The Liver X Receptor Ligand T0901317 Down-regulates APOA5 Gene Expression through Activation of SREBP-1c*

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Alterations in the expression of the recently discovered apolipoprotein A5 gene strongly affect plasma triglyceride levels. In this study, we investigated the contribution of APOA5 to the liver X receptor (LXR) ligand-mediated effect on plasma triglyceride levels. Following treatment with the LXR ligand T0901317, we found that APOA5 mRNA levels were decreased in hepatoma cell lines. The observation that no down-regulation of APOA5 promoter activity was obtained by LXR-retinoid X receptor (RXR) co-transfection prompted us to explore the possible involvement of the known LXR target gene SREBP-1c (sterol regulatory element-binding protein 1c). In fact, we found that co-transfection with the active form of SREBP-1c down-regulated APOA5 promoter activity in a dose-dependent manner. We then scanned the human APOA5 promoter sequence and identified two putative E-box elements that were able to bind specifically SREBP-1c in gel-shift assays and were shown to be functional by mutation analysis. Subsequent suppression of SREBP-1 mRNA through small interfering RNA interference abolished the decrease of APOA5 mRNA in response to T0901317. Finally, administration of T0901317 to APOA5 transgenic mice revealed a significant decrease of APOA5 mRNA in liver tissue and circulating apolipoprotein AV protein in plasma, confirming the described down-regulation also occurs in vivo. Taken together, our results demonstrate that APOA5 gene expression is regulated by the LXR ligand T0901317 in a negative manner through SREBP-1c. These findings may provide a new mechanism responsible for the elevation of plasma triglyceride levels by LXR ligands and support the development of selective LXR agonists, not affecting SREBP-1c, as beneficial modulators of lipid metabolism.

Numerous epidemiological studies have revealed that, in addition to elevated low density lipoprotein and reduced high density lipoprotein levels, elevated plasma triglyceride (TG) level is an independent risk factor for coronary heart disease (1, 2). Thus, understanding the regulation of genes that influence triglyceride levels is of prime interest and may aid in the development of therapies to reduce hypertriglyceridemia and the associated risk of atherosclerosis. The apolipoprotein gene family represents one class of molecules that affect plasma lipid levels. Apolipoprotein AV (apoAV) is a newly described member of the apolipoprotein family that was recently identified through human-mouse comparative sequence analysis (3). The APOA5 gene appears mainly expressed in the liver, where its gene product (apoAV) is exported into plasma and associates with high density lipoprotein and very low density lipoprotein particles. Studies in mice have revealed a crucial role of apoAV in plasma triglyceride metabolism with over- and underexpression in animals leading to decreased and increased triglyceride levels, respectively (3). Furthermore, in humans, several single nucleotide polymorphisms across the APOA5 locus were found to be significantly associated with high plasma triglyceride levels and very low density lipoprotein mass in several ethnic groups (4–8).

It has been reported that the APOA5 gene is regulated by peroxisome proliferator-activated receptor-α and farnesoid X receptor ligands, both nuclear receptors implicated in triglyceride metabolism (9, 10). However, although administration of liver X receptor (LXRs) ligands is known to affect triglyceride metabolism in mice, their relationship to APOA5 has not been described (11). LXRs are members of the nuclear receptor family that play a crucial role in cholesterol, fatty acid, and glucose metabolism (12, 13). The availability of synthetic LXR agonists such as T0901317 has provided additional insight into metabolic consequences of LXR activation. Oral administration of LXRs ligands to low density lipoprotein receptor−/− and apolipoprotein E−/− mice revealed their potential antiatherogenic role (14, 15). Treatment of C57BL/6 mice with T0901317 induced expression of a panel of genes involved in liver fatty acid biosynthesis and caused hepatic TG accumulation. An increase of high density lipoprotein cholesterol, phospholipids, and very low density lipoprotein triglycerides in the plasma was also reported (11, 16). The lipogenic effect of LXR ligands in the liver was mainly attributed to the activation of sterol regulatory element-binding protein 1c (SREBP-1c) (12, 17).

SREBP's are members of the basic-helix-loop-helix-leucine zipper family of transcription factors. Three different SREBP's have been described. SREBP-1a and SREBP-1c are produced from a single gene through the use of alternative promoters that produce transcripts with different first exons. The third isoform, SREBP-2, is produced from a separate gene (18). SREBP-1c is a transcription factor with preferential specificity for fatty acid and triglyceride metabolism and is the only member of this family being activated by LXRs and insulin (17, 19, 20).
Although SREBP-1c is mainly expressed in hepatic cell lines, SREBP-1c is predominantly found in liver and thus plays a central role in human physiology (21). Activation of SREBP-1c induces de novo lipogenesis in the liver through induction of lipogenic genes such as acetyl-CoA-carboxylase, fatty acid synthase, and glycerol-3-phosphate acyltransferase (22–24). SREBPs are synthesized as precursors bound to the endoplasmic reticulum and the nuclear membranes. Proteolytic cleavage of the precursor generates a mature, active form of the transcription factor that migrates into the nucleus, where it can bind to both sterol regulatory elements (5′-TCACCCGCCA-3′) and E-box elements (5′-CTTGGTCTCCAGCTCAG-3′) of target genes (25–27).

In this study, we investigated the effect of LXR ligands on APOA5 gene regulation since LXR ligands influence very low density lipoprotein-TG metabolism. We show that the widely used LXR ligand T0901317 down-regulates APOA5 gene expression via an indirect pathway involving its target gene SREBP-1c. Thus, our findings provide new insights into the transcriptional regulation of APOA5 and the mechanism by which LXR ligands may elevate plasma TG levels.

**EXPERIMENTAL PROCEDURES**

**Plasmid Cloning and Site-directed Mutagenesis—**Human APOA5 promoter fragments −845/+63, −304/+63, and −146/+63 were cloned in the pGL3 luciferase vector as reported previously (10). The −61/+63 APOA5 promoter fragment was amplified by PCR using a APOA5 genomic bacterial artificial chromosome clone as template using the forward oligonucleotide 5′-CAAGTGGTGCCAGAAGGTCTAC-3′ and the reverse oligonucleotide 5′-AATTGCCTCCCTTATAGGACTGTGAC-3′ and was also cloned in the luciferase-driven pGL3 basic vector. The same vector was used to produce the plasmid containing E-box 1 in a three tandem repeats in sense orientation (5′-CATGCCTCCCTTATAGGACTGTGAC-3′). Site-directed mutagenesis (Stratagene) were done with following primers (mutated bases are indicated in bold): E-box 1, 5′-CTG TCC GCA GAC GGT GCC GGC GAC CAG CGT GG-GG-3; E-box 2, 5′-GTG TCG AAC TCC CCC CCG GGT TTT ACT CAG CGT-3; SP1, 5′-GAG CAG CTA AGA AAG AGG GAT GGG-3.

**Cell Culture and Transfection Assays—**Hepatoma HepG2 and HuH7 cells were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, streptomycin/penicillin, sodium pyruvate, glutamine and nonessential amino acids (Invitrogen) at 37 °C in a humidified 5% CO2 atmosphere. The medium was changed every 48 h.

HepG2 cells were treated with 2 μM T0901317 for 12, 24, and 48 h, and HuH7 cells were treated for 24 h with the indicated ligands. Total mRNA was subsequently isolated. T0901317 was provided by Genfit SA (Loos, France). HepG2 cells were transfected with the calcium phosphate precipitation method in 24-well plates as described (28). 300 ng of the indicated firefly luciferase reporter plasmids was co-transfected with 1, 10, 25, and 50 ng of the nuclear form of human SREBP-1c, SREBP-1a, or pCDNA3 (Invitrogen). After 3 h, cells were washed with phosphate-buffered saline and incubated for 48 h in 10% fetal calf serum. Transfection efficiency was monitored by co-transfecting 300 ng of a cytomegalo-virus-driven β-galactosidase expression plasmid. Transfection experiments were done at least three times in triplicate. Data were represented as fold-induction over the normalized activity of the reporter plasmid in the absence of nuclear receptor co-transfection and agonist supplementation. The data were expressed as means ± S.D.

**Preparation of Nuclear Protein Extracts and Western Blot Analysis—**Nuclear extracts from HuH7 cells were prepared as described previously (30). Protein concentration was determined with the Bio-Rad protein assay. 10 μg of nuclear proteins was analyzed by Western blot using the SREBP-1 antibody (Santa Cruz) and detected by enhanced chemiluminescence (ECL) (Amersham Biosciences).

**Animal Studies—**Female human APOA5 transgenic mice (3) in a C57BL/6 background (Charles River Laboratories) as well as C57BL/6 wild-type mice were divided into two groups randomized on serum TG levels (n = 6 for each subgroup). The mice were maintained on a standard rodent chow diet and dosed with 20 mg/kg T0901317 or vehicle alone (0.5% methylcellulose) for 3 days. Animals were sacrificed after a 4-h fasting period. Blood was obtained by retro-orbital puncture, and livers were collected and snap-frozen for further mRNA analysis.

RNA analysis of liver samples used for RNA extraction and cDNA synthesis was carried out by using a mirVana mRNA isolation kit (Ambion). cDNA synthesis was performed with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). PCR amplification was performed using the Brilliant quantitative PCR core reagent kit as recommended by the manufacturer (Stratagene) in a volume of 25 μl containing 200 nM each primer and 2.5 mM MgCl2. The following parameters were employed for PCR amplification: 95 °C for 10 min, 40 cycles of 30 s at 95 °C, 30 s at 55 °C, and 30 s at 72 °C. APOA5 and SREBP-1c levels were subsequently normalized to 36B4. Independent experiments were done at least three times in triplicate. mRNA levels were expressed relative to nontreated cells as means ± S.D. Statistical significance was assessed by Student’s t test.

Comparison of APOA5 mRNA expression between different cell types was done by semiquantitative RT-PCR. APOA5, LXRα, as well as SREBP-1c mRNA was amplified with the above described primers and normalized to 28 S ribosomal protein. The resulting products were separated on a 2% agarose gel, stained with ethidium bromide, and quantified with the Gel Doc 2000 apparatus (Bio-Rad).

**Synthesis and Transfer of Small Interfering RNAs (siRNAs)—**The following DNA oligonucleotide templates were synthesized (QiaGen) for use in a T7 polymerase-based in vitro transcription reaction (Silencer siRNA construction kit, Ambion) to generate double-stranded 21-bp RNA with dideoxynucleotide overhangs complementary to a genomic bacterial artificial chromosome clone as template using the forward oligonucleotide 5′-CAAGTGGTGCCAGAAGGTCTAC-3′ and the reverse oligonucleotide 5′-AATTGCCTCCCTTATAGGACTGTGAC-3′ and was also cloned in the luciferase-driven pGL3 basic vector. The same vector was used to produce the plasmid containing E-box 1 in a three tandem repeats in sense orientation (5′-CATGCCTCCCTTATAGGACTGTGAC-3′). Site-directed mutagenesis (Stratagene) were done with following primers (mutated bases are indicated in bold): E-box 1, 5′-CTG TCC GCA GAC GGT GCC GGC GAC CAG CGT GG-GG-3; E-box 2, 5′-GTG TCG AAC TCC CCC CCG GGT TTT ACT CAG CGT-3; SP1, 5′-GAG CAG CTA AGA AAG AGG GAT GGG-3.

**RESULTS**

T0901317 Decreases APOA5 mRNA Levels in Human Hepatoma HepG2 and HuH7 Cells—To test the effect of LXR ligands on APOA5 mRNA expression, human hepatoma cells were incubated over a time course with 1 or 2 μM T0901317, and APOA5 mRNA levels were determined by real-time PCR (Figs.
1 and Fig. 2A). We found that treatment of HepG2 cells for 12, 24, and 48 h with 1 μM T0901317 resulted in a 20–25% decrease of APOA5 mRNA levels (Fig. 1). The repressive effect of T0901317 on APOA5 mRNA levels was confirmed in HuH7 cells (Fig. 2A). Thus, after 24 h of treatment with 1 and 2 μM T0901317, a dose-dependent decrease of APOA5 mRNA levels was obtained (63 and 78% decrease, respectively). Treatment with 2 μM T0901317 and 10 μM 9-cis-retinoic acid resulted in a further repression of APOA5 mRNA levels (88%), suggesting that the heterodimer LXR/RXR is involved in this regulation (Fig. 2A). Additionally, we measured SREBP-1c mRNA (Fig. 2B) and protein levels (Fig. 2C), and consistent with previous reports, we found that they were significantly elevated after treatment with T0901317. These data demonstrate that APOA5 mRNA levels are decreased by the LXR ligand T0901317 in both HepG2 and HuH7 cells, accompanied by an elevation of mature SREBP-1c protein.

Expression of APOA5 and SREBP-1c in Human Cells—Previous data indicate that APOA5 mRNA expression is restricted to liver (3, 32). We analyzed APOA5 mRNA expression by semiquantitative RT-PCR (Fig. 3) in APOA5 Tg mice, human primary hepatocytes, hepatoma HepG2, and HuH7 cells (duplicates are from independent experiments). The highest levels of APOA5 mRNA were detected in APOA5 Tg mice. Human primary hepatocytes expressed APOA5 mRNA at higher levels than the hepatoma cell lines HepG2 and in HuH7 (1/3 and 2/3 lower expression, respectively). Consistent with the specific hepatic expression, no APOA5 mRNA was detected in smooth muscle cells and human primary macrophages. LXRα (Fig. 3B), SREBP-1c (Fig. 3C), as well as 28 S mRNA levels (Fig. 3D) were amplified in the same samples.

Thus, our analysis revealed both hepatoma cell lines to be a suitable model for the study of APOA5 gene regulation. The study of both cells lines was of further interest since the ratio of APOA5 mRNA expression as compared with SREBP-1c and LXRα expression is different.

Co-transfection of SREBP-1c Down-regulates APOA5 Promoter Activity—To determine the mechanism responsible for T0901317-mediated down-regulation of APOA5 mRNA, we first performed co-transfection assays with LXR and RXR expression plasmids. Surprisingly, no down-regulation of the APOA5 promoter was detected (data not shown). Thus, we tested whether the LXR target gene SREBP-1c may be responsible for this down-regulatory mechanism. We transiently transfected HepG2 cells with the −304/+63 luciferase-driven APOA5 promoter and different concentrations of a plasmid encoding the active nuclear form of SREBP-1c. Indeed, we observed a significant dose-dependent down-regulation of APOA5 promoter activity after co-transfection with 1, 10, and 50 ng of SREBP-1c (30, 50, and 60% down-regulation, respectively) (Fig. 4A). No further down-regulation was obtained with 100 ng of SREBP-1c. Similar negative effects were obtained when HuH7 cells were transfected or when the SREBP-1c rat homologue, ADD1, was employed (data not shown).

Further, we addressed the question of whether this down-regulation is restricted to the SREBP-1c isoform. Co-transfection assays indicate that APOA5 promoter activity is also down-regulated by SREBP-1a (Fig. 4B) in a dose-dependent manner (7, 20, 45, and 60% down-regulation by 1, 10, 50, and 100 ng of SREBP-1a, respectively). These results suggest that APOA5 is negatively regulated by SREBP-1 at the transcriptional level.
SREBP-1c Is Necessary for Trans-repression of APOA5 mRNA after Treatment with T0901317—

To further examine whether SREBP-1c may mediate the negative effect of T0901317 on APOA5 mRNA levels, we attempted to suppress the SREBP-1c gene product through RNA interference. We produced siRNAs specific for SREBP-1 since the small difference between the two isoforms did not allow the design of a siRNA specific for SREBP-1c. The fact that SREBP-1a is not regulated by T0901317 supports that this would not confound the interpretation of this approach. As shown in Fig. 5A, transfection with SREBP-1 siRNA significantly decreased the expression of its respective mRNA in the absence and presence of T0901317 (about 70 and 60%, respectively). We confirmed that APOA5 mRNA levels were significantly decreased after treatment with T0901317 when cells were transfected with nonspecific control siRNA (Fig. 5B). In contrast, when SREBP-1c mRNA levels were reduced by the SREBP-1-specific siRNA, treatment with T0901317 did not further decrease APOA5 mRNA levels (Fig. 5B). Taken together, these results support the hypothesis that SREBP-1c is responsible for the repressive effect of T0901317 on APOA5 gene expression.

Identification of Two SREBP-response Elements in the Human APOA5 Promoter—

To determine the region that confers transcriptional responsiveness to SREBP-1c, APOA5 deletion constructs were co-transfected with SREBP-1c into HepG2 cells. The activity of all promoter fragments was markedly repressed by SREBP-1c (Fig. 6A), suggesting a proximal localization of the response element. Through sequence analysis of the promoter, we identified two potential E-box elements, which are known SREBP binding sites. APOA5 E-box 1 (EB1-A5) resides at −61/+63, whereas a second E-box (EB2-A5) was found at −76/-81 in the APOA5 promoter. A diagram of the APOA5 promoter region and its transcription factor binding sites is available in Fig. 6B.

Binding of SREBP-1c to Two Potential E-box Elements in the APOA5 Promoter—

Electromobility shift assays were performed to determine whether the two identified E-box elements are capable of binding SREBP-1c (Fig. 7). Both oligonucleotides, containing E-box 1 (EB1-A5) or E-box 2 (EB2-A5), bound SREBP-1c, with a higher affinity binding for E-box 1. In contrast, the oligonucleotides containing mutated E-box 1 or E-box 2 were unable to bind SREBP-1c (Fig. 7A). The binding speci-
ficity of SREBP-1c to these E-boxes was further demonstrated by the competitive inhibition of 10-, 50-, and 100-fold excess of unlabeled consensus E-box designed for SREBPs (Fig. 7, B and C, lanes 3–5). Oligonucleotides containing the mutated E-box consensus sequence did no more inhibit binding to both E-box elements (Fig. 7, B and C, lanes 6–8). Furthermore, isofrom SREBP-1a was also capable of binding both E-box elements (data not shown). Thus, gel-shift assays revealed a physical interaction between SREBP-1 proteins and two E-box motifs in the proximal APOA5 promoter.

**SREBP-1c Mediates Down-regulation of APOA5 Promoter Activity via E-box Elements—** To test whether the identified E-box elements are responsible for SREBP-1c-mediated down-regulation of the APOA5 promoter, HepG2 cells were co-transfected with a reporter plasmid driven by the APOA5 promoter fragment −146/+63 in which E-box 1 or E-box 2 were mutated (Fig. 8A). In contrast to the native promoter construct, SREBP-1c had only a minor residual effect on APOA5 promoter activity when E-box 1 was mutated. The construct bearing the mutated E-box 2 was not affected when 10 ng of SREBP-1c was tested, whereas 50 ng of SREBP-1c reduced repression by approximately one-third. These results support that SREBP-1c-dependent down-regulation of APOA5 promoter activity occurs via two E-box elements, and identification of E-box 1 as the main site is in accordance with results of gel-shift assays.

Further, we were interested in testing whether the presence of co-regulatory transcription factors is required for the SREBP-1c-mediated repressive effect on the APOA5 promoter. For this purpose, we first mutated a potential SP1 binding site located nearby E-box 1 (Fig. 6B). However, mutation of this site did not affect SREBP-1c-mediated down-regulation (data not shown), indicating that SP1 is not implicated in the SREBP-1c-dependent down-regulation of the APOA5 promoter.

Next, we decided to test the effect of SREBP-1c on the isolated E-box 1, as E-box 1 is the principal site. For this purpose, this E-box was cloned in three tandem repeats into the pGL3 basic vector. As shown in Fig. 8B, a dose-dependent down-regulation of APOA5 promoter activity was observed with 50 ng of SREBP-1c.
regulation by SREBP-1c was obtained when co-transfected with the plasmid containing the isolated E-box element, as compared with the empty pGL3 control vector, which was not affected (Fig. 8C). As a positive control, we tested the low density lipoprotein receptor-response element, and consistent with the literature, we obtained activation by SREBP-1c co-transfection (Fig. 8D). These results suggest that the E-box motif EB1-A5 is capable of conferring repression after SREBP-1c overexpression, in contrast to the well documented SRE elements that are activated by SREBPs (33).

**Fig. 7.** SREBP-1c specifically binds to two E-box elements in the APOA5 promoter. A, electromobility shift assays were performed with in vitro transcribed/translated human SREBP-1c or unprogrammed reticulocyte lysate (indicated by lysat) and the end-labeled APOA5 E-box 1 (lanes 1–3), APOA5 E-box 2 (lanes 5–7), and consensus (cons) E-box sequence as described for SREBPs (lanes 9–11). For mutation analysis, end-labeled mutated (mut) APOA5 E-box 1 (lane 4) and mutated APOA5 E-box 2 (lane 8) were employed. n.sp., nonspecific; Ab, antibody. B and C, competition analyses were performed by adding 10-, 50-, and 100-fold excess of cold SREBP consensus E-box (lanes 3–5) or 10-, 50-, and 100-fold excess of cold mutated SREBP consensus E-box (lanes 6–8) before adding labeled APOA5 E-box 1 (B) or APOA5 E-box 2 (C), respectively.

**Fig. 8.** Functional analysis of the APOA5 E-box elements. A, mutation analysis of the APOA5 promoter. HepG2 cells were transfected with the wild-type APOA5 promoter (−164/+63) or promoter constructs containing mutations of E-box 1 or E-box 2 along with 10 or 50 ng of SREBP-1c expression vector or the control vector pC DNA3. LUC, luciferase. B–D, luciferase activity of the isolated EB1-A5 (B), the empty pGL3 control vector (C), and the isolated low density lipoprotein receptor SRE (D) were monitored after SREBP-1c co-transfection. Luciferase activity was measured and expressed as described under “Experimental Procedures.” *, p < 0.05; **, p < 0.001.
A

![Diagram A](http://www.jbc.org/)

B

![Diagram B](http://www.jbc.org/)

C

![Diagram C](http://www.jbc.org/)

D

![Diagram D](http://www.jbc.org/)

**Fig. 9.** T0901317 decreases APOA5 mRNA in liver and apoAV protein in serum of hAPOA5 Tg mice. Human APOA5 Tg mice were dosed with 20 mg/kg T0901317 or vehicle (a = 6/group) for 3 days. A, triglycerides were measured in serum after a 4-h fasting period. DMSO, Me₂SO. B, total RNA was isolated from liver and human APOA5 (B), and mouse SREBP-1c (D) levels were quantified by real-time PCR with 36B4 as internal standard. C, serum APOA5 protein was quantified by enzyme-linked immunosorbent assay. Values are expressed as means ± S.D. *, p < 0.05; **, p < 0.001; ***, p < 0.0001.

accordance with our in vitro results, APOA5 mRNA in liver (Fig. 9B) and apoAV protein in plasma (Fig. 9D) were significantly decreased (by 40 and 35%, respectively). In contrast, SREBP-1c liver mRNA levels (Fig. 9C) were strongly elevated (9-fold). These results support that APOA5 is indeed down-regulated by the LXR ligand T0901317 in vivo in hAPOA5 Tg mice.

**DISCUSSION**

Since the identification of APOA5 several years ago, numerous studies have indicated its significant effect on plasma TG levels (3, 34). However, the effect of SREBP-1c, a key transcription factor of hepatic lipogenesis, on APOA5 transcription is unknown. In this report, we show that SREBP-1c influences APOA5 promoter activity in a negative manner. Treatment of hepatoma cell lines with the LXR ligand T0901317, which elevates SREBP-1c mRNA and protein levels, results in down-regulation of APOA5 mRNA. A stronger down-regulation was obtained in HuH7 cells. This finding could be explained by the occurrence of less APOA5, but more SREBP-1c and LXRα mRNA, when compared with HepG2 cells. Since transfection assays suggested that LXR is not directly implicated in this repressive effect, we focused on its target gene SREBP-1c. Suppression of the SREBP-1 mRNA by siRNA assays indicate that SREBP-1c is, in fact, involved in T0901317-dependent repression of APOA5 mRNA. Treatment of APOA5 Tg mice with the same ligand revealed that down-regulation of APOA5 also occurs in vivo in mouse liver and plasma, accompanied by highly up-regulated SREBP-1c mRNA levels. Our results suggest that the well-established inverse relationship between apoAV and triglyceride levels is also true after LXR ligand treatment, and we propose that down-regulation of APOA5 may contribute to the elevation of TG levels after administration of LXR ligands such as T0901317.

SREBP-1 preferentially regulates genes involved in fatty acid metabolism (20, 35). These genes are commonly up-regulated via SRE-like or E-box sequences (36–38), whereby the latter seem to be less powerful (33). In this study, we identified APOA5, an apolipoprotein with a close relationship to plasma TG levels, as an SREBP-1c target gene. As demonstrated by gel-shift and mutation analysis, two E-box elements are implicated in SREBP-1c-mediated down-regulation of this promoter, although E-box 2 emerged as less important. Cloning of the isolated, more potent E-box 1 in tandem repeats suggest that the E-box sequence alone is capable of conferring repression after SREBP-1c transfection. Thus, APOA5 was found, besides microsomal triglyceride transfer protein (MTP), phosphoenolpyruvate carboxykinase (PEPCK), 12α-hydroxysterol 7α-hydroxylase, and o xo-steryl 7α-hydroxylase (39–42), to be one of the rare genes being down-regulated by SREBPs.

Earlier described mechanisms of SREBP-dependent down-regulation include mainly indirect mechanisms such as interaction of SREBP with Sp1, fetoprotein transcription factor, or HNF4 (41–43) and competition for an overlapping binding site between SREBP and Sp1 (44). Here we propose that SREBP-1 binding to E-box 1 is responsible for the repression of APOA5 promoter activity. Gene regulation of SREBPs via E-box elements is not well understood. A limited number of genes such as acetyl-CoA carboxylase-α, Spot 14, and δ-6-desaturase are reported to be up-regulated by SREBPs via E-box elements (36, 45, 46). However, the presence of E-boxes in the promoter does not necessarily predict transactivation by SREBP. Comparative analyses of E-box and classical SRE elements by Amemiya-Kudo and co-workers (33) show that SREBPs activate a promoter fragment of the Spot 14 gene (containing an E-box, a carbohydrate-response element, and an inverted E-box) cloned in six copies. However, SREBPs had no significant effect on an enhancer construct containing a single copy of an isolated E-box; SREBP-1c transfection resulted even in repression. Interestingly, mutant SREBP activation of this construct had a strong positive effect, which lets the authors suggest that an unknown basic helix leucine zipper protein might be present (33). Further, they showed that SREBP activation did not change in the presence or absence of neighboring NF-Y or Sp1 sites (33). These findings are in line with our hypothesis that certain isolated E-box elements may conduct to repression in response to SREBP-1. The constitutive fixation of another, more active helix leucine zipper protein on the APOA5 E-box elements and its replacement by SREBP-1c may help to explain this repressive effect. The finding that the basal promoter
activity of constructs containing mutated E-box 1 is decreased when compared with wild type promoters supports this hypothesis. However, previous reports indicate that down-regulation of genes by SREBPs is likely to be complex. Further analysis should shed more light on it.

Besides APOA5, other apolipoprotein genes such as APOC3, APOA2, and APOB have been reported to contain E-box elements that bind SREBPs. However, APOA5 is the only apolipoprotein to be down-regulated by SREBP-1c, although the APOC3 and APOB promoters have been shown to be unaffected and the APOA2 promoter has been shown to be activated after transfection with SREBP-1 (47–49).

The finding that SREBP-1c plays an important role in hepatic nutritional regulation of lipogenic enzyme genes (50) lets us speculate that APOA5 may be regulated by nutritional stimuli via SREBP-1c. After a high carbohydrate diet or fasting/refeeding, SREBP-1c is implicated in the coordination of hepatic lipogenesis and gluconeogenesis by activating lipogenic enzymes. Their activity of constructs containing mutated E-box 1 is decreased when compared with wild type promoters supports this hypothesis. However, previous reports indicate that down-regulation of genes by SREBPs is likely to be complex. Further analysis should shed more light on it.

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