZeneca, Mölndal, Sweden). The plasmid ph-371luc, bearing the human CYP7A1 promoter/luciferase fusion gene, was described elsewhere (22). The antibody against HNF-4α, the expression plasmid for HA-tagged CBP, SRC-1, TIF2, for the Gal4 rat HNF-4α chimera (pBX-HNF-4) and its mutant 130-455 lacking the N-terminal region of the receptor and Gal4-VP16 (pBX-VP16) were generous gifts from Dr. Iannis Talianidis (Institute of Molecular Biology and Biotechnology, Herakleion, Crete, Greece). The plasmid pBX-HNF-4/1-249 carrying the N-terminal portion of HNF-4α fused to the Gal4 DNA-binding domain has already been described (16). The plasmid pGL3-PEPCK-Luciferase and its mutants in the AF1, AF2 and AF3 sequences were generous gifts from Dr. Daryl Granner (Vanderbilt University School of Medicine, Nashville, Tennessee, USA). The expression vector for the HA-tagged human PGC-1α in pcDNA3 was donated by Dr. Anastasia Kralli (University of Basel, Switzerland). The plasmid pFlag-HNF-4α was prepared by standard PCR methods and cloned into the pcDNA3 vector.

*Cell Cultures and Transient Transfection Assays*

HepG2 cells were maintained in DMEM/F12 medium supplemented with 10% heat inactivated dextran-charcoal-stripped fetal calf serum. Cells were transfected by the calcium phosphate coprecipitation technique as previously described (16). Typically, coprecipitates contained 100 ng of reporter plasmid, 50 ng of receptor vector, 250 ng of coactivator expression vector or an
equivalent amount of empty carrier vector (pcDNA3) and 300 ng of pCMVβ (Clontech, Palo Alto, California, USA) in 24-well plates. Transfected cells were incubated for 16 h in serum-free medium in the presence of the 25 μM CDCA or 10 μM FXR ligand GW4064 (kindly provided by Dr. Krister Bamberg) or an equivalent amount of vehicle (0.1% ethanol or DMSO, respectively). In some experiments, transfected cells were also treated with 1 mM 8-Bromo-cAMP for 8 hours. As a negative control for the specificity of CDCA effect on gene transcription we also performed experiments treating transfected cells with 25 μM ursodeoxycholic acid (UDCA). Luciferase and β-galactosidase assays were performed as described previously (16) and are expressed as mean (± standard deviation) of triplicate samples. Each experiment was performed at least three times.

Reverse Transcription Real Time Quantitative PCR

HepG2 cultures were treated with the indicated combinations of 25 μM CDCA and 1 mM 8-Bromo cAMP for 8 hours in serum-free medium. Triplicate samples were pooled and total RNA was extracted with the Absolutely RNA RT-PCR Miniprep Kit (Stratagene, La Jolla, California, USA) according to the manufacturer’s instructions. About 1 μg of total RNA from each sample was reverse transcribed with Superscript II following the manufacturer’s instructions (Invitrogen srl, Milano, Italia) and aliquots of the cDNAs (corresponding to about 10 ng of the original RNA) were subjected to real time quantitative PCR with SYBR Green kit (Qiagen SpA Milano, Italia) following the manufacturer’s instructions to detect CYP7A1, PEPCK, HNF-4α, SHP, and PGC-1α mRNA. 18S rRNA was used as the housekeeping gene for sample normalization and was amplified in separate wells within the same plate. Primers for real time PCR reactions were designed with Primer Express Software (Applied Biosystems, Monza-Milano, Italy) and optimized to work in a two-step protocol (denaturation at 95°C for 15 seconds, annealing-
extension at 60°C for 60 seconds, 40 cycles). The oligonucleotides used for real time PCR were synthesized by Eurogentec (Seraing, Belgium) and their sequences are available upon request. The specificity of the amplified products was monitored by performing melting curves at the end of each amplification. All the amplicons generated a single peak, thus reflecting the specificity of the primers. Experiments were repeated at least twice with different cell preparations.

**Co-immunoprecipitations and Western Blots**

HepG2 cultures were transfected with either pcDNA3-HNF-4 full length or pBX-HNF-4/1-249 or pBX-HNF-4/130-455 and either pcDNA3-HA-PGC-1α or pcDNA3-HA-CBP. Transfected cells were treated with 25 μM CDCA or 0.1% ethanol in serum-free medium for 8 hours. Cells were lysed in modified RIPA buffer (50 mM TrisHCl pH 7.4, 1% NP-40, 0.25% Na-deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM Na3VO4, 1 mM NaF, 1 μg aprotinin/ml, 1 μg leupeptin/ml, 1 μg pepstatin/ml, 1 Protease Inhibitor Cocktail tablet/10 ml). Cell lysates containing equivalent amounts of total proteins were precleared with protein-G Sepharose (Amersham Biosciences Europe, Milano, Italia) and then incubated with anti-HA monoclonal antibody (Roche Diagnostics SpA, Monza-Milano, Italia) for 3 h, followed by an overnight incubation with protein-G Sepharose. The beads were washed three times with the same buffer and once with 50 mM TrisHCl pH 8 and immunoprecipitates were analyzed by SDS-PAGE. For Western blot analysis, proteins were probed with anti-HNF-4α antibody (kindly provided by Dr. Iannis Talianidis) or antibodies against Gal4 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-HA antibody. Whole cell extracts were analyzed by SDS-PAGE followed by Western blot with anti-HNF-4α antibody to detect the total amount of HNF-4α with
ECL Plus kit (Amersham Biosciences Europe, Milano, Italia) according to the manufacturer’s instructions. Similar experiments performed without cotransfecting the HNF-4α expression plasmid and detecting the endogenous receptor, gave identical results. To study protein–protein interactions between PGC-1α or CBP and *in vitro* synthesized HNF-4α, lysates from cells transfected with the HA-tagged coactivators and treated with 25 μM CDCA or 0.1% ethanol were mixed with 20 μl of [35S]-methionine labeled Flag-HNF-4α for 4 hours, immunoprecipitated as described above, analyzed by SDS-PAGE and detected by autoradiography.

*Chromatin Immunoprecipitations*

HepG2 cells transfected with HA-PGC-1α plasmid were treated with 25 μM CDCA or vehicle (3 plates/group) for 12 hours in serum-free medium and chromatin was cross-linked with 1% formaldehyde for 10 min at room temperature. Cross-linking was stopped with 125 mM glycine, cells were washed with ice-cold phosphate-buffered saline (PBS), scraped and swollen on ice 10 minutes in PBS containing protease inhibitors (Roche, Milano, Italia). Cell extracts were prepared in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1, protease inhibitors). Extracts were sonicated with a microtip on ice to obtain DNA fragments ranging from 200 to 1000 bp (3 pulses for 30 s, power set to 5, in a Heat Systems sonicator). Soluble chromatin fragments were centrifuged, diluted 10 times with ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl), precleared with protein G-sepharose containing 200 μg sonicated salmon sperm DNA/ml and immunoprecipitated with the indicated antibodies for 14 hours at 4 °C. Immunocomplexes were captured on protein G-sepharose, washed sequentially with low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 250 mM NaCl, 0.5% Nonidet P-40), 2× SDS-PAGE sample buffer, boiled and analyzed by SDS-PAGE and detected byautoradiography.
X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), LiCl wash buffer (0.25 M LiCl, 1% NP40, 1% deoxycholate, 1 mM EDTA 10 mM Tris-HCl, pH 8.1) and twice with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8). Samples were eluted with freshly prepared 50 mM Tris-HCl, 1 mM EDTA, 1% SDS, pH 8.1 buffer at 65 °C for 10 minutes and, after adjusting the concentration of NaCl to 200 mM, cross-linking was reverted at 65 °C for 5 hours. Following treatment with 10 μg RNase A and 20 μg proteinase K/ml for 1 hour at 45 °C, genomic DNA fragments were extracted with phenol/chloroform and precipitated with ethanol. Specific genomic DNA fragments from immunoprecipitated samples and inputs were quantitated by real time PCR with SYBR Green as indicated above. As a negative control for the specificity of CDCA effect on recruitment of coactivators on CYP7A1 and PEPCK gene we also performed experiments treating HepG2 cells with 25 μM UDCA.

The antibodies used were:

anti-CBP, C-terminal (Upstate Biotechnology, Lake Placid, NY, USA)

anti-HA (Roche Diagnostics SpA, Monza-Milano, Italia)

anti-RNA polymerase II (Santa Cruz Biotechnology, Santa Cruz, CA, USA)

Primer sets were designed to amplify the following human genomic DNA regions:

CYP7A1 BARE (from nt -252 to nt –113 relative to the cap site)

CYP7A1 upstream region (from nt –1935 to nt –1833 relative to the cap site)

CYP7A1 TATA box (from nt -90 to nt +26 relative to the cap site)

CYP7A1 3’-UTR (from nt +2009 to nt +2141 relative to the cap site)

PEPCK HRU (from nt –518 to nt -263 relative to the cap site)

PEPCK upstream region (from nt -1318 to nt -1236 relative to the cap site)

PEPCK TATA box (from nt -77 to nt +72 relative to the cap site)
**PEPCK 3’-UTR** (from nt +2298 to nt +2408 relative to the cap site)

Primer sequences are available upon request.

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*Animal studies*

Male mice (C57BL/6J, 8 week old, 5 to 9 animals per group) were maintained on normal light cycle (7:00 am-7:00 pm) on standard chow diet or treated with diet supplemented with 1% cholic acid for 8 days. Both groups had free access to food and water. To test the effect of fast on gene expression, mice under standard chow diet were starved for 15 hours and compared to mice fed the same diet. Both groups had free access to water. Livers were collected between 9:00 and 10:00 am and total RNA was extracted with RNeasy midi kit (Qiagen) and treated with RNase-free DNase to remove possible contaminating genomic DNA following the manufacturer’s instructions. Specific mRNA quantitation was performed by real time quantitative PCR as described above. Experiments were conducted in accordance of the Animal Research Regulations of the Università degli Studi di Milano and of the policy of the European Commission.

*Statistical analyses*

Statistical analyses were performed with Student’s *t* test, setting the significance at *P*<0.05.
Results

Bile acids repress the HNF-4α transcriptional activity and CYP7A1 expression via an FXR-independent pathway

We have previously shown (16) that bile acids repress the transcription of CYP7A1 by affecting the transactivation potential of the liver-enriched orphan nuclear receptor HNF4α via a MAPK-dependent pathway. We wanted to verify whether the inhibitory effect of bile acids on HNF-4α activity is secondary to the initiation of the FXR/SHP cascade. To this end, we used a Gal4-based assay in HepG2 cells with the DNA-binding domain (DBD) of the yeast transcription factor Gal4 fused with HNF-4α and observed that chenodeoxycholic acid (CDCA) repressed the activity of this nuclear receptor (Figure 1A, left). Surprisingly, the FXR agonist GW4064 (23) did not affect the transactivation potential of HNF-4α (Figure 1A, left). The specificity of this effect was assessed in a similar assay with the Gal4-VP16 construct, which was affected neither by CDCA nor GW4064 (Figure 1A right). Control experiments showed that CDCA and GW4064 did activate the FXR-dependent transcription (Figure 1B) and repress the transcription of CYP7A1 (Figure 1C). In parallel transfection assays the more hydrophilic bile acid UDCA did not affect the transcription of CYP7A1 or the activity of Gal4-HNF-4α (data not shown). These results establish that bile acids downregulate CYP7A1 transcription via both FXR-dependent and -independent pathways, the latter typically involving the nuclear receptor HNF-4α.

Bile acids repress CYP7A1 transcription by targeting the HNF-4α/PGC-1α complex

To assess how bile acids affect the transcription machinery of CYP7A1 we studied the role of transcription coactivators. We hypothesized that bile acids affect the transcription machinery of CYP7A1 by impairing the formation of the HNF-4α/coactivator complex. As a candidate coactivator involved in this process we considered PGC-1α, a stress-inducible coactivator.
strongly interacting with HNF-4α, which is implicated in the transcription regulation of genes playing a key role in liver, skeletal muscle and adipose tissue metabolism (21,24,25). We asked whether bile acids affect the recruitment of PGC-1α by HNF-4α on CYP7A1 promoter when they reach threshold concentration in hepatocytes. To test the hypothesis we studied protein–protein interaction by co-immunoprecipitation experiments. HepG2 cells were transfected with an expression vector for hemagglutinin (HA)-tagged PGC-1α and treated with CDCA or vehicle; whole cell extracts were immunoprecipitated with a monoclonal antibody against HA. Captured complexes were analyzed by SDS-PAGE and Western blot with an antibody against HNF-4α to evaluate the effect of CDCA on the association between PGC-1α and HNF-4α. CDCA strongly hindered the physical association of HNF-4α with PGC-1α (Figure 2A upper panel). Control experiments show that CDCA did not change the protein levels of HA-PGC-1α and HNF-4α (Figure 2A middle and lower panel). As expected from previous data (21), when the immunoprecipitation was performed with the N-terminal portion of HNF-4α no interaction was detected (Figure 2B), whereas PGC-1α interacted with the C-terminal part of HNF-4α and CDCA prevented this interaction. To further assess whether bile acids exert their action at the level of HNF-4α or other cofactors, we modified the co-immunoprecipitation experiment by adding in vitro synthesized HNF-4α to HepG2 cell extracts. In this case we observed no change in HNF-4α/PGC-1α interaction with lysates from HepG2 cells treated with CDCA (Figure 2C), which suggests that bile acids may elicit a structural or post-translational modification of the nuclear receptor that alters its affinity to PGC-1α.

We next tested in transient transfection assays the functional relevance of the inhibition of HNF-4α/PGC-1α interaction by bile acids. HepG2 cells were transfected with the human CYP7A1 promoter/luciferase chimeric gene (22) and then challenged with CDCA, 8-bromo-
cAMP, a stable analog of cyclic AMP, or a combination of the two stimuli. CDCA strongly repressed the activity of the human CYP7A1 promoter (Figure 2D). In contrast, cAMP strongly stimulated the transcription of luciferase driven by CYP7A1 promoter (Figure 2D). However, when transfected HepG2 cells were incubated in the presence of both stimuli, CDCA failed to repress the promoter activity of the CYP7A1 construct (Figure 2D). The SV40-driven luciferase gene did not respond to any of the tested stimuli. These observations suggested that cAMP, by inducing the expression of PGC-1α (see below Figure 4C), may stimulate the transcription of CYP7A1 and overcome the bile acid-mediated downregulation of CYP7A1 promoter activity caused by the disruption of the HNF-4α/PGC-1α complex.

To confirm this hypothesis, we measured the transcription output of the CYP7A1 promoter/luciferase gene cotransfected with the expression vector for PGC-1α in the presence or absence of bile acids. As expected, CDCA robustly depressed the CYP7A1 promoter-driven transcription of the luciferase reporter gene (Figure 2E). However, the ectopic expression of PGC-1α prevented the effect of CDCA on CYP7A1 transcription (Figure 2E) and stimulated the basal promoter activity of CYP7A1, consistently with the previous experiment showing a stimulation of CYP7A1 promoter in the presence of cAMP. To assess whether PGC-1α mediates these effects directly on HNF-4α, we performed Gal4-based assays, cotransfecting the PGC-1α expression vector. CDCA repressed the transcription activity of the Gal4-HNF4 full-length protein (Figure 2F upper left). The overexpression of PGC-1α boosted the Gal4-HNF-4-dependent transcription and, most importantly, completely abolished the effect of bile acids (Figure 2F upper left). However, PGC-1α failed to stimulate the basal transcription activity of the C-terminal deletion Gal4-HNF-4/1-249, which lacks the interface for the interaction with coactivators recruited by the ligand-binding domain (LBD) (21), and did not prevent the
repression by CDCA (Figure 2F upper right). Conversely, CDCA inhibited the activity of the N-terminal deletion of HNF-4[], Gal4-HNF-4/130-455, whereas the overexpression of PGC-1[] prevented the effect of the bile acid on the transcriptional activity of the receptor and stimulated the basal activity of the truncated receptor (Figure 2F lower left). This result is consistent with the ability of the LBD of the receptor to interact with PGC-1[] (21). Taken together, these experiments indicate that bile acids repress the transcription of CYP7A1 by impairing the protein-protein interaction of PGC-1[] with the LBD of HNF-4[], thus preventing the assembly of factors that promote the transcription initiation of CYP7A1. These data also suggest that two regions of HNF-4[] could bestow the responsiveness of the receptor to bile acids, the C-terminal domain being PGC-1[]-dependent and the N-terminal domain PGC-1[]-independent.

**Bile acids impair the recruitment of CBP by HNF-4[]**

Since the N-terminal region of HNF-4[] (aa 1-249) maintained the response to CDCA, we looked into the possibility that another coactivator interacting with this domain of the receptor may also be involved in this phenomenon. The coactivator CBP/p300 apparently (26,27) interacts with both the N-terminal and C-terminal regions of HNF-4[], and we therefore tested the effect of bile acids on the HNF-4[]/CBP complex in similar co-immunoprecipitation assays in HepG2 cells cotransfected with HA-tagged CBP expression vector. CDCA strongly decreased the interaction of CBP with HNF-4[] (Figure 3A, upper panel). Control Western blots show that CDCA affected neither the levels of HA-CBP nor the expression of HNF-4[] (Fig 3A, middle and lower panel). We then analyzed the interaction of CBP with the N-terminal and C-terminal domain of HNF-4[] by immunoprecipitations performed with Gal4/HNF-4[] fusion proteins and confirmed that they both interacted with CBP (Figure 3B); most importantly, however, we found that CDCA
prevented the interaction of CBP with both portions of the receptor (Figure 3B). As with PGC-1α, co-immunoprecipitation of in vitro synthesized HNF-4α with HA-CBP from control and CDCA-treated cells showed no change in the HNF-4α/CBP complex (Figure 3C).

Transcription analysis in HepG2 cells demonstrates that the overexpression of CBP prevented the feedback regulation of CYP7A1 gene by CDCA but, unexpectedly, did not affect the basal activity of CYP7A1 promoter (Figure 3D). On the other hand, the ectopic expression of CBP blocked the effect of CDCA on all the Gal4-HNF-4α constructs (Figure 3E) as distinct from PGC-1α. CBP therefore also seems to be involved in the regulation of CYP7A1 transcription by bile acids. CBP stimulated the basal transcription activity of full-length HNF-4α and the C-terminal deletion bearing the amino acids 1–249 (Figure 3E upper panels) but not that of the N-terminal deletion containing the amino acids 130–455 (Figure 3D lower left).

Bile acids and cAMP regulate the mRNA levels of CYP7A1

To confirm the physiological significance of our observations we measured the mRNA levels of CYP7A1, SHP, PGC-1α and HNF-4α in HepG2 cells treated with CDCA, 8-Br-cAMP or a combination of the two by real time quantitative PCR. CDCA reduced the levels of CYP7A1 mRNA (Figure 4A) and at the same time enhanced the expression of SHP as previously reported (9,10) (Figure 4B); CDCA did not decrease the levels of PGC-1α (Figure 4C) and HNF-4α (Figure 4D) mRNA. Conversely, cAMP increased PGC-1α mRNA (Figure 4C) due to the cAMP Response Element (CRE) in the promoter of this coactivator (20). Consistent with the increase of PGC-1α expression, cAMP also enhanced the mRNA levels of CYP7A1, indicating that cholesterol 7α-hydroxylase gene is a target of the glucagon/cAMP cascade in liver cells (Figure 4A). Remarkably, when CDCA was added in combination with cAMP it failed to repress the
transcription of CYP7A1, confirming the importance of PGC-1α in the feedback regulation of CYP7A1 by bile acids (Figure 4A). As expected, the FXR ligand, GW4064, decreased the mRNA level of CYP7A1 and at the same time increased that of SHP but did not reduce the level of mRNA for PGC-1α and HNF-4α.

Bile acids regulate the rate-limiting enzyme of gluconeogenesis in the liver
The demonstration that bile acids target the protein–protein interaction between HNF-4α and the coactivators PGC-1α and CBP/p300 prompted us to ask whether this type of regulation may also apply to other hepatic genes. The liver gene phosphoenolpyruvate carboxykinase (PEPCK), which encodes the enzyme catalyzing the rate-limiting step of gluconeogenesis, is under the control of the HNF-4α/PGC-1α complex, which is recruited to a composite hormone response unit (HRU) (20,21). We found that CDCA strongly reduced the mRNA level of PEPCK whereas cAMP increased it (Figure 5A). However, CDCA did not decrease the level of PEPCK mRNA when cells were simultaneously treated with cAMP (Figure 5A). We confirmed these results by measuring the transcription output of the PEPCK promoter in the presence or absence of bile acids. CDCA strongly repressed the promoter activity of the PEPCK promoter/luciferase fusion gene (Figure 5B); however, when PGC-1α was cotransfected, the basal promoter activity increased 3-fold and CDCA no longer affected the PEPCK promoter (Figure 5B). Similarly, the overexpression of CBP prevented the inhibition of CDCA on PEPCK transcription (Figure 5B), although CBP did not stimulate the basal promoter activity of PEPCK, as in the case of the CYP7A1 promoter (Figure 3D). As expected, the FXR agonist GW4064 did not decrease the mRNA levels (Figure 5A) and transcription (Figure 5C) of PEPCK, which demonstrates that bile acids can affect gene transcription also through an FXR-independent signaling pathway. In
parallel transfection assays the more hydrophilic bile acid UDCA did not affect the transcription of \textit{PEPCK} (data not shown).

To prove that HNF-4\[\alpha\] was the target of the signaling cascade elicited by CDCA that leads to the repression of \textit{PEPCK} transcription, we performed reporter gene assays with mutants of the \textit{PEPCK} promoter, in which the AF1 and AF3 sequences, the binding sites for HNF-4\[\alpha\], were substituted by Gal4-binding sites (21). This substitution blunted the CDCA-mediated repression of transcription (Figure 5D, mutants gAF1, gAF3, gAF1/gAF3). However, the ectopic expression of Gal4-HNF-4\[\alpha\], which can bind to the Gal4-binding sites introduced in the \textit{PEPCK} promoter, restored the repression by bile acids (Figure 5D). The mutant of the sequence AF2, which brings about a negative response of \textit{PEPCK} to insulin (28), retained the response to bile acids. It is noteworthy that the basal transcription activity of the gAF1 and gAF3 mutants was lower than that of the wild-type promoter and that the cotransfection of Gal4-HNF-4\[\alpha\] strongly elevated the basal transcription of these constructs. Altogether, our results indicate for the first time that bile acids returning to the liver also regulate \textit{PEPCK} and that, by analogy with the \textit{CYP7A1} promoter, the nuclear receptor HNF-4\[\alpha\] and the coactivators PGC-1\[\alpha\] and CBP play a crucial role in this type of regulation.

\textbf{Bile acids decrease the recruitment of PGC-1\[\alpha\], CBP and RNA polymerase II to \textit{CYP7A1} and \textit{PEPCK} promoters in the context of chromatin}

To definitely prove that CDCA downregulates the transcription of \textit{CYP7A1} and \textit{PEPCK} by selectively impairing the recruitment of PGC-1\[\alpha\] and CBP by HNF-4\[\alpha\] binding to these two promoters in the native chromatin environment, we carried out chromatin immunoprecipitation assays with extracts from HepG2 cells treated with CDCA or vehicle. Cell extracts were
immunoprecipitated with antibodies selective for PGC-1α and CBP and the amount of immunoprecipitated DNA fragments containing the BARE in *CYP7A1* and the HRU in *PEPCK* was quantitated by real time PCR. CDCA significantly decreased the amount of PGC-1α and CBP recruited to the BARE and HRU of *CYP7A1* and *PEPCK* respectively (Figure 6A and B, top panels). The more hydrophilic bile acid UDCA did not affect the recruitment of PGC-1α and CBP on *CYP7A1* and *PEPCK* promoter (data not shown). No signal was detected with the upstream sequences of the two genes (data not shown), indicating that these antibodies immunoprecipitated selective regions of these two genes associated to PGC-1α or CBP. Moreover, CDCA decreased the amount of RNA polymerase II, PGC-1α and CBP recruited to the core promoters (Figure 6A and B, middle panels) as well as the amount of these proteins found downstream in the 3’-untranslated regions (Figure 6A and B, bottom panels), reflecting the reduction of gene transcription. Therefore, these results demonstrate that bile acids act upon the transcription of these two genes also in the native context of chromatin by decreasing the amount of these coactivators and RNA polymerase II associated to specific regions of these genes.

**In vivo regulation of Cyp7a1 and Pepck by bile acids and by the fasted state in mice**

To substantiate the *in vivo* relevance of the results obtained with cell cultures, we performed experiments in mice fed 1% cholic acid diet and in mice undergoing an overnight fast. In the first experiment, the analysis of mouse *Cyp7a1* and *Pepck* gene expression, performed by real time quantitative PCR, demonstrated that bile acids decreased the mRNA levels of these two genes *in vivo* (Figure 7A) and at the same enhanced the expression of SHP (data not shown). In the second experiment, the mRNA levels of *Cyp7a1* and *Pepck* in fasted mice were strongly increased (Figure 7B) along with *Pgc-1α* (data not shown), confirming therefore that these two genes are positively regulated *in vivo* by the glucagon/cAMP cascade triggered during the fasted state.
These data provide strong *in vivo* evidence that bile acids negatively regulate the expression of key genes in cholesterol and glucose metabolism and establish for the first time that *Cyp7a1* is stimulated in fasted mice along with *Pepck*. Taken together these results suggest that the coordinated regulation of these two genes by bile acids may be linked to the fasted-to-fed cycle.
Discussion

In the present paper, we provide multiple evidences for a novel molecular mechanism of transcription regulation of key genes in cholesterol catabolism and in gluconeogenesis (Figure 8). According to this model bile acids affect the recruitment of the coactivators PGC-1α and CBP by HNF-4α on the promoter of CYP7A1 and PEPCK.

Based on these observations, it is necessary to reconsider and broaden our view on the way bile acids affect gene transcription. Bile acids tightly control their own synthesis by multiple signaling cascades that converge on the promoter of the gene CYP7A1, which encodes the rate-limiting enzyme of the classic pathway. Such stringent control is necessary because an excessive output of bile acids may be detrimental to several tissues, so that multiple biochemical mechanisms have evolved to fine-tune their synthesis. The FXR/SHP/FTF and HNF-4α/PGC-1α/CBP pathways may complement each other for this purpose.

The other remarkable finding in this study is that bile acids not only affect their own synthesis but also control the transcription of genes involved in other metabolic pathways such as gluconeogenesis (Figure 8). Considering that glucose output from the liver is often increased in type 2 diabetes (29) contributing to exacerbate this disease, our results on the inhibition of PEPCK transcription by bile acids provide an opportunity to exploit this signaling cascade as a potential target for novel antidiabetic agents.

The regulation of CYP7A1 and PEPCK by opposing stimuli such as bile acids and cAMP, the first mimicking postprandial conditions, the latter the fasted state, suggests an intriguing hypothesis according to which the fasted-to-fed cycle regulates apparently unrelated metabolic pathways in a coordinated fashion. In the light of these observations, we propose that the regulation of gene transcription by bile acids should be viewed dynamically in the context of the
fasted and fed state. After a prolonged fasting period the transcription of CYP7A1 and PEPCK raises probably as a combination of the stimulation by the glucagon/cAMP cascade and of the concomitant decrease in the concentration of bile acids returning to the liver. On one hand, this may help to prepare the gastrointestinal tract for the digestion and absorption of fats in a subsequent meal and, on the other, to increase gluconeogenesis and buffer the falling plasma concentration of glucose during the fasted state. Conversely, in the fed state, as the concentration of bile acids fluxing through the enterohepatic circulation increases, the reduction of CYP7A1 and PEPCK transcription may be secondary to the drop of glucagon level and to the direct inhibition elicited by bile acids, which are massively secreted into the duodenum and return to the liver at higher concentrations than during a prolonged fasting period.

This is the first clear evidence demonstrating that CYP7A1 transcription is enhanced during the fasted state. Previous reports showed contradicting results that may be explained by the different experimental systems used in those studies (30,31). However, our in vitro and in vivo results strongly argue for this type of regulation. In this respect, PGC-1α is the key factor contributing to the stimulation of CYP7A1 and the other target gene, PEPCK, because it is strongly induced by the glucagon/cAMP cascade (20).

Besides PGC-1α, CBP is also involved although the two coactivators play different roles on the feedback regulation of CYP7A1 and PEPCK by bile acids. PGC-1α interacts exclusively with the C-terminal domain of HNF-4α and is displaced upon exposure of hepatocytes to bile acids. Conversely, CBP interacts with both the N-terminal and C-terminal regions of the receptor. The involvement of PGC-1α and CBP in this type of regulation appears to be specific since the overexpression of other coactivators like SRC-1 and TIF2 did not overcome the effect of bile acids on the HNF-4α-dependent transcription of these genes (data not shown). At present, we still
cannot explain how bile acids can induce the dissociation of these coactivators from HNF-4α. However, the observation that *in vitro* synthesized HNF-4α does not dissociate from coactivators present in extracts from cells challenged with bile acids argues for possible posttranslational or structural modifications of the receptor induced by bile acids. At this regard, it should be mentioned that the interaction of CBP with the N-terminal domain of HNF-4α determines the acetylation of lysine residues within the nuclear localization sequence, which is crucial for nuclear localization, DNA binding and interaction with CBP itself (32). It is possible that bile acids may prevent the acetylation of these lysine residues and consequently decrease the activity of the receptor as well as its affinity for CBP. It will be interesting to verify whether similar or other posttranslational modifications can affect the recruitment of coactivators in the C-terminal domain of HNF-4α and if bile acids can somehow cause these modifications.

It was somewhat surprising to find that the overexpression of CBP could not enhance the transcription of these two genes, as opposed to PGC-1α. This may reflect the requirement of PGC-1α docking to the LBD of HNF-4α as a prerequisite for the proper interaction of CBP with and coactivation of HNF-4α through the LBD (33). The endogenous levels of PGC-1α in HepG2 cells may therefore be insufficient to cooperate with CBP in the coactivation of HNF-4α via the LBD. On the other hand, the interaction of CBP with the N-terminal fragment HNF-4α does not require the presence of PGC-1α (27), thus it could be activated by CBP in the Gal4-based assay. However, in the context of *PEPCK* and *CYP7A1* promoters the interaction of CBP with the N-terminal domain of HNF-4α does not seem to be sufficient itself to enhance the expression of these two genes but requires the presence of PGC-1α.

The data presented here open the question which of the two bile acid-elicited signaling pathways is more relevant for the negative regulation of *CYP7A1* and *PEPCK* gene transcription. The
following considerations suggest that the HNF-4α pathway may be more critical for these two genes. First, bile acids can still decrease the mRNA levels of Cyp7a1 in Shp⁻/⁻ mice. Second, PEPCK does not contain LRH-1/FTF binding sites, and its inhibition by bile acids relies solely on HNF-4α binding to the AF1 and AF3 sequences in the promoter. Thus, the PEPCK promoter may be considered as a natural “variant” of the CYP7A1 Bile Acid Responsive Element that responds to bile acids exclusively via HNF-4α (Figure 8). This would also justify why the selective FXR ligand, GW4064, did not repress the transcription of PEPCK since FTF, the target of SHP, does not interact to this promoter. This result is in conflict with a recent report (34), showing that PEPCK is a novel target for SHP inhibition. However, it should be recalled that the negative effect of SHP was observed in cells stimulated by glucocorticoid hormones whereas here we studied the effect of bile acids and the selective FXR agonist on PEPCK transcription under basal conditions. Third, since inhibition via FXR/SHP/FTF requires de novo synthesis of SHP through ligand-bound FXR, it is likely that the inhibition mediated by HNF-4α precedes others in time because it is modulated by rapid events involving a MAPK pathway (16). This view is supported by the indication that HNF-4α seems to undergo regulation of its own activity via a yet unidentified posttranslational modification, as an essential prerequisite for the bile acid-induced dissociation of the HNF-4α/coactivator complex. Several groups have already shown that posttranslational modifications can affect the activity of HNF-4α in different ways (32,35-37). The FXR/SHP pathway may however have a role in reinforcing and supporting the repression already established through HNF-4α.

In conclusion, we have described a novel mechanism of transcription regulation affecting key genes in bile acid synthesis and gluconeogenesis, which explains why Shp⁻/⁻ mice are still responsive to bile acids. The discovery that bile acids also repress the transcription of PEPCK,
the rate-limiting enzyme of gluconeogenesis, will stimulate new studies on the coordinated control of different metabolic pathways. This study discloses new target mechanisms for the design of novel treatments of metabolic diseases such as hyperlipidemia and diabetes.

**Acknowledgments**

This paper is dedicated to the memory of Prof. Giovanni Galli, our mentor and the master who inspired these investigations and kept encouraging us during our careers. We wish to thank Drs. Krister Bamberg, Iannis Talianidis, Daryl K. Granner and Anastasia Kralli for the generous gifts of the FXR ligand GW4064, the HNF-4α antibody and some of the plasmids used in this study. We are also grateful to Drs. Luca Ruocco and Elisabetta Mozzon for their advice on real time quantitative PCR and Miss Elda Desiderio Pinto for her valuable administrative support. We also wish to thank Miss Ana Vigil Chacon (University of Granada, Spain) for critically reading the manuscript and for help with animal studies.
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Footnotes

1This work was supported by grants from the European Community (NORTh, QLG1-CT-2001–01513) and the Italian Ministry of University and Research (COFIN 2002062991 and FIRST 2002 ex MURST 60%).

2EDF and NM contributed equally to this paper therefore they should be both considered as first author.

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4Abbreviations used are: BARE, bile acid responsive element; CBP, CREB Binding Protein; CDCA, chenodeoxycholic acid; ChIP, chromatin immunoprecipitation; CMV, cytomegalovirus; COUP-TFII, chicken ovalbumin upstream promoter-transcription factor II; CPF, CYP7A1 promoter binding factor; CRE, cAMP Response Element; CREB, cAMP Response Element Binding Protein; CYP7A1, cholesterol 7α-hydroxylase gene; DBD, DNA-binding domain; DMSO, dimethylsulphoxide; FTF, α-fetoprotein transcription factor; FXR/BAR, farnesoid X receptor; FXRE, FXR response element; GTFs, general transcription factors; HA, hemagglutinin; HNF-4α, hepatocyte nuclear factor 4α; HRU, hormone response unit; LBD, ligand-binding domain; LRH-1, liver receptor homologue-1; MAPK, mitogen-activated protein kinase; PEPCK, phosphoenolpyruvate carboxykinase gene; PGC-1α, PPARα coactivator-1α; PIC, preinitiation complex; RT-PCR, reverse transcription-polymerase chain reaction; PPARγ, peroxisome proliferator-activated receptor γ; SHP, small heterodimer partner; SRC-1, steroid receptor
coactivator-1; TIF2, transcription intermediary factor 2; UDCA, ursodeoxycholic acid; UTR, untranslated region.

While this manuscript was under revision, Holt J. A. et al. (2003) Genes Dev. 17, 1581-1591, reported a novel mechanism of transcription regulation by bile acids mediated by FXR and fibroblast growth factor-19 (FGF-19), a secreted growth factor that signals through the FGFR4 cell-surface receptor tyrosine kinase and in turn strongly suppresses the expression of CYP7A1 in hepatocytes through a c-Jun N-terminal kinase (JNK)-dependent pathway.
**Figure Legends**

**Figure 1.** Bile acids repress CYP7A1 transcription via HNF4α in an FXR-independent manner. (A) Left, Gal4-based assay using Gal4-HNF-4α full length in HepG2 cells cotransfected with the human FXR expression vector. After transfection, cells were treated with either CDCA or the FXR ligand GW4064. Right, control experiments with Gal4-VP16 fusion protein. (B) HepG2 cells were cotransfected with the plasmid carrying two copies of the FXRE in front of the SV40 early promoter and luciferase reporter gene, and the FXR expression vector. Transfected cells were treated with CDCA or GW4064 as in panel A. (C) Transfection of HepG2 cells with the human CYP7A1/luciferase fusion gene (ph-371luc) and treatment as in panel A. *P < 0.05 vs. control vehicle, Student’s t test.

**Figure 2.** Bile acids downregulate CYP7A1 transcription by impairing the HNF-4α/PGC-1α complex. (A) Upper panel, determination of HNF-4α/PGC-1α interaction by coimmunoprecipitation analysis of HepG2 cells treated with CDCA or vehicle. Immunoprecipitation was performed with an anti-hemagglutinin antibody (αHA) to capture HA-tagged PGC-1α and HNF-4α was detected by Western blot with an anti-HNF-4α antibody (αHNF-4). Middle panel, filter shown in the upper panel was stripped and incubated with anti-HA antibody (αHA). Lower panel, Western blot analysis of whole cell lysates with anti-HNF-4α antibody (αHNF-4). (B) Immunoprecipitation was performed as in (A) except that either Gal4-HNF-4/1-249 or Gal4-HNF-4/130-455 plasmid was cotransfected and bands were detected with an anti-Gal4 antibody (αGal4). (C) Immunoprecipitation with anti-HA IgG of lysates from HepG2 cells transfected with HA-PGC-1α and treated with CDCA or vehicle and incubated with *in vitro* synthesized [35S]-labeled HNF-4α; bands were detected by autoradiography. Similar
results were obtained with smaller amounts of in vitro synthesized receptor. Input, 10% of added labeled HNF-4α. (D) HepG2 cells were transfected with either the human CYP7A1/luciferase (hCYP7A1) or the SV40/luciferase construct and treated with CDCA and 8-Br-cAMP in the indicated combinations. (E) Transfection of HepG2 cells with hCYP7A1/luciferase with or without the expression vector for PGC-1α and treatment with CDCA. The specificity of CDCA effect was assessed by using the SV40/luciferase construct. (F) Gal4-based assay in HepG2 cells by cotransfecting PGC-1α or the empty vector pcDNA3 with full-length HNF-4α (upper left), its C-terminal deletion 1–249 (upper right) and N-terminal deletion 130–455 (lower left). Transfected cells were treated with CDCA. The specificity of the CDCA effect was assessed by using the Gal4-VP16 construct (lower right). *P and **P < 0.05 vs. control vehicle, Student’s t test.

Figure 3. Bile acids downregulate CYP7A1 transcription by impairing the HNF-4α/CBP complex. (A) Upper panel, determination of HNF-4α/CBP interaction by coimmunoprecipitation analysis of HepG2 cells treated CDCA or vehicle. Immunoprecipitation was performed with an anti-hemagglutinin antibody (αHA) to capture HA-tagged CBP and HNF-4α was detected by Western blot with an anti-HNF-4α antibody (αHNF-4). Middle panel, filter shown in the upper panel was stripped and incubated with anti-HA antibody (αHA). Lower panel, Western blot analysis of whole cell lysates with anti-HNF-4α antibody (αHNF-4). (B) Immunoprecipitation was performed as in (A) except that either Gal4-HNF-4/1-249 or Gal4-HNF-4/130-455 plasmid was cotransfected and bands were detected with an anti-Gal4 antibody (αGal4). (C) Immunoprecipitation with anti-HA IgG of lysates from HepG2 cells transfected with HA-CBP and treated with CDCA or vehicle and incubated with in vitro synthesized [35S]-labeled HNF-4α;
bands were detected by autoradiography. Similar results were obtained with smaller amounts of \textit{in vitro} synthesized receptor. Input, 10\% of added labeled HNF-4\[%. (D) Transfection of HepG2 cells with \textit{hCYP7A1}/luciferase with or without the expression vector for CBP and treatment with CDCA or vehicle. The specificity of CDCA effect was assessed by using the SV40/luciferase construct. (E) Gal4-based assay in HepG2 cells by cotransfecting CBP or the empty vector pcDNA3 with full-length HNF-4\[% (upper left), its C-terminal deletion 1–249 (upper right) and N-terminal deletion 130–455 (lower left). Transfected cells were treated with CDCA or vehicle. The specificity of the CDCA effect was assessed by using the Gal4-VP16 construct (lower right). \*P and **P < 0.05 vs. control vehicle, Student’s \(t\) test.

\textbf{Figure 4. Regulation of mRNA levels of CYP7A1, SHP, PGC-1 and HNF-4\[\% in HepG2 cells.} Real time quantitative RT-PCR analysis of the \textit{CYP7A1} (A), SHP (B), PGC-1\[% (C) and HNF-4\[% (D) mRNA levels in HepG2 cells exposed to CDCA, 8-Br-cAMP, GW4064 in the indicated combinations. Numbers above the bars indicate levels of mRNA relative to controls.

\textbf{Figure 5. Downregulation of PEPCK transcription by bile acids via HNF-4\[\% is FXR-independent.} (A) Real-time quantitative RT-PCR analysis of \textit{PEPCK} mRNA levels in HepG2 cells exposed to CDCA, 8-Br-cAMP, GW4064 in the indicated combinations. Numbers above the bars indicate levels of mRNA relative to the control. (B) HepG2 cells were transfected with the \textit{PEPCK} promoter/luciferase fusion gene with or without the expression vector for PGC-1\[% or CBP and treated with CDCA. \*P and **P < 0.05 vs. control vehicle, Student’s \(t\) test. (C) Transfection of HepG2 cells with the \textit{PEPCK} promoter/luciferase fusion gene and treatment with either GW4064 (vehicle DMSO) or CDCA (vehicle ethanol). \*P < 0.05 vs. control vehicle,
(D) Mutation analysis of the hormone response unit in *PEPCK* promoter. The AF1, AF2 and AF3 sites in the *PEPCK* promoter were replaced by a Gal4 binding site and tested for response to CDCA in HepG2 cells. The specificity of CDCA effect was assessed by using the SV40/luciferase construct. *P* and #*P* < 0.05 vs. Gal4 cotransfected cells and control vehicle, respectively, Student’s *t* test.

**Figure 6. Bile acids decrease the recruitment of coactivators and RNA polymerase II on CYP7A1 and PEPCK.** (A) ChIP assay was performed in HepG2 cells transfected with HA-PGC-1α expression vector and treated with CDCA or vehicle. Chromatin fragments were immunoprecipitated with anti-HA antibody or anti-CBP antibody or anti-RNA polymerase II antibody and promoter sequences containing BARE (upper panel), TATA box (middle panel) and 3’-UTR (lower panel) of CYP7A1 were amplified and quantitated by real time PCR. (B) ChIP assay was performed as in (A) using the same antibodies and amplifying the HRU (upper panel), TATA box (middle panel) and 3’-UTR (lower panel) of PEPCK. The amplification of upstream sequences from CYP7A1 and PEPCK promoters immunoprecipitated with anti-HA, anti-RNA polymerase II or anti-CBP antibodies gave no signal. Experiments were performed with triplicate samples and data are expressed as the mean ± S.D. *P* < 0.05 vs. control vehicle, Student’s *t* test.

**Figure 7. Regulation of Cyp7a1 and Pepck by bile acids and in the fasted state in mice.** (A) C57BL/6J mice (n= 5) were fed with normal diet or 1% cholic acid-supplemented diet (CA) for 8 days as indicated. mRNA of Cyp7a1 and Pepck were quantitated by real time quantitative PCR on single liver samples using 18S rRNA as internal standard for normalization. (B) Another group of mice (n= 9) were fed normal diet and fasted for 15 hours as indicated. mRNA of
Cyp7a1 and Pepck were quantitated as in (A). Results are expressed as mean ± SEM. (*) indicates statistical significance with P<0.05 (Student’s t test).

Figure 8. The model of CYP7A1 and PEPCK transcriptional repression by bile acids. (A) Under basal conditions HNF-4α binds to the Bile Acid Responsive Element (BARE) of CYP7A1 and PEPCK and triggers gene transcription by recruiting the coactivators PGC-1α and CBP. The interaction of HNF-4α with CBP is facilitated by the presence of PGC-1α. The HNF-4α/coactivator complex recruits the general transcription factors (GTFs) and activates RNA polymerase II. (B) Bile acid-induced repression of CYP7A1 and PEPCK transcription is secondary to the dissociation of HNF-4α/coactivator complex that causes the faulty recruitment of GTFs and activation of RNA polymerase II. According to this model the effect of bile acids does not depend on the activation of CYP7A1 transcription by oxysterols via LXR (9) or PEPCK transcription by glucocorticoid hormones (34).
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Figure 8
Coordinated control of cholesterol catabolism to bile acids and of gluconeogenesis via a novel mechanism of transcription regulation linked to the fasted-to-fed cycle
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J. Biol. Chem. published online July 15, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M305079200

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