Hypertriglyceridemic VLDL Downregulates Tissue Plasminogen Activator Gene Transcription Through \textit{cis}-Repressive Region(s) in the Tissue Plasminogen Activator Promoter in Cultured Human Endothelial Cells

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\textbf{Abstract}—The relationship between tissue plasminogen activator (tPA) levels and the potential regulation by hypertriglyceridemic very low density lipoprotein (HTG-VLDL) was examined in a human umbilical vein endothelial cell (HUVEC) culture model system. HUVEC cultures were incubated in the absence/presence of HTG-VLDL or normal (NTG)-VLDL (0 to 50 \(\mu\)g/mL) at 37°C for various times (0 to 24 hours), followed by analyses of tPA antigen (ELISA), mRNA (reverse transcription–polymerase chain reaction), endothelial cell surface–localized plasmin generation assays, and nuclear transcription run-on assays. Secreted tPA antigen levels decreased \(\sim\)53\% (3.3 \(\pm\)0.14 versus 6.97 \(\pm\)0.42 \(\mu\)g/mL) and mRNA levels decreased \(\sim\)70\% in HTG-VLDL–treated HUVECs compared with NTG-VLDL–treated and culture medium control cells. Decreased tPA antigen and mRNA expression was associated with a concomitant \(\sim\)98\% decrease in tPA-mediated plasmin generation in HTG-VLDL–treated HUVEC cultures. Nuclear transcription run-on assays demonstrated that HTG-VLDL decreased tPA gene transcription \(\sim\)73\% (tPA mRNA/GAPDH mRNA) in cultured HUVECs. To identify and localize the repressive element(s) in the tPA promoter responsive to HTG-VLDL, a tPA promoter/luciferase construct (ptPA222/luc) was generated. HUVECs transiently transfected with this construct were incubated in the absence/presence of HTG-VLDL or NTG-VLDL (20 \(\mu\)g/mL). HTG-VLDL decreased promoter activity \(\sim\)52\% to 57\% in the ptPA222/luc-transfected cells compared with NTG-VLDL–treated or buffer control cells. These results indicate that the 2.2-kb fragment of the promoter and 5\'-flanking region of the tPA gene contains the repressive sequences that direct the transcriptional downregulation of the tPA promoter. Data from these studies suggest that the repression of tPA gene expression by HTG-VLDL may contribute to the impaired fibrinolysis often associated with hypertriglyceridemia. (\textit{Arterioscler Thromb Vasc Biol}. 2000;20:1675-1681.)

\textbf{Key Words:} tissue plasminogen activator \(\square\) gene regulation \(\square\) hypertriglyceridemic VLDL \(\square\) fibrinolysis \(\square\) tissue plasminogen activator promoter \(\square\) \textit{cis}-repressive elements

Impaired fibrinolysis plays an important role in the early pathogenesis of atherosclerosis, coronary artery disease (CAD), and eventual myocardial infarction (MI).\textsuperscript{1–3} Fibrinolysis involves the endothelial cell (EC) surface–localized conversion of plasminogen (Pmg) by receptor-bound Pmg activators (PAs), tissue-type PA (tPA) and urokinase-type PA (uPA), to the serine protease, plasmin. Plasmin, in turn, degrades fibrin, which is associated with clot formation. ECs synthesize tPA and uPA as well as the major physiological regulator and inhibitor of PA-mediated fibrinolytic activity, Pmg activator inhibitor type (PAI)-1.\textsuperscript{4,5} Therefore, it is conceivable that perturbation of the expression or activity of \(\geq\)1 of these fibrinolytic proteins may alter the hemostatic balance on the EC surface, thus promoting early initiation of fibrin deposition, atherogenesis, CAD, and eventual MI.\textsuperscript{1} Several atherogenic lipoproteins may cause such a perturbation; eg, Lp(a),\textsuperscript{6} oxidized LDL,\textsuperscript{7,8} and acetylated LDL\textsuperscript{9} increase PAI-1 levels and decrease tPA expression in cultured human ECs.\textsuperscript{8,10} In addition, abnormal triglyceride-rich lipoproteins, including VLDL (hypertriglyceridemic VLDL [HTG-VLDL]), have been shown to increase PAI levels\textsuperscript{11,12} and decrease surface localized plasmin generation.\textsuperscript{13} Conversely, normotriglyceridemic VLDL (NTG-VLDL) has been shown to be less potent in creating a prothrombotic state.\textsuperscript{11,14} The differences in these molecules may be due to differences in composition, conformation, and receptor binding properties; eg, HTG-VLDL, but not NTG-VLDL, binds to \(\beta\)-VLDL and LDL receptors, which are also found in ECs.\textsuperscript{15} Furthermore, HTG-VLDL has a more variable and higher lipid load than does NTG-VLDL.\textsuperscript{15}

Altered tPA antigen concentrations may be of important consequence in preclinical atherosclerosis and may be a marker for risk of future MI.\textsuperscript{16–18} Basal tPA antigen levels are increased in plasma from young post-MI patients compared

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with control subjects, whereas postocclusion tPA concentrations are decreased, a finding that has been confirmed in most cross-sectional studies of CAD patients.1

The exact molecular regulatory mechanisms underlying the repression of tPA by atherogenic lipoproteins have not been elucidated. Other studies have demonstrated that tPA is transcriptionally regulated by a variety of agents, including thrombin,19 cAMP,20 ethanol,21 and transforming growth factor.22 The present study demonstrates that HTG-VLDL decreases tPA antigen and mRNA levels in cultured HUVECs, that this downregulation also occurs at the level of gene transcription, and that a specific region of the tPA promoter is responsive to repression by HTG-VLDL. In addition, the transcriptional repression of tPA gene expression by HTG-VLDL observed in the present study is associated with a decreased net expression of surface-localized EC fibrinolytic activity and, hence, may contribute, in part, to the substantial thrombotic risk associated with hypertriglyceridemia.

Methods

Materials

Human Gls-Pmg was obtained from Enzyme Research Products, Inc; collagenase (type I, CLS), from Boehringer-Mannheim Biochemicals; FBS, from Intergen Corp; heparin (porcine intestinal mucosa), human fibronectin, and BSA, from Sigma Chemical Co; [α-32P]dCTP, [α-32P]dUTP (3000 Ci/mmol), and sodium 125I (specific activity 14.0 mCi/g), from Amersham Corp; Iodo-Beads, from Iodo-Beads, from Pierce Chemical Co; Sephadex G-25 column (PD-10), from Pharmacia; aprotinin (Trasylol), from Mobay Corp; 1,1′-diacetoxy-1,3,3′,3′-tetramethylinodocarboxylic perchlorate (DiI)-labeled acetylated LDL (DiI-Ac-LDL), from Biomedical Technologies, Inc; medium 199 (M199), TRIZol reagent, and M-MLV reverse transcriptase, from GibCO-BRL; tPA ELISA kits (TintElize), from BioPool; BglII, KpnI, AccI, BamHI, Drel, SacI, and Smal, from Boehringer-Mannheim; calf intestinal phosphatase, T4 DNA ligase, Klenow fragment, Gene Light vector pGL3-basic expression, Dual-Luciferase Reporter Assay System, pRL-TK, and agarose, from Promega Inc; [α-32P]dCTP (300 Ci/mmol), from Amersham; and Taq DNA polymerase and oligo(dT) primers, from Promega, Inc. Specific primer pairs for tPA and GAPDH (constitutive control) used for polymerase chain reactions (PCRs) were obtained from DNA International, Inc. Lipofectamine, Opti-MEM-1 reduced serum medium (F12), and M199 were from GibCO-BRL. Frozen bovine hypothalamus, from which endothelial cell growth factor was isolated,25 were obtained from Pel-Freeze Inc.

Isolation and Characterization of Lipoproteins

Purified VLDL was obtained freshly isolated from Drs William A. Bradley and Sandra H. Gianturco, University of Alabama at Birmingham (UAB), and was isolated and characterized as described in detail previously.24 Briefly, plasma was obtained from fasting subjects with normal lipid values for isolation of NTG-VLDL or from fasting patients with types 4 and 5 lipoprotein profiles for HTG-VLDL. The diagnoses were based on commonly used criteria.25 NTG-VLDL and HTG-VLDL were subfractionated through a discontinuous NaCl gradient from a density of 1.063 to 1.006 g/mL by the cumulative flotation methods of Lindgren et al.26 as previously detailed.24 The VLDLs used had Svedberg flotation rates of 100 to 400. The defined flotation cut precludes comparison of lipid-rich with lipid-poor particles, ie, large versus small particles that could occur in comparing NTG-VLDL and HTG-VLDL. HTG-VLDL, but not NTG-VLDL, has been shown to bind with high affinity to bovine adrenal LDL receptors25 and to the monocyte-macrophage receptor for triglyceride-rich lipoproteins.26 Binding to the LDL receptor was correlated with the presence of accessible apoE, as found in HTG-VLDL but not in NTG-VLDL.24 Total protein contents of the lipoproteins were obtained by a modified method of Lowry et al.,28 and triglyceride contents were obtained by using a kit from Boehringer-Mannheim.27 All lipoproteins were also routinely analyzed by Western blotting to identify their apoprotein contents.

Cell Culture

Human umbilical vein ECs (HUVECs) were obtained from fresh (discarded) umbilical cords by mild collagenase treatment,4,5 seeded into human fibronectin–coated plastic T-25 or 960-mm2 Petri dishes, and grown to confluence in complete culture medium as we have previously described.29,30 Cultures were refed every 48 hours with complete culture medium and maintained in a 95% air/5% CO2– humidified atmosphere. All experiments were carried out with pooled (4 to 6 umbilical veins), confluent, serially subcultured HUVECs (passages 1 to 4). Cells were routinely counted by use of phase-contrast microscopy and a 0.5-mm×0.5-mm counting reticle.

Cell cultures were routinely characterized as HUVECs, and their purity was established by their uniform uptake of the EC-specific fluorescent probe DiI-Ac-LDL21 and their typical monolayer “cobblestone” tight-packaging growth morphology.31,32 Only individual cultures with >95% identifiable ECs were used in these experiments.

tPA antigen/mRNA analyses were carried out with postconfluent HUVEC cultures grown in 24-well multilwell plates (200 m/L well). Cultures were incubated (0 to 24 hours) in serum-free culture medium (500 mL per well, the same as for complete culture medium, except FBS is replaced with 0.25% BSA) in the absence/presence of HTG-VLDL or NTG-VLDL (0 to 50 m/mL), and each culture (in triplicate) was then analyzed for its secreted tPA antigen and coincident mRNA levels, as described below.

Nuclear transcription run-on assays were carried with postconfluent cultures grown in T-75 (7500-mm2) flasks. Cultures were incubated in serum-free culture medium in the absence/presence of HTG-VLDL or NTG-VLDL (20 m/mL) for 8 hours before the isolation of nuclei, as described below.

tPA Antigen Analysis (ELISA)

Measurement of total secreted tPA antigen levels (free plus complex forms) were performed (in duplicate) in serum-free conditioned culture media by use of a commercial tPA (TintElize, BioPool) kit. Determination of antigen concentration was made with a Dynatech plate reader, appropriate standards, and BioLinx software (Dynatech) to measure absorption at 405 nm. Total secreted tPA levels were calculated in nanograms per millilitre per well (∼1.8×105 cells per well) per 24 hours.

RNA Isolation

Total cytoplasmic RNA was isolated from the confluent monolayer of each HUVEC culture (in duplicate) that had been incubated for varying times (0, 2, 4, and 8 hours) in the absence/presence of HTG-VLDL or NTG-VLDL (20 m/mL). Cell monolayers were washed twice in Dulbecco’s PBS, and total RNA was extracted by a single-step method, with the use of TRIZol reagent, according to the manufacturer’s instructions.

Measurement of tPA mRNA Level by Reverse Transcription–PCR

Total RNA (1 m) in a 20 mL reaction mixture was reverse-transcribed with M-MLV reverse transcriptase (200 U) for 10 minutes at room temperature, followed by 1 hour at 42°C, with oligo(dT) used as a primer. The resulting cDNA (5 mL) was used as a template, and a 234-bp segment of the tPA cDNA was amplified by use of a 24-mer upstream primer (5′-GTCGTCGTTGACACCATTCCCCATC-3′) identical to positions 177 to 200 and a 24-mer downstream primer (5′-TTTGTCAATCATCATTCCATC-3′) complementary to positions 400 to 377 of the human tPA mRNA.33 A 600-bp segment of an internal standard, GAPDH cDNA, was simultaneously amplified by use of a 24-mer upstream primer (5′-CCACCCATGCGCAATCCGAGC-3′) identical to positions 212 to 235 and a 24-mer downstream primer (5′-TTTGTCAATCATCATTCCATC-3′) complementary to positions 809 to 786 of the human GAPDH mRNA.34 Amplification was carried out in a Techne Thermal Cycler PHC-3 for 28 cycles (1 cycle consisted of 94°C for 45 seconds, 60°C for 45 seconds, and 72°C for 45 seconds). PCR products were analyzed on a 1.2%
agaro–ethidium bromide gel. The gels were photographed, and the intensity of each IP and GAPDH mRNA band was measured by laser densitometric scanning with a Molecular Dynamics Personal Densitometer and expressed as a relative ratio of the GAPDH mRNA band intensity in each lane.

Fibrinolytic (Plasmin) Activity Assay

Surface-localized fibrinolytic activity was measured, with the use of live confluent cultured HUVECs (passages 1 and 2), by the direct conversion of EC-bound single-chain $^{125}$I-labeled Glu-Pmg by receptor-bound tPA to 2-chain $^{125}$I-labeled plasmin and quantification of either $^{125}$I-labeled plasmin M, 20-kDa light chain or $M$, 60-kDa heavy chain, after SDS-PAGE under reducing conditions, according to the method of Mussoni et al, as modified in our laboratory. Because early-passage (passages 1 and 2) HUVECs do not synthesize urokinase, the results of this particular assay are largely attributed to the effects of tPA. Postconfluent cultured HUVECs in 96-well plates (in triplicate) were incubated at 37°C for 8 hours in the absence/presence of HTG-VLDL or NTG-VLDL (20 mg/mL) in serum-free culture medium, followed by removal of lipoproteins and further incubation in complete culture medium for an additional 16 hours. The cultures were then washed (3 times) with 10 mmol/L HEPES and 0.1 mol/L sodium acetate, pH 7.4, containing 1% BSA (buffer A) and equilibrated with buffer A (50 μL per well) at 4°C for 20 minutes. $^{125}$I-labeled Glu-Pmg (2 μmol/L) in buffer A containing 1000 kallikrein inhibiting units per milliliter aprotinin (40 μg/mL) for 8 hours at 37°C, and the $^{125}$I-labeled nuclear RNA was isolated on a 5.7 mol/L CsCl gradient by centrifugation at 100 000g for 18 hours at 20°C and then hybridized with cDNAs for tPA and GAPDH (constitutive control) immobilized on nitrocellulose filters. Preparation of nitrocellulose filters containing the cDNAs, hybridization, and washing of filters were carried out as previously described. The radioactivity corresponding to each individual filter was quantified by phosphorimaging autoradiography with the Molecular Dynamics Series 425F PhosphorImager in combination with ImageQuant software (Molecular Dynamics).

Amplification 5' Promoter and Flanking Regions (~2220-bp Fragments) of the tPA Gene

A 2220-bp segment of the promoter and 5' flanking region of the tPA gene containing the start site of transcription at +1 and the TATA box at −24 to −29 were amplified by PCR as previously described. The PCR was carried out with use of an upstream primer (5' CGATCGATTCTCCATTGTCACCTTATACGCTGGCC 3') identical to positions 1473 to 1497 and downstream primer (5' GATCACTTTCCTGCAGAGTTTCTCTTCCTCAGC 3') complementary to positions 3692 to 3668 of the human tPA gene. Both these primers had a CGATC clamp and a KpnI site in the upstream primer (underlined) and a BglII site in the downstream primer (underlined) to aid in the subsequent cloning into the luciferase reporter gene (luc, P63, L3, basic expression vector, Promega Corp). Detailed sequencing analyses were carried out with duplicate PCR clones from single PCR amplification of 8 individual (16 sequences) promoter fragments to rule out errors due to PCR cloning or sequencing, as described previously. Sequencing was carried out on both strands by the UAB Automated DNA Sequencing Core Facility.

Construction of the tPA Promoter/Luc Construct

The amplified IPA promoter fragments (2.2 kb) were purified by electrophoresis on 1% agarose, digested with KpnI and BglII, and ligated into the KpnI/BglII site of the luciferase reporter gene (Gentype vector, Promega Corp) to generate the tPA reporter construct. The ligated mixture was transformed into JM 109 competent cells to generate pTA222/ luc constructs. IPA/luc constructs were purified by ultracentrifugation through a cesium chloride/ethidium bromide gradient before transfection. The tPA fragment in the plasmid construct was sequenced on both strands by the UAB Automated DNA Sequencing Core Facility for sequence verification. Sequences were aligned and compared with published human tPA sequences by using the Genetics Computer Group sequence analysis software provided by the UAB Biological Computing Resource Core Facility.

Transient Transfection of Cultured HUVECs With ptPA/luc and Promoter and Measurement of Luciferase Activity

Transient transfection experiments were carried out with semiconfluent (60% to 75%) subcultured HUVECs grown in 6-well multiwell plates by use of lipofectamine, as we have previously described with minor modifications. Briefly, DNA-lipofectamine com-
plexes were preformed for 45 minutes at room temperature by use of 1 μg per well of DNA, 0.05 μg per well internal control vector, a thymidine kinase promoter–driven Renilla luciferase construct (pRL-TK), and 10 μg per well lipofectamine in Opti-MEM-1 reduced serum medium, according to the manufacturer’s instructions. Cultured HUVECs were transfected (in triplicate) with the ptPA/luc constructs and cotransfected with pRL-TK (internal control).31,42 Transfection mixtures were incubated in Opti-MEM-1 reduced serum medium for 1 hour at 37°C. At the end of the incubation, the medium was removed, and cultures were rinsed twice with M199 and then incubated in fresh M199 containing 0.25% BSA for 18 hours in the absence/presence of HTG-VLDL or NTG-VLDL (20 μg/mL). Finally, cultures were rinsed twice with Dulbecco’s PBS, lysed by the addition of 200 μL lysis buffer (Dual-Luciferase kit, Promega), and centrifuged at 16 000g for 1 minute, and the cell supernatants were assayed for their dual luciferase activity (firefly and Renilla) by use of the Dual-Luciferase Reporter Assay System according to manufacturer’s instructions. Activities were measured, and final luciferase activities were normalized to the corresponding Renilla activities to correct for transfection efficiency.

Analysis of Data
All of the data were expressed as the mean±SD of triplicate experiments performed in each assay and analyzed by the Student t test. Data with P<0.05 were taken to represent statistically significant differences in experimental results.

Results

Effect of tPA Antigen and mRNA Expression by HTG-VLDL Cultured HUVECs
Confluent cultured HUVECs were analyzed for their expression of tPA antigen and coincident mRNA levels in the absence/presence of HTG-VLDL or NTG-VLDL (control) at 0 to 50 μg/mL. tPA antigen levels showed a significant decrease in HTG-VLDL–treated HUVEC cultures compared with the NTG-VLDL–treated and culture medium control cells. After incubation for 24 hours in the absence/presence of these lipoproteins, secreted tPA antigens levels were decreased up to ~53% in HTG-VLDL–treated HUVEC cultures compared with NTG-VLDL–treated and culture medium control cells (Figure 1). Interestingly, the relative changes in tPA antigen between different doses of HTG-VLDL were small, suggesting an all-or-none response at these levels of lipoprotein.

Simultaneous reverse transcription–PCR analysis of these HUVEC cultures for their coincident expression of relative tPA mRNA levels (tPA mRNA/GAPDH mRNA ratios) also indicated a decline in steady-state levels of tPA after HTG-VLDL treatment. After incubation for 8 hours in the presence of HTG-VLDL (20 μg/mL), tPA mRNA levels decreased by 70% compared with baseline levels (Figure 2). In addition, after incubation for 8 hours, tPA mRNA levels decreased in the presence of HTG-VLDL (20 μg/mL) compared with NTG-VLDL or control medium (data not shown). These studies were carried out in 16 separate experiments in which 3 or 4 different individual HUVEC cultures were pooled, examined, and compared; similar results were obtained, providing strong evidence that HTG-VLDL downregulates tPA expression.

Effect of HTG-VLDL on Fibrinolytic Activity in Cultured HUVECs
Although urokinase is an efficient convertase, tPA is the enzyme responsible for the conversion of plasminogen to plasmin; fibrinolytic activity is largely due to tPA.34 These experiments were carried out to establish whether the decrease in tPA levels induced by HTG-VLDL may additionally exert an inhibitory effect on the net expression of fibrinolytic activity. Confluent cultured HUVECs preincubated in the presence of HTG-VLDL (20 μg/mL), followed by incubation with 125I-labeled Glu-Pmg, showed a significant decrease (≈98%) in net expressed fibrinolytic activity (2±2 pmol per well) compared with activity in NTG-VLDL–treated or culture medium control cells (≈120±20 pmol per well), as seen in Figure 3. As stated earlier, because early-passage HUVECs do not synthesize urokinase, the results of this particular assay are attributed largely to the effects of tPA.35 These studies on the effects of HTG-VLDL on fibrinolytic activity were repeated in 16 separate experiments that used different pools of cultured HUVECs (in triplicate) with similar results.

Effect of HTG-VLDL on tPA Gene Transcription Rates
Nuclear transcription run-on assays were carried out to establish whether the observed decrease in tPA mRNA in
response to HTG-VLDL was due to a decrease in the rate of tPA transcription. Nuclei were isolated from postconfluent cultured HUVECs (in 7500-mm² flasks) and incubated in the absence/presence of HTG-VLDL for 8 hours at 37°C. Newly synthesized 32P-labeled nuclear transcripts were hybridized to tPA and GAPDH cDNAs immobilized on nylon membranes, and the nuclear run-on assay results were measured by phosphorimaging autoradiography. These results indicated transcriptional repression of the tPA gene by HTG-VLDL, as evidenced by a significant decrease (~72%) in new 32P-labeled tPA mRNA (tPA mRNA/GAPDH mRNA ratio) in the cultured HUVECs treated with HTG-VLDL compared with NTG-VLDL–treated controls (Figure 4). These assays were carried out in 6 separate experiments with at least 3 pooled different individual HUVEC cultures; similar results were obtained.

Generation of ptPA/luc Promoter Construct and Transient Transfection of Cultured HUVECs With the ptPA/luc Promoter Construct

Because the present study demonstrated that HTG-VLDL but not NTG-VLDL decreased tPA levels in cultured HUVECs and that this repression occurred at the transcriptional level, we sought to further confirm and localize this repression of tPA expression by HTG-VLDL; a 2220-bp segment of the tPA promoter and 5′ flanking region, containing the start +1 site as well as the TATA box, was PCR-amplified (Figure 5). This was followed by ligation into a promoterless/enhancer-less luciferase to generate the promoter/luciferase construct (ptPA222luc) to be used in transient transfection studies. HUVEC cultures were transiently transfected with the ptPA222/luc construct. The cells were concomitantly transfected with Renilla to correct for differences in DNA uptake. The transfected cells were subsequently incubated in the absence/presence of HTG-VLDL or NTG-VLDL (20 μg/mL) for 18 hours, then harvested, and assayed for luciferase and Renilla activity. Compared with NTG-VLDL or control medium, HTG-VLDL decreased promoter activity (luciferase activity) ~52% to 57% in the ptPA222/luc construct (Figure 6). In addition, no luciferase activity was generated in the reverse orientation undeleted construct (negative control).

Therefore, these results demonstrate that the 2.2-kb fragment of the promoter and 5′ flanking region of the tPA gene contains the repressive sequences that direct the transcriptional downregulation of the tPA promoter.

Discussion

The purpose of the present study was to examine the interrelationship between tPA expression and HTG-VLDL in a cultured HUVEC model system. We have demonstrated that HTG-VLDL, but not NTG-VLDL, decreases tPA antigen and mRNA expression and suppresses tPA-mediated fibrinolytic activity. In addition, nuclear transcription run-on assays in these studies demonstrate that HTG-VLDL downregulates tPA expression at the level of gene transcription. These findings complement previous in vitro and clinical observations. For example, it is known that other atherogenic lipoproteins decrease tPA secretion and tPA-mediated Pla-mediated fibrinolytic activity in cultured ECs, and that compared with control subjects, patients with CAD and elevated Lp(a) levels have a marked reduction in their ability to release tPA in response to venous occlusion. Whether this type of regula-
tion also occurs with HTG-VLDL would be of additional clinical importance given the association between HTG and atherosclerosis.

HTG-VLDL is known to transcriptionally regulate other fibrinolytic proteins. For example, prior studies in our laboratory have demonstrated that HTG-VLDL transcriptionally upregulates PAI-1 expression in a genotype-specific manner. In addition, Eriksson et al recently identified a VLDL response element in the PAI-1 promoter, which is located between −672 bp and −657 bp in the promoter region. However, to our knowledge, no description of regulatory elements in the tPA gene responsive to HTG-VLDL exists. In the present study, we add to the prior body of knowledge by reporting for the first time the transcriptional repression of tPA gene expression by HTG-VLDL. Our transient transfection studies with the pTFA222/luc construct indicate that the cis-repressive element(s), which specifically directs the transcriptional downregulation of the tPA gene by HTG-VLDL, is located within the PCR-amplified 2.2-kb region of the tPA promoter used in these studies.

Repression of gene expression may occur by transcriptional interference. This results when a transcription factor is blocked from successfully interacting with the transcription initiation complex through direct or indirect interactions with another factor. Transcriptional interference of the general transcription factor(s) has been observed for other fibrinolytic protein genes, including the urokinase gene. Whether this type of interference contributes to the observed repression of the tPA gene by HTG-VLDL is not known.

Additional studies to identify the specific site(s) of the HTG-VLDL repressive element(s) within the tPA promoter and HTG-VLDL inducible transcription/repressive factor(s) that bind to this region are in progress.

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