Identification of Rev-erbα as a physiological repressor of apoC-III gene transcription[1]

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Abstract **Elevated serum levels of triglyceride-rich remnant lipoproteins (TRL) are a major risk factor predisposing a subject to atherosclerosis. Apolipoprotein C-III (apoC-III) is a major constituent of TRL that impedes triglyceride hydrolysis and remnant clearance and, as such, may exert pro-atherogenic activities. In the present study, transient cotransfection experiments in rat hepatocytes in primary culture and rabbit kidney RK13 cells demonstrated that overexpression of Rev-erbα specifically decreases basal and HNF-4 stimulated human apoC-III promoter activity. A Rev-erbα response element was mapped by promoter deletion, mutation analysis, and gel-shift experiments to a AGGTCA half-site located at position −23/−18 (downstream of the TATA box) in the apoC-III promoter. Finally, Rev-erbα-deficient mice displayed elevated serum and liver mRNA levels of apoC-III together with increased serum VLDL triglycerides. Taken together, our data identify Rev-erbα as a regulator of apoC-III gene expression, providing a novel, physiological role for this nuclear receptor in the regulation of lipid metabolism.**—Raspé, E., H. Duez, A. Mansén, C. Fontaine, C. Fiévet, J-C. Fruchart, B. Vennström, and B. Staels. **Identification of Rev-erbα as a physiological repressor of apoC-III gene transcription.** ***J. Lipid Res.*** **2002.** 43: **2172–2179.**

Supplementary key words apolipoprotein C-III • triglycerides • nuclear receptors

Triglyceride-rich lipoprotein remnants (TRL) are positively correlated with the progression of atherosclerosis (1, 2). Moreover, elevated serum triglyceride concentrations, in addition to elevated LDL-cholesterol (LDL-C) and reduced HDL-C levels, are now considered as an independent risk factor for coronary heart disease (CHD) (3–6). ApoC-III is a 79-amino acid glycoprotein synthesized in the liver and intestine that plays a key role in serum triglyceride metabolism by delaying the catabolism of triglyceride-rich particles (7, 8). Hence, apoC-III is a potential target gene for the identification of hypolipidemic drugs.

ApoC-III gene expression is tightly regulated, being repressed by hormones such as insulin (9, 10) or thyroid hormones (11), cytokines such as interleukin-1 (12) or tumor necrosis factor α (13), as well as hypolipidemic drugs such as fibrates (14, 15), or β-blocked fatty acids (16, 17). By contrast, its expression is increased by retinoids (18). The apoC-III gene is located on chromosome 11q23 between the apoA-I and apoA-IV genes (19). Regulatory sequences determining the tissue-specific expression pattern of apoC-III have been delineated (20). They include a distal regulatory enhancer that determines the level and tissue specificity of expression of apoC-III, apoA-I, and apoA-IV, as well as a proximal promoter. The C3P site located in the proximal promoter plays a key role in the control of apoC-III promoter activity. It contains a direct repeat (DR) of two PuGGTCA half-sites separated by one nucleotide (DR-1) to which bind the nuclear receptors HNF-4, PPAR, RXR, RAR, and the transcription factors USF1 and 2a that activate transcription, as well as, Ear2, TRβ, COUPTF-I, and COUPTF-II that repress apoC-III promoter activity. The proximal promoter also comprises binding sites for C/EBPδ, ATF-2, NFκB, and Jun. Recently, we identified RORα1, a member of the retinoic acid receptor related orphan receptor (ROR) subfamily of orphan nuclear receptors, as a new physiological transcriptional activator of apoC-III gene expression (21). RORα1 acts through at least two response elements located at positions −83/−78 (within

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the C3P site) and −23/−18 (downstream of the TATA box) in the apoC-III promoter. This latter site, which plays a dominant role in RORα1 action, is functionally conserved between the rodent and human apoC-III gene promoter sequences. Finally, initiator-like elements directly involved in the transactivation of the apoC-III promoter by USF and necessary to the combined effect between USF and HNF-4 were recently localized between the TATA box and the C3P site (22).

The Rev-erb orphan receptors are a subfamily of nuclear receptors consisting of two different genes, Rev-erbα (also termed ear1 or NR1D1) and Rev-erbβ (also termed RVR, BD73 or NR1D2), the ligands of which are presently unknown (23). The Rev-erbα gene is located on human chromosome 17q21 and encoded on the opposite strand of the TRα2 receptor (24–26). Rev-erbα, initially reported to activate transcription (27), actually acts as a strong repressor of transcription (28). Rev-erbα binds as monomer to response elements consisting of the halfcore PuGGTCA motif preceded by a 6-bp AT-rich sequence (27, 29) or as dimer on response elements consisting of a tandem repeat of two PuGGTCA motifs spaced by two nucleotides and preceded by a 6-bp AT-rich sequence (28, 30). Rev-erbα is widely expressed, especially in muscle (29) and liver (29, 31). Expression of Rev-erbα is induced in rat liver after chronic exposure to fibrates (31) while it is downregulated after liver exposure to glucocorticoids (32). Based on the presence of putative response elements in their promoter and on in vitro data, several target genes for Rev-erb family members were proposed (30, 33–37). A transgenic mouse line has been developed that carries a deleted Rev-erbα gene and presented alterations mainly in cerebellar development (38).

Interestingly, Rev-erbα was shown to bind to similar response elements as RORα, although with opposite effects on transcription, indicating the existence of crosstalk between both nuclear receptor signaling pathways (29). Thus, we hypothesized that the RORα1 response element recently localized in the human and mouse apoC-III gene promoter could also be a target site for Rev-erbα. A marked reduction in both basal and HNF4-stimulated activity of the human apoC-III promoter was observed upon over-expression of Rev-erbα. The Rev-erbα response element was located at the −23/−18 AGGTCA half-site downstream of the TATA box that is also involved in RORα1 action data which are in line with observations reported while this work was in progress (39). In addition, we report here an increase in serum and liver mRNA levels of apoC-III that accompanied elevated triglycerides in male Rev-erbα-deficient mice. Taken together, these data identify Rev-erbα as a novel, physiological regulator of apoC-III expression.

MATERIALS AND METHODS

Cloning of recombinant plasmids

The plasmids containing wild type or mutated (−33/−16mut: $-22^{G\rightarrow C}$, $-21^{G\rightarrow A}$) fragments the human apoC-III gene promoter cloned in front of the luciferase reporter gene were described previously (21). The construct pCDNA3-hRORα1 was a gift of A. Shevelev. The pRenConT+ construct used to evaluate transfection efficiency containing the Renilla luciferase gene under the control of the SV40 promoter and enhancer was previously described (21). The pSG5-hRev-erbα and pSG5-hHNF4 plasmids were kindly provided by V. Laudet and B. Laine.

Cell culture and transient transfection assays

Rat hepatocytes were isolated by collagenase perfusion of livers from male rats (150 to 250 g) (17). Cells were seeded in Williams medium (Gibco, Paisley, UK), supplemented with UltroserSF (2% by vol) (Biosepra, Cergy St Christophe, France), penicillin (100 U/ml), streptomycin (100 μg /ml) (Invitrogen, Carlsbad, CA), fatty acid-free BSA (0.2% mass/vol), L-glutamine (2 mM), dexamethasone (1 μM), T_3 (100 nM), and insulin (100 nM) (Sigma, St Louis, MO). After 4 h, the culture medium was switched to the same Williams medium without Ultroser and BSA. Cells were transfected overnight using lipofectine (Invitrogen) with reporter plasmids (50 ng/well), expression vectors (100 ng/well), and the pRenCont+ transfection efficiency control plasmid (1 ng/well). After transfection, the medium was removed, and cells were quickly washed with ice-cold phosphate-based saline (PBS, 0.15 M NaCl, 0.01 M sodium phosphate buffer; pH 7.2) and incubated for additional 24 h in Williams medium supplemented as above. At the end of the experiment, the cells were washed once with ice-cold PBS and the luciferase activity was measured with the Dual-Luciferase™ Reporter Assay System (Promega, Madison, WI) according to the manufacturer's instructions. All transfection experiments were performed at least three times. Protein content of the extract was evaluated by the Bradford assay using the kit from Bio-Rad (Bio-Rad, München, Germany).

RK13 cells, obtained from ECACC (Porton Down, Salisbury, England), were maintained in standard culture conditions (Dulbecco's modified Eagle's minimal essential medium supplemented with 10% fetal calf serum at 37°C in a humidified atmosphere of 5% CO_2/95% air). Medium was changed every 2 days. Cells were seeded in 24-well plates at a density of 5×10^4 and incubated at 37°C for 16 h prior to transfection. Cells were transfected using the cationic lipid RPR 120535B as previously described (21) with reporter plasmids (50 ng/well), expression vectors (100 ng/well), and the control plasmid (1 ng/well). At the end of the experiment, the cells were washed with ice-cold PBS, lysed, and reporter gene activity was measured as described above.

Gel retardation assays

Rev-erbα was in vitro transcribed from the pSG5-hRev-erbα plasmid using T7 polymerase and subsequently translated using the TNT coupled transcription/translation system (Promega, Madison, WI) following the manufacturer's instructions. DNA-protein binding assays were conducted as described (21). Double stranded oligonucleotides were end-labeled using T4 polynucleotide kinase and γ^{32}P-ATP and used as probe. For competition experiments, 5, 10, and 50-fold excess of cold oligonucleotide were included 15 min before adding labeled oligonucleotides. DNA/protein complexes were resolved by nondenaturating poly-acrylamide gel electrophoresis.

Animals

Nine to sixteen weeks of age, 20–30 g weighing wild-type, and homozygous Rev-erbα-deficient male mice littermates in a Sv129OlaHsd × BALB/c background as previously described (38) were used. The mice were fed a standard rodent chow. Blood drawn from the tail vein was collected after a 4 h fasting period. Serum was isolated by centrifugation at 1,200 rpm for 25 min at 4°C, stored at 4°C, and subsequently used for serum apoC-

III, triglycerides, and lipoprotein analyses. After carbon dioxide anesthesia, the mice were decapitated and tissue samples were recovered, frozen on dry ice, and then stored at −80°C until RNA analysis.

Triglycerides, apoC-III, and lipoprotein analyses. Serum apoC-III levels were measured by an immunonephelometric assay using a specific polyclonal antibody as previously described (40). Serum triglyceride concentrations were determined by enzymatic assays using commercially available reagents (Boehringer, Mannheim, Germany). Lipoprotein triglyceride profiles were obtained by fast protein liquid chromatography (FPLC) and triglyceride concentration measurement in the eluted fractions as previously described (41).

RNA analysis. RNA extractions and Northern blot hybridizations were performed as described previously (15).

RESULTS

hRev-erbα represses the activity of the human apoC-III gene promoter

We reported recently that RORα is a positive physiological regulator of hepatic apoC-III transcription (21). In order to determine whether Rev-erbα, which is also expressed in liver, controls the transcription of the human apoC-III gene, transient transfection experiments were performed. In primary rat hepatocytes, cotransfection of a human (h)Rev-erbα expression plasmid resulted in a decreased activity of a luciferase reporter gene driven by the −1415/+24 fragment of the human apoC-III promoter (**Fig. 1A**). A strong repression of apoC-III promoter activity was also observed in rabbit kidney RK13 cells (Fig. 1B). The effect of hRev-erbα overexpression was promoter-dependent as the promoterless vector pGL3 was unaffected in both cells. The effect of hRev-erbα depended on the amount of expression vector transfected (**Fig. 2**). In addition to RORα, other members of the nuclear receptor family (in particular HNF-4) enhance apoC-III gene promoter activity (20). In order to establish the extent to which hRev-erbα overexpression influences the action of such other transcription factors, RK13 cells were cotransfected with a reporter plasmid driven by the −1415/+24 fragment of the apoC-III gene promoter in the presence of a fixed amount of hHNF-4 expression vector and increasing amounts of hRev-erbα expression vector. Overexpression of hRev-erbα reduced the hHNF-4-stimulated activity of the reporter gene in a dose-dependent manner (Fig. 2). Similar results were obtained with hRORα1 (data not shown).

Mapping of the human apoC-III promoter sites conferring responsiveness to hRev-erbα

To identify the response element(s) required for hRev-erbα repression of the apoC-III promoter, 5′-nested deletions of this promoter were cotransfected with the hRev-erbα expression vector in RK13 cells. Though, as previously described (21), deletion of the promoter led to a decrease in its basal activity (**Fig. 3**), even the shortest construct tested (−108/+24WTpGL3) was still repressed by hRev-erbα, indicating that the first 108 nucleotides of the apoC-III promoter are sufficient to confer hRev-erbα responsiveness (Fig. 3). To identify sequences to which hRev-erbα directly binds, radiolabeled overlapping oligonucleotides corresponding to portions of the −108/+24 fragment of the apoC-III promoter were used as probes in gel shift assays. hRev-erbα protein binding as monomer was observed only on the −33/−16 fragment of the apoC-III gene promoter (**Fig. 4**). This fragment contains the previously described AGGTCA half-site preceded by an A/T-rich region that responds to hRORα1 (21). Binding of hRev-erbα to the −33/−16 fragment of the apoC-III promoter was lost after mutation of the AGGTCA half-site present in position −23/−18 (Fig. 4). The binding of hRev-erbα to the −33/−16 fragment of the apoC-III promoter was displaced by increasing amounts of a cold double-stranded

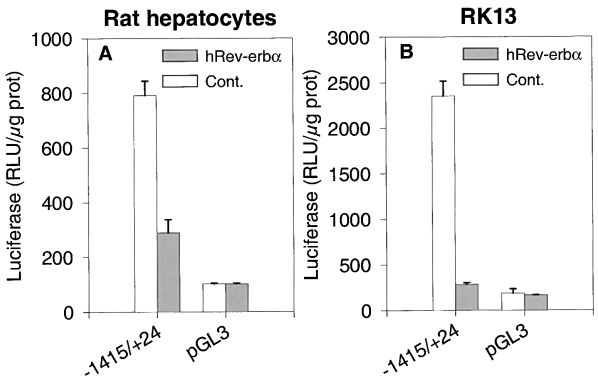

Fig. 1. hRev-erbα represses the activity of the human apolipoprotein C-III (apoC-III) gene promoter. Rat hepatocytes (A) or RK13 cells (B) were transiently cotransfected with the −1415/+24wtpGL3 reporter plasmid (50 ng) containing the −1415/+24 fragment of the human apoC-III promoter cloned in front of the luciferase reporter gene or the empty pGL3 vector as control (50 ng), and the expression plasmid (100 ng) pSG5-hRev-erbα (hRev-erbα) or the empty pSG5 vector as control (Cont). Cells were transfected and luciferase activity measured and expressed as described in Materials and Methods.

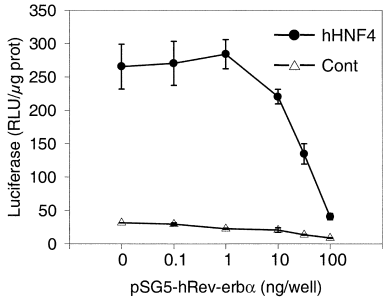

Fig. 2. Human Rev-erbα negatively interferes with the activation of the human apoC-III gene promoter by hHNF4. RK13 cells were transiently cotransfected with the −1415/+24 wtpGL3 reporter plasmid (50 ng) and with increasing amounts of the expression plasmid pSG5-hRev-erbα (hRev-erbα) in the presence or absence of 100 ng of the expression plasmids pSG5-hHNF4α (hHNF4). Cells were transfected and luciferase activity measured and expressed as described in Materials and Methods.

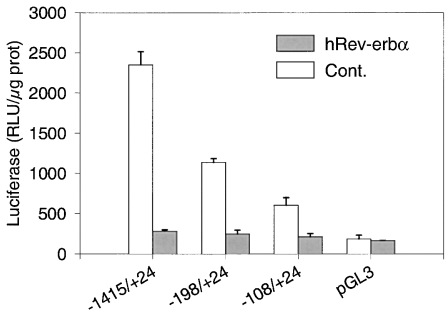

Fig. 3. Identification of the human apoC-III promoter elements conferring its responsiveness to hRev-erbα. RK13 cells were cotransfected with pSG5-hRev-erbα expression vector (100 ng) (hRev-erbα) or the empty pSG5 vector as control (Cont) and reporter constructs (50 ng) containing the indicated nested fragments of the apoC-III promoter cloned in front of the luciferase reporter gene. The empty pGL3 vector (50 ng) was used as control. Cells were transfected and luciferase activity measured and expressed as described in Materials and Methods.

oligonucleotide that contains one copy of the hRev-erbα consensus binding site (Fig. 4). It was also displaced by increasing amounts of the cold wild-type −33/−16 double-stranded oligonucleotide but unaffected by increasing amounts of the mutated cold −33/−16 double-stranded oligonucleotide (data not shown). Taken together, our results suggest the presence of a binding site for hRev-erbα on the proximal human apoC-III promoter, downstream of the TATA box (−23/−18).

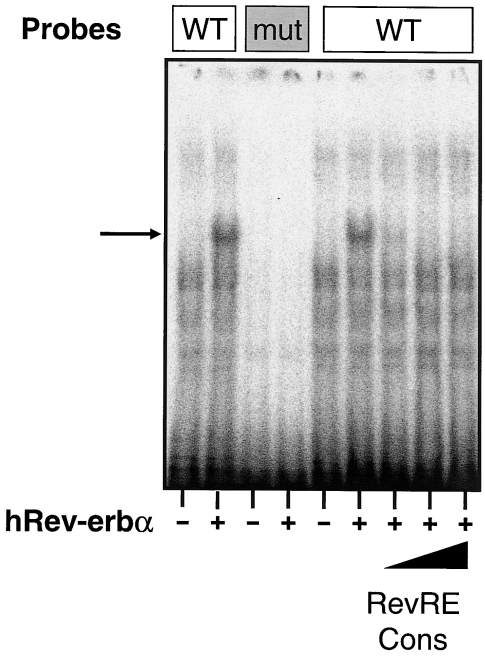

Fig. 4. hRev-erbα binding to labeled probes covering the −33/−16 region of the human apoC-III promoter. Double stranded oligonucleotide probes corresponding to the wild type or mutated −33/−16 fragment of the apoC-III promoter were labeled, incubated as indicated with in vitro translated hRev-erbα protein or unprogrammed lysate as control, and analyzed as described in Materials and Methods. In addition, in vitro translated hRev-erbα protein or unprogrammed lysate were also pre-incubated 15 min with 5, 10, and 50-fold excess of unlabeled double stranded oligonucleotide corresponding to a consensus Rev-erbα response element before the addition of the labeled wild type −33/−16 probe. Specific complexes not observed with unprogrammed lysate are indicated by an arrow.

Functional characterization of the hRev-erbα response element in the proximal human apoC-III promoter

To evaluate whether this putative response element is functional in the context of the proximal apoC-III promoter, the AGGTCA half-site present downstream of the TATA box in position −23/−18 of the apoC-III promoter was mutated by site-directed mutagenesis in the −1415/+24WTpGL3 construct. This mutation enhanced the basal activity of the apoC-III promoter in rat hepatocytes and abrogated hRev-erbα responsiveness (**Fig. 5**). In RK13 cells, this mutation resulted in a loss of the hRev-erbα-mediated repression (**Fig. 6A**). These data indicate that the −23/−18 half-site plays the major role in the hRev-erbα responsiveness of the apoC-III promoter in hepatocytes and RK13 cells.

To evaluate whether the −23/−18 half-site could confer Rev-erbα responsiveness to a heterologous promoter, the −33/−16 fragment of the apoC-III promoter was cloned in front of a thymidine kinase (Tk) promoter-driven luciferase reporter gene. The luciferase activity of RK13 cells transfected with the $(-33/-16)_{3S}$TkpGL3 construct was strongly repressed by hRev-erbα over-expression (Fig. 6B). To evaluate the specificity of hRev-erbα action, the mutated construct $(-33/-16\text{mut})_{3S}$TkpGL3 was cotransfected with a hRev-erbα expression vector in RK13 cells. In contrast to the wild-type construct, the luciferase activity from RK13 cells transfected with the mutated constructs was unaffected by hRev-erbα (Fig. 6B). To exclude that the −108/+24 fragment of the apoC-III promoter contains other hRev-erbα responsive elements, overlapping fragments of the apoC-III promoter (covering the −100/−16 region of the apoC-III promoter) were cloned in

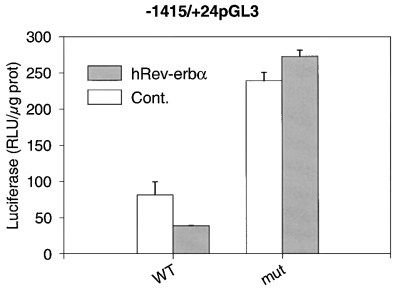

Fig. 5. Functional evaluation of the hRev-erbα response element present in the −1415/+24 fragment of the human apoC-III promoter in rat hepatocytes. Primary rat hepatocytes were cotransfected with pSG5-hRev-erbα expression vector (100 ng) (hRev-erbα) or the empty pSG5 vector as control (Cont), and reporter constructs (50 ng) containing the wild type or a site-directed mutated −1415/+24 fragment of the human apoC-III promoter cloned in front of the luciferase reporter gene. Twenty-four hours afterwards, cells were lysed and luciferase activity measured and expressed as described in Materials and Methods.

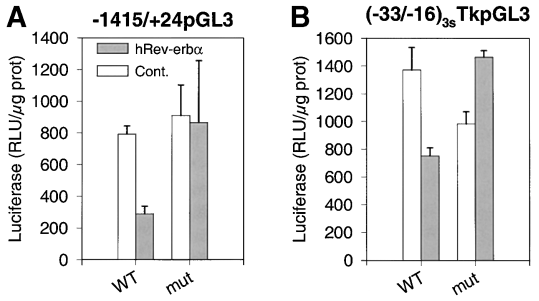

Fig. 6. Functional evaluation of the hRev-erbα response element present in the −1415/+24 fragment of the human apoC-III promoter in RK13 cells. RK13 cells were cotransfected with pSG5-hRev-erbα expression vector (100 ng) (hRev-erbα) or the empty pSG5 vector as control (Cont), and reporter constructs (50 ng) containing the wild type or a site-directed mutated −1415/+24 fragment of the human apoC-III promoter cloned in front of the luciferase reporter gene (A). The empty pGL3 vector was used as negative control. B: RK13 cells were similarly cotransfected with pSG5-hRev-erbα expression vector, pSG5 vector, and reporter constructs (50 ng) containing three copies of the wild type or the mutated −33/−16 fragment of the human apoC-III promoter inserted in front of the Herpes simplex thymidine kinase promoter cloned upstream of the luciferase reporter gene as described in Materials and Methods. Cells were transfected, luciferase activity measured, and expressed as described in Materials and Methods.

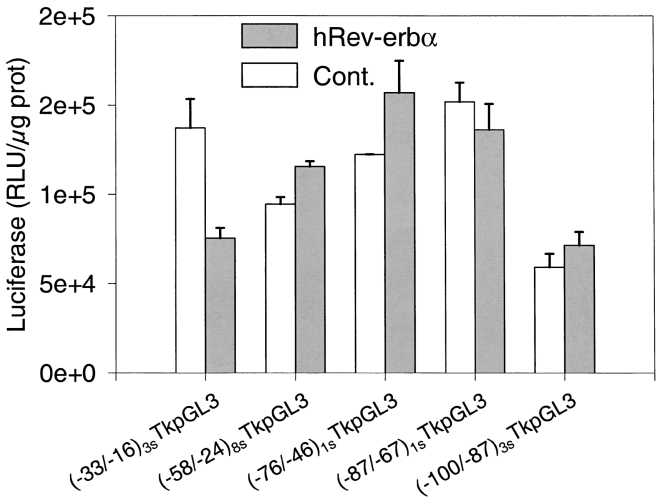

Fig. 7. Evaluation of the presence of other hRev-erbα response elements present in the −108/+24 fragment of the human apoC-III promoter. RK13 cells were cotransfected with pSG5-hRev-erbα expression vector (100 ng) (hRev-erbα) or the empty pSG5 vector as control (Cont) and reporter constructs (50 ng) containing the indicated fragments of the human apoC-III promoter inserted in front of the Herpes simplex thymidine kinase promoter cloned upstream of the luciferase reporter gene as described in Materials and Methods. Cells were transfected and luciferase activity measured and expressed as described in Materials and Methods.

front of a thymidine kinase (Tk) promoter-driven luciferase reporter vector. The luciferase activity of cellular extracts from RK13 cells cotransfected with these constructs and the hRev-erbα expression vector was not affected by hRev-erbα over-expression (**Fig. 7**). These data suggest that the AGGTCA half-site to which hRev-erbα binds in the proximal human apoC-III promoter is also functional in the context of a heterologous promoter and that the −100/−16 region of the apoC-III promoter does not contain an additional hRev-erbα response element.

Rev-erbα-deficient mice display elevated serum and liver mRNA levels of apoC-III as well as elevated VLDL triglyceride levels

To determine whether Rev-erbα plays a physiological role in the regulation of apoC-III expression, apoC-III levels were compared between male Rev-erbα-deficient and wild-type mice (38). Liver apoC-III mRNA levels were increased in male mutant mice, whereas hepatic 36B4 mRNA levels measured as control were similar in both groups (**Fig. 8A**). Furthermore, Rev-erbα-deficient male mice exhibited a statistically significant 30% increase in apoC-III concentration compared to wild-type littermates (Fig. 8B). Finally, this increase in apoC-III concentration was associated with a significant increase in serum triglyceride levels (143 ± 18 vs. 214 ± 17 mg/dl, $P < 0.01$) that appeared to be almost exclusively confined to the VLDL fraction as evidenced by FPLC fractionation (Fig. 8C). Preliminary data suggest that serum triglycerides are also increased in female Rev-erbα-deficient mice (data not shown). These data strongly support the idea that Rev-erbα acts as a physiological regulator of apoC-III expression.

DISCUSSION

TRL are considered major risk factors contributing to the pathogenesis of atherosclerosis (1, 2). Since apoC-III is a major determinant of serum triglyceride and remnant lipoprotein metabolism (7, 8), reducing apoC-III gene transcription is a possible therapeutic strategy to reduce serum concentrations of TRL.

In the present work, we identified Rev-erbα as a dominant repressor of apoC-III promoter activity. A Rev-erbα response element was located in the −23/−18 position of the human apoC-III promoter, data which are in line with similar in vitro findings reported by Coste et al. (39) while this work was in progress. This site, which coincides with the previously identified RORα1 response element (21), consists of a perfect AGGTCA half-site preceded by an A/T rich region that deviates from the optimal consensus only by a C in position −1 (27). Our results demonstrate that this sequence is transcriptionally active in the context of a natural promoter. As in the rat apoA-I and mouse apoC-III promoters, the human apoC-III ROR/Rev-erb response element is located downstream of the TATA box, which provides the AT-rich region required to confer RORα1 and Rev-erbα responsiveness to the PuGGTCA half site. This indicates that the apoC-III ROR/Rev-erb response site lies in a particular context with a potentially strong functional impact. It remains to be determined whether modulation of transcription by Rev-erbα or RORα1 involves interaction with TATA-Box-binding proteins and whether the presence of a ROR/Rev-erb response element downstream of the TATA box is a frequent feature.

The significant increase in serum apoC-III concentra-

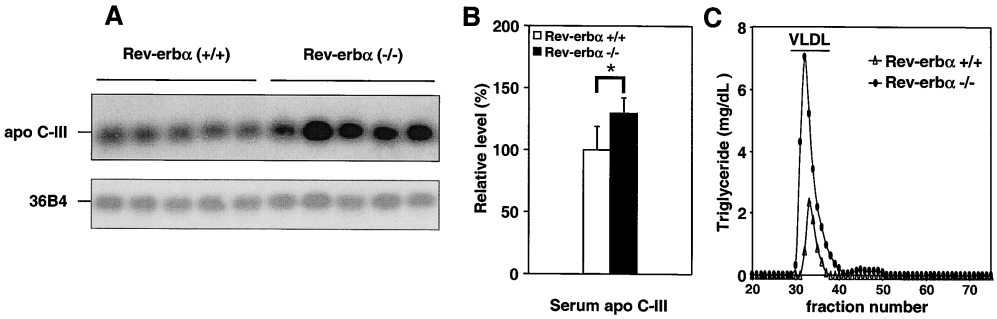

Fig. 8. Rev-erbα-deficient mice have elevated serum triglyceride and apoC-III concentrations and apoC-III liver mRNA levels. Male Sv129OlaHsd × BALB/c homozygous wild-type and Rev-erbα deficient mice previously described (38) received a standard rodent chow. A: Representative Northern blot analysis showing liver apoC-III mRNA levels in Rev-erbα wild-type (+/+) and deficient (−/−) mice. The 36B4 cDNA was used as control probe. B: Serum apoC-III concentrations of Rev-erbα wild-type (n = 17) and deficient (n = 21) mice were measured as described in Materials and Methods. The results are expressed in percent as compared to the control mice. Each value represents the mean ± SD. Statistically significant differences between the two genotypes are indicated by asterisk (Mann-Whitney test, $*P < 0.05$). C: Representative triglyceride lipoprotein distribution profiles of pooled plasma from Rev-erbα wild-type (n = 17) and Rev-erbα deficient (n = 21) mice receiving a standard rodent chow as described in Materials and Methods.

tions and in liver apoC-III mRNA levels of Rev-erbα-deficient mice as compared to wild-type mice indicates that Rev-erbα controls apoC-III gene expression in vivo, suggesting that the effect of Rev-erbα on apoC-III gene expression is physiologically relevant and extends the data reported by Coste et al. to the in vivo situation (39). Although apoC-III plays an important role in intravascular triglyceride metabolism (7, 8), other genes contribute to the control of their synthesis or degradation and thus influence serum triglyceride concentrations. Further studies are required to determine whether, in addition to its effects on apoC-III expression, Rev-erbα modulates serum triglyceride metabolism via such additional, complementary mechanisms. Since the sequence of the −33/−16 fragment of the human apoC-III promoter that binds Rev-erbα is functionally conserved in the mouse promoter (42), it is likely that Rev-erbα also plays a role as physiological repressor of apoC-III expression in man. The Rev-erb orphan receptor subfamily consists of two different genes, Rev-erbα and Rev-erbβ, that both bind similar response elements (29). We observed that human Rev-erbβ can also repress human apoC-III promoter activity (E. Raspé, unpublished observations). Our results demonstrating altered serum apoC-III concentrations in Rev-erbα-deficient mice suggest that mouse Rev-erbβ is not able to fully substitute for Rev-erbα, even though both receptors are expressed in the liver (29). Nevertheless, it is anticipated that double knockout mice will display an even more severe phenotype.

The apoC-III gene is located on chromosome 11q23 between the apoA-I and apoA-IV genes (19). ApoA-I, the major protein constituent of HDL and apoA-IV, also present in HDL (43), are both involved in reverse cholesterol transport and have a protective impact on atherosclerosis (44). Due to the protective roles of apoA-I and apoA-IV against atherosclerosis, normolipidemic treatments should therefore aim at reducing apoC-III levels without negatively affecting apoA-I and apoA-IV expression. Interestingly, although RORα1 activates rat apoA-I gene expression (45), whereas Rev-erbα represses it via the same response element, the corresponding site is not conserved in the human apoA-I promoter (37). Therefore, human apoA-I promoter activity remains unaffected by RORα1 or Rev-erbα (21, 37). Since the repression of the apoC-III promoter activity by Rev-erbα is dominant and since basal activity of the human apoC-III promoter in rat hepatocytes is increased when the Rev-erbα response element is mutated, our results suggest that Rev-erbα is a valuable therapeutic target that will reduce the human apoC-III expression without adverse effect on human apoA-I expression.

Fibrates or other β-blocked fatty acids that activate PPARα are potent hypolipidemic drugs used in the treatment of hypertriglyceridemia. In addition to other pleiotropic effects, these compounds were shown to reduce apoC-III expression in vivo and in vitro (14–17). The mechanism by which fibrates downregulate apoC-III gene transcription is not known but clearly involves PPARα (41). PPARα/RXRα heterodimers bind to the C3P site of the proximal human apoC-III promoter (16). However, this site, when cloned in front of a heterologous promoter, is activated by PPARα/RXRα heterodimers in the presence of their ligands (18). Therefore, the negative effect of fibrates on apoC-III gene transcription is probably indirect. Bar Tana and colleagues proposed that HNF-4 expression is reduced following PPARα activation and that PPARα/RXRα heterodimers could compete with binding of HNF-4 to the C3P site, thereby reducing the activity of this site (16). However, we did not observe any down regulation of HNF-4 expression by fibrates (37). Since Rev-erbα expression is induced by fibrates via a PPARα-response element in the Rev-erbα gene promoter (31), our results suggest that PPARα may indirectly repress apoC-III gene transcription at least in part by increasing liver Rev-erbα gene expression. Further studies are required to address these issues.

ApoC-III gene promoter activity is controlled by a variety of transcription factors acting in concert, amongst which several nuclear receptors (20). Interestingly, RORα has been shown to be a positive regulator of hepatic apoC-III transcription binding to the same response element in the apoC-III promoter as Rev-erbα (21), suggesting that the relative activity levels of both receptors determine a balance controlling apoC-III expression. Moreover, Rev-erbα also decreases apoC-III promoter activation by the nuclear receptor HNF-4, a key regulator of apoC-III transcription that binds to distinct sites in the promoter (20). Altogether, these observations suggest a contributing role of Rev-erbα in apoC-III regulation. Hence, the crosstalk between several nuclear receptor pathways might be physiologically important for the control of apoC-III. In the same line, the expression of rat apoA-I is controlled by both RORα (45) and Rev-erbα (37). Unbalanced action of any of these receptors could therefore play a role in the pathogenesis of the dyslipidemia predisposing to atherosclerosis as already observed with RORα (46).

So far, no natural ligand has been identified for Rev-erbα. The lack of an AF2 transactivation domain in the hRev-erbα ligand-binding domain rather suggests that it is unlikely that such ligand exists (23). Hence, its activity will probably be defined mainly by its expression level or by post-transcriptional modifications. Further characterization of the mechanisms regulating its expression or activity, e.g. via phosphorylation, will therefore be of great interest to identify factors influencing serum triglyceride levels.

In conclusion, our observations that human apoC-III promoter activity is decreased by hRev-erbα and that Rev-erbα-deficient mice display increased liver mRNA and serum apoC-III levels identify Rev-erbα as a modulator of apoC-III expression in mice and humans. These data suggest that hRev-erbα would be a valuable target for the development of hypotriglyceridemic agents.

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