Transcriptional Regulation of Apolipoprotein C-III Gene Expression by the Orphan Nuclear Receptor RORα*

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Triglyceride-rich remnant lipoproteins are considered as major risk factors contributing to the pathogenesis of atherosclerosis. Because apolipoprotein (apo) C-III is a major determinant of plasma triglyceride and remnant lipoprotein metabolism, it is important to understand how the expression of this gene is regulated. In the present study, we identified the orphan nuclear receptor RORα as a regulator of human and mouse apo C-III gene expression. Plasma triglyceride and apo C-III protein concentrations in staggerer (sg/sg) mice, homozygous for a deletion in the RORα gene, were significantly lower than in wild type littermates. The lowered plasma apo C-III levels were associated with reduced apo C-III mRNA levels in liver and intestine of sg/sg mice. Transient transfection experiments in human hep- atoma HepG2, human colonic CaCO2, and rabbit kidney RK13 cells demonstrated that overexpression of the human RORα isoform specifically increases human apo C-III promoter activity, indicating that RORα enhances human apo C-III gene transcription. RORα response elements were mapped by promoter deletion analysis and gel shift experiments to two AGGTCA half-sites located at positions −83/−78 (within the C3P site) and −23/−18 (downstream of the TATA box) in the human apo C-III promoter, with the −23/−18 site exhibiting the highest binding affinity. Transfection of site-directed mutated constructs in HepG2 cells indicated that the RORα effect is predominantly mediated by the −23/−18 site. This site is conserved in the mouse apo C-III gene promoter. Moreover, RORα binds to the equivalent mouse site and activates constructs containing three copies of the mouse site cloned in front of an heterologous promoter. Taken together, our data identify RORα as a transcriptional regulator of apo C-III gene expression, providing a novel, physiological role for RORα in the regulation of genes controlling triglycereal metabolism.

Several epidemiological studies support the idea that, in addition to elevated low density lipoprotein and reduced high density lipoprotein cholesterol, elevated triglycerides constitute an independent risk factor for coronary heart disease (1–4). More specifically, triglyceride-rich lipoprotein remnants are positively correlated to the progression of atherosclerosis (5, 6). Identifying the factors or genes controlling triglyceride metabolism is therefore of major importance and may provide means for pharmacological intervention in dyslipidemic patients.

Apolipoprotein (apo) C-III is a 79-amino acid glycoprotein synthesized in the liver and, to a lesser extent, in the intestine, that plays a key role in plasma triglyceride metabolism as evidenced by pharmacological (7–9), clinical (10, 11), genetic (12), and experimental data in transgenic animal models (13). Apo C-III concentrations in plasma are positively correlated with plasma triglyceride levels, both in the normal population as well as in hypertriglyceridemic patients (10, 11, 14) or in transgenic animals (15). Moreover, apo C-III deficiency in humans (16) or apo C-III gene disruption in transgenic mice (17) results in increased catabolism of very low density lipoprotein particles, whereas increased apo C-III synthesis occurs in hypertriglyceridemic patients (18). Results from both in vivo (19–22) and in vitro (23–25) studies indicate that apo C-III delays the catabolism of triglyceride-rich particles. Several potential mechanisms may participate in the inhibitory effect of apo C-III on triglyceride catabolism. These include inhibition of lipolysis by lipoprotein (16, 26) or hepatic lipase (27), inhibition of triglyceride-rich particle binding to glycosaminoglycans (22), as well as interference with apo E-mediated receptor clearance of remnant particles from plasma (19, 20, 22, 25).

Apo C-III gene expression is tightly regulated, being down-regulated by hormones such as insulin (28, 29) or thyroid hormones (30), cytokines such as interleukin-1 (31) or tumor necrosis factor α (32), as well as hypolipidemic drugs such as fibrates (7, 33) or β-blocked fatty acids (8, 34). By contrast, its expression is increased by retinoids (9). Regulatory sequences determining the tissue-specific expression pattern of apo C-III have been delineated in its gene (35–38). The C3P site located at position −87/−67 relative to the transcription start site is a major determinant of apo C-III promoter activity (35, 36). It contains a direct repeat of two AGGTCA half-sites separated by one nucleotide (DR-1) to which the nuclear hormone receptors HNF-4, PPAR, RXR, Ear2, COUP-TF-1, and COUP-TF-II are binding (8, 9, 37, 39, 40). Whereas Ear2, COUP-TF-1, and COUP-TF-II repress apo C-III promoter activity via these sites (37, 39), HNF-4 activates it (35–37, 39, 40). PPAR/RXR heterodimers also enhance the activity of reporter construct containing the C3P site cloned in front of an heterologous promoter (9). In addition, the −592/−792 fragment of the apo C-III

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†‡ The abbreviations used are: apo, apolipoprotein; CAT, chloramphenicol acetyltransferase; PAGE, polyacrylamide gel electrophoresis.
promoter acts as an enhancer that potentiates the strength of the proximal apo C-III promoter (38). Functional positive HNF-4 and Sp1 binding sites as well as negative COUP-TF-I and COUP-TF-II binding sites have been mapped in this region (38, 40). In addition, a CCAAT/enhancer binding protein δ binding site located in the proximal apo C-III promoter (−171/−137) seems to be involved in the negative regulation of apo C-III expression by interleukin-1 (31). Finally, T,Rβ (41), ATF-2 (42), NFκB (43), and Jun (42) regulatory elements have also been identified in the human apo C-III promoter.

The ROR (retinoic acid receptor related orphan receptor; also termed RZR) orphan receptors (44–46) are a subfamily of orphan nuclear receptors consisting of three different genes RORα, β, and γ (44, 46, 47). RORs were initially reported to bind as monomers to response elements consisting of a 6-base pair AT-rich sequence preceding the half-core PuGGTCA motif (44, 48, 49), but more complex response elements have also been described (50, 51). Because of alternative splicing and promoter usage, the RORa gene gives rise to four isoforms: α1, α2, α3, and RZRα (44–46), which differ in their N-terminal domains and display distinct DNA recognition and transcriptional properties (44). In contrast to RORβ, the expression of which is restricted to brain, retina, and pineal gland (52), both RORα and RORγ are widely expressed in peripheral tissues (44, 45, 47, 49). Based on the presence of putative response elements in their promoter, several target genes for ROR subfamily members were proposed and analyzed in vitro (53–56). A role for RORα1 has been proposed in muscle differentiation (57), whereas RORγ expression is induced during adipose tissue differentiation (58). Transgenic mice have been developed that carry a deleted RORα gene (59, 60). Their phenotype is similar to the one of staggerer mice, which carry a natural deletion in the RORα gene that prevents the translation of its putative ligand-binding domain, thereby presumably disrupting the normal function of this transcription factor (61). These mice exhibit deficient intestinal apo A-I expression (62), suggesting that the mouse apo A-I gene is an in vivo target of RORα. Moreover, when maintained on a high fat atherogenic diet staggerer (sg/sg) mice develop a severe hypo-alphalipoproteinemia and atherosclerosis, suggesting an important role for RORα in cardiovascular and metabolic diseases (63).

In the present study, we investigated the regulation of apo C-III expression and triglyceride metabolism by the orphan nuclear receptor RORα in vivo using the staggerer mouse model. We observed a striking reduction in both triglyceride and apo C-III plasma levels in mutant compared with wild type mice. Next, we studied in vitro the molecular mechanisms regulating apo C-III gene transcription by RORα. Our results indicate that RORα enhances the activity of the human −1415/+24 apo C-III promoter. Furthermore, a RORα response element was identified at position −23/−18 that confers RORα responsiveness to the human apo C-III promoter. This response element is preserved in both the mouse and the human apo C-III gene promoters and confers RORα responsiveness to a heterologous promoter. Taken together, our results identify RORα as a positive regulator of apo C-III gene transcription and support a role of RORα as a regulator of lipid and lipoprotein metabolism.

**MATERIALS AND METHODS**

*Mice—staggerer* mutant mice were obtained by crossing heterozygote (+/sg) mice maintained in a C57BL/6 genetic background and identifying homozygous offspring by polymerase chain reaction genotyping and by their clinical ataxia. Mice were maintained on chow diet purchased from UAR (France) as described previously (62). Wild type littermates of the same age as the homozygous mutants were used as control. 10-week-old mice fasted overnight were killed by ether overdose. Blood, liver, and intestine samples were taken and stored for further analysis.

**RNA Analysis**—RNA extractions, Northern blot hybridizations, and measurements of mRNA levels were performed as described previously (33) using rat apo C-III (33) and 36B4 control probes (64). Autoradiograms were analyzed by quantitative scanning densitometry (Bio-Rad Gel Documentation system) as described (33).

**Lipid and Lipoprotein Analysis**—Plasma triglyceride concentrations were determined by enzymatic assays using commercially available reagents (Roche Molecular Biochemicals), whereas plasma levels of apo C-III were measured by an immunonephelometric assay using a specific polyclonal antibody as described previously (65).

**Cloning of Recombinant Plasmids**—The plasmid containing the −117 to −89 region of the mouse apo C-III gene promoter cloned in front of the chloramphenicol acetyltransferase (CAT) reporter gene (−1415/+24WT-CAT) has been described previously (9). The luciferase gene from the plasmid pGL3 (Promega, Madison, WI) (Saci/BamHI) was subcloned between the corresponding sites of the vector pBKCMV (Stratagene, La Jolla, CA) (pBKCMV-Luc+). The CAT reporter gene of the plasmid −1415/+24WT-CAT was then excised by digestion with Kpn1 and BamHI and replaced by the luciferase-containing Kpn1/BgII fragment of the plasmid pBKCMV-Luc+ plasmid. The −1415/+24 fragment of the apo C-III promoter was excised from the resulting construct by HindIII digestion and cloned in the corresponding site of the vectors pGL3 (construct −1415/+24WTpGL3) and pSL301 (Amersham Pharmacia Bio- tech) (construct pSL301−1415/+24HiCII). The pSL301−1415/+24HiCII construct was partially digested with EcoO109I and the resulting construct was digested with XhoI and HindIII. The insert was cloned in the corresponding sites of pGL3 to create the construct −108/+24WTpGL3. The plasmid −1415/+24WTpGL3 was then used as template to polymerase chain reaction amplify fragments of different length of the human apo C-III promoter using forward primers annealing to specific parts of the promoter sequence and containing a Nhel restriction site and a reverse primer annealing downstream of the pGL3 polylinker. The polymerase chain reaction products were cut with Nhel and HindIII and cloned in the corresponding sites of the pGL3 vector. Site-directed mutagenesis of the construct −1415/+24WTpGL3 was performed using the Quick Change site-directed mutagenesis kit (Stratagene) following the manufacturer’s instructions. The constructs (−58/−27)TKpGL3 and (−47/−79)TKpGL3 were obtained following the described previously strategy (66) based on intermediary cloning in the BamHI and BglII sites of the vector pIC20H using double-stranded oligonucleotides with sequences corresponding to the indicated fragment of the apo C-III promoter flanked by protruding ends compatible with BamHI and BglII sites. The oligonucleotide multimers were excised from pIC20H with SaIl and XhoI and cloned in the XhoI site of the described previously vector TkpGL3 (34). Alternatively, these oligonucleotide multimers were directly cloned into the BglII site of the pTKpGL3 vector after digestion of the BamHI site by Klenow blunting. The construct pCNDNA3-hRORα containing the hRORα cDNA cloned in the Kpn1 and XhoI sites of the pCNDNA3 vector was a gift of Dr. A. Shevelev. The Renilla luciferase gene of the pRenLull construct (Promega) was excised by the enzymes Nhel and XhoI and cloned in the XhoI site of the plasmid pBKCMV. The resulting construct was cut by HindIII and XhoI, and the insert was cloned in the corresponding sites of the pGL3 control vector (Promega) to yield the pRenConT+ construct used to evaluate transfection efficiency.

**Cell Culture and Transient Transfection Assays**—Human hepatoma HepG2, CaCo2 and RK13 cells were obtained from European Collection of Animal Cell Cultures (Porton Down, Salisbury, UK). Cell lines 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% CO2/95% air). Medium was changed every 2 days.

Cells were seeded in 24-well plates at a density of 5 × 104, 6 × 104, or 105 cells/well for RK13, HepG2, or CaCo2, respectively, and incubated at 37 °C for 16 h prior to transfection. Cells were transfected using the cationic lipid RPR 120535B as described previously (34) with reporter plasmids (at 50 ng/well), expression vectors (pCNDNA3 or pCNDNA3-hRORα1 at 100 ng/well), and the control plasmids (pRen-Cont+ at 1 ng/well or pSV-β-gal at 50 ng/well). At the end of the experiment, the cells were washed once with ice-cold 0.15 m NaCl, 0.01 m sodium phosphate buffer, pH 7.2, and the luciferase activity was measured with the Dual-Luciferase™ Reporter Assay System (Promega) according to the manufacturer’s instructions. All transfection experiments were performed at least twice. The β-galactosidase activity was measured as described previously (66). Protein content of the extract was evaluated by the Bradford assay using the kit from Bio-Rad.

**Gel Retardation Assays**—RORα was in vitro transcribed from the pCNDNA3-hRORα plasmid using T7 polymerase and subsequently translated using the TNT-coupled transcription/translation system.
transient cotransfected with the −1415/+24 wtPL3 reporter plasmid (50 ng) containing the −1415/+24 fragment of the human apo C-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) pCDNA3. hRORα (hRORα) or the empty pCDNA3 vector as control (Cont.). Cells were transfected and luciferase activity measured and expressed as described under “Materials and Methods.” The fold induction above control level is indicated for the −1415/+24 wtPL3 construct.

**RESULTS**

**Plasma Triglyceride and apo C-III Concentrations as Well as Hepatic and Intestinal apo C-III mRNA Levels Are Decreased in staggerer Mice**—To determine whether staggerer mice display altered triglyceride metabolism, plasma triglycerides were measured in overnight fasted female staggerer mice and compared with age-matched wild type C57BL/6 littermates (Fig. 1). Interestingly, staggerer mice exhibited 50% lower blood triglyceride levels compared with wild type littermates (Fig. 1A). Because apo C-III is a major determinant of plasma triglyceride levels (13), its plasma concentrations were measured next. A 70% decrease of plasma apo C-III concentration was observed in mutant mice (Fig. 1B). To determine whether this reduction was associated with a decreased expression of the apo C-III gene, hepatic and intestinal apo C-III mRNA levels were analyzed by Northern blotting. Both intestinal and liver apo C-III mRNA levels were reduced in mutant mice compared with wild type mice (Fig. 1C). Hepatic and intestinal 36B4 mRNA levels measured as control were similar in both groups.

**Overexpression of hRORα Enhances the Activity of the Human apo C-III Gene Promoter**—Because staggerer (sg/sg) mice carry a nonfunctional RORα gene (61), the above data suggest that RORα is a positive regulator of apo C-III transcription. Transient transfection assays were performed to determine whether human RORα controls the transcription of the human apo C-III gene. In hepatoma HepG2 cells, which produce apo C-III, cotransfection of a human nuclear receptor RORα expression plasmid resulted in an increased activity of the luciferase reporter gene driven by the −1415/+24 fragment of the human apo C-III gene promoter (Fig. 2A). An activation was also observed in rabbit kidney RK13 cells that do not express apo C-III (Fig. 2B) and in human intestinal CaCO2 cells (Fig. 2C) that produce apo C-III. The effect of hRORα overexpression was promoter-dependent because the promoter-less vector pGL3 was unaffected in all cell lines studied.

**Mapping of the Human apo C-III Promoter Sites Confering Responsiveness to hRORα**—To identify the response element(s) required for hRORα activation of the apo C-III gene promoter, 5’ nested deletions of the apo C-III promoter were cotransfected with the hRORα expression vector in HepG2 cells. Deletion of the promoter led to a decrease in its basal activity (Fig. 3), corroborating previous observations that the −792/−592 fragment of the apo C-III promoter acts as a strong hepatic enhancer (38). However, hRORα activation was still observed with the shortest construct −108/+24wtPl3, indicating that the first 108 nucleotides of the human apo C-III promoter contain sequence determinants sufficient to confer hRORα responsiveness (compare the 3.1-fold induction of the −1415/+24 wtPl3 construct with the 2.6-fold induction of the −108/+24 wtPl3 construct). To verify whether hRORα directly binds to the proximal apo C-III promoter, radiolabeled overlapping oligonucleotides corresponding to portions of the −108/+24 fragment of the apo C-III promoter were used as probes in gel shift assays. Specific binding of in vitro translated hRORα protein was observed only on the −33/−16 and −90/−64 fragments (Fig. 4A). Both fragments contain an AGGTCA half-site preceded by a degenerated AT-rich region that could function as hRORα response element. Binding of hRORα to the −33/−16 fragment of the apo C-III promoter was lost after mutation of the AGGTCA half-site present in position −23/−18 (−33/−16mt: −22g−c, −21g−a) (Fig. 4B). The binding of hRORα to the −33/−16 fragment of the apo C-III promoter was displaced by increasing amounts of either the cold −33/−16 double-stranded oligonucleotide or a cold double-stranded oligonucleotide that contains one copy of the hRORα consensus binding site (Fig. 5A). This binding was not displaced by the mutated cold −33/−16 double-stranded oligonucleotide (Fig. 5A). Binding of hRORα to the −90/−64 fragment corresponding to the C3P site of the human apo C-III gene promoter was specific because it could be displaced by increasing amounts of either cold −90/−64 (Fig. 5B) or

**Fig. 1.** staggerer mice have decreased plasma triglyceride and apo C-III levels associated with decreased hepatic and intestinal apo C-III mRNA levels compared with wild type littermates. Overnight fasted 11-week-old homozygous female staggerer mice carrying a nonfunctional RORα gene and their wild type littermates were killed by ether overdose. Plasma triglyceride (A) and apo C-III levels (B) were measured as described under “Materials and Methods” (four animals/group). Total RNA was extracted from liver and intestinal tissues and analyzed by Northern blotting as described under “Materials and Methods.” C shows the relative apo CIII mRNA levels (three animals/group) as evaluated by quantitative scanning densitometry (Bio-Rad GS670 Densitometer) of the Northern blot presented. The data are expressed as the mean values ± S.D. (Mann-Whitney nonparametric test). *, p < 0.05.

**Fig. 2.** hRORα enhances the activity of the human apo C-III gene promoter. HepG2 (A), RK13 (B), or CaCO2 (C) cells were transiently cotransfected with the −1415/+24 wtPL3 reporter plasmid (50 ng) containing the −1415/+24 fragment of the human apo C-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) pCDNA3. hRORα (hRORα) or the empty pCDNA3 vector as control (Cont.). Cells were transfected and luciferase activity measured and expressed as described under “Materials and Methods.” The fold induction above control level is indicated for the −1415/+24 wtPL3 construct.
hRORα consensus binding site double-stranded oligonucleotides (data not shown). By contrast, binding of hRORα to the −90/−64 fragment of the human apo C-III gene promoter was abrogated when the AGGTCA half-sites located at position −82/−77 was mutated (−90/−64mt: −78G−C, −79G−A; Fig. 4B). No significant binding was observed to other fragments of the proximal human apo C-III promoter (Fig. 4A). Taken together, our results suggest the presence of two binding sites corresponding to the wild type or mutated −33/−16 (−22G−c, −21G−A), and −90/−64 (−78G−c, −79G−A) fragments of the human apo C-III promoter were labeled and incubated with in vitro translated hRORα1 protein or unprogrammed lysate as control. DNA/protein complexes were resolved by nondenaturating PAGE as described under “Materials and Methods.” Specific complexes not observed with unprogrammed lysate are indicated by arrows.

Functional Characterization of hRORα Response Elements Present in the Proximal Human and Mouse apo C-III Promoters—To evaluate whether these two putative response elements were functional in the context of the proximal human apo C-III promoter, the half-sites present downstream of the TATA box in position −23/−18 (−22G−c, −21G−A) or in the C3P site in position −82/−77 (−78G−c, −79G−A) of the human apo C-III promoter were mutated by site-directed mutagenesis in the −1415/+24WTpGL3 construct either alone (−82/−77mt, −23/−18mt, respectively) or in combination (−82/−77mt + −23/−18mt). Mutation of the −82/−77 half-site reduced the basal activity of the apo C-III promoter but did not prevent its activation by hRORα1 (Fig. 6). Mutation of the −23/−18 half-site enhanced the basal activity of the apo C-III promoter in HepG2 cells and abrogated hRORα1 responsiveness. The activity of the promoter and its hRORα1 responsiveness were lost when both half-sites were mutated simultaneously. These data suggest that the −23/−18 half-site plays a major role in the hRORα1 responsiveness of the apo C-III promoter in HepG2 cells.

To evaluate whether these two sites could confer RORa responsiveness to an heterologous promoter and to exclude that the −108/+24 fragment of the apo C-III promoter contains other hRORa1-responsive elements, overlapping fragments of the apo C-III promoter (covering the −100/−16 region of the apo C-III promoter) were cloned in front of a thymidine kinase (Tk) promoter-driven luciferase reporter vector. These constructs were cotransfected with a hRORα1 expression vector in HepG2 cells. The (−33/−16)G5TkGL3 construct was strongly
activated by hRORα, whereas the (−83/−67)4TkplGL3 construct was weakly stimulated (Fig. 7A). The other constructs were not activated by hRORα (Fig. 7A). Both the −33/−16 and −83/−67 fragments contain an AGGTCA half-site preceded by a degenerate A/T-rich region. To evaluate the specificity of RORα action, these half-sites were next mutated to create the constructs (−33/−16mt)4TkplGL3 (−22g−c; −21c−a) and (−87/−67mt)4TkplGL3 (−78g−c; −79c−a) and their wild type homologues were cotransfected in HepG2 cells with pCDNA3-hRORα expression vector (100 ng; hRORα) or empty pCDNA3 vector as control (Cont. B). Cells were transfected and luciferase activity measured and expressed as described under “Materials and Methods.”

Because the sequence of the −33/−16 fragment of the human apo C-III promoter is almost fully conserved in the mouse (67), hRORα binding to the mouse sequence corresponding to this region was analyzed. As shown in Fig. 8A, hRORα bound with similar affinity to the −33/−14 fragment of the mouse apo C-III gene promoter as to the −33/−16 fragment of the human apo C-III gene promoter. To compare its activity with the corresponding human promoter sequence, three copies of the wild type −33/−14 fragment of the mouse apo C-III promoter were cloned in front of the thymidine kinase promoter and tested in cotransfection assay. As shown in Fig. 8B, the human (−33/−16)3TkplGL3 and the mouse (−33/−14)3TkplGL3 were similarly activated by hRORα overexpression in HepG2 cells. This indicates that the AGGTCA half-sites located downstream of the TATA box both in the mouse and human apo C-III promoters are equally functional in the context of a heterologous promoter.

**DISCUSSION**

In the present study, we report that staggerer mice lacking functional orphan nuclear receptor RORα (61) have significantly reduced plasma triglyceride levels compared with wild type controls. These data indicate a physiological role of this receptor in the regulation of plasma triglyceride metabolism in mice.

Because apo C-III plays an important role in intravascular triglyceride metabolism (13), we subsequently evaluated the role of RORα in the control of apo C-III expression. Our observation that the decrease in plasma triglyceride levels observed in staggerer mice is associated with a strong decrease in apo C-III plasma concentrations and hepatic as well as intestinal gene expression provides a possible mechanistic explanation of the phenotype and suggests that apo C-III is a RORα target in mice. Despite the severe phenotype of staggerer mice, few RORα target genes have been identified to date. To the best of our knowledge, beside rat apo A-I, apo C-III is the second apo C-III promoter and cloned upstream of the luciferase reporter gene as described under “Materials and Methods.” The empty TkplGL3 reporter plasmid was used as negative control. Cells were transfected and luciferase activity measured and expressed as described under “Materials and Methods.”
a natural response element with hRORα and demonstrate that this sequence is transcriptionally active in the context of a natural promoter. The −82/−77 site also consists of a perfect AGGTTCA half-site preceded by an A in position −1 and two Gs in positions −3 and −4. This larger divergence from the consensus sequence likely explains the weaker binding of hRORα to this sequence and the weaker transactivation of a construct containing three copies of this sequence cloned in front of a heterologous promoter. Site-directed mutagenesis of both sites confirmed that the −23/−18 half-site plays a major role in hRORα responsiveness of the apo C-III promoter in HepG2 cells. Further, the observation that human apo C-III promoter is unaffected by hROR1/elevated serum triglycerides are considered as an independent genetic diet develop more severe atherosclerosis than wild type (69). This might explain why, despite their decreased hepatic apo C-III levels associated with lowered hepatic apo C-III ex-pressed by overexpressing hROR1, apo C-III promoter activity and, hence, a valuable tar-get of therapy.

The better sensitivity of the apo A-I promoter is unaffected by hROR1/elevated serum triglycerides are considered as an independent genetic diet develop more severe atherosclerosis than wild type (69). This might explain why, despite their decreased hepatic apo C-III levels associated with lowered hepatic apo C-III ex-pressed by overexpressing hROR1, apo C-III promoter activity and, hence, a valuable tar-get of therapy.

The sequence of the −33/−16 fragment of the human apo C-III promoter is almost fully conserved in the mouse promoter (67). We observed hRORα binding to the −33/−14 fragment of the apo C-III promoter and activation of a reporter construct containing three copies of this site cloned in front of an heterologous promoter. This suggests that RORα could also act via this site on the mouse promoter.

Although elevated triglycerides likely affect atherosclerosis in humans, the effect of hypertriglyceridemia in mice is modest (69). This might explain why, despite their decreased hepatic apo C-III gene expression (63), staggerer mice fed an athero-genic diet develop more severe atherosclerosis than wild type mice. In humans, a different picture may be expected because elevated serum triglycerides are considered as an independent risk factor for coronary heart disease and because the human apo A-I promoter is unaffected by hRORα (data not shown). The enhancement of human apo C-III gene promoter activity by overexpressing hRORα in HepG2 cells suggests that it acts at the transcriptional level. Hence, hRORα could be a valuable target for the development of hypotriglyceridemic agents.

In conclusion, the strong decrease in plasma triglyceride and apo C-III levels associated with lowered hepatic apo C-III expression observed in staggerer mice lacking functional RORα identifies RORα as a modulator of triglyceride levels in mice. Furthermore, the observation that human apo C-III promoter activity was also enhanced by hRORα extends the mice data to humans and suggests that hRORα is another modulator of human apo C-III promoter activity and, hence, a valuable target for the development of hypotriglyceridemic agents.
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