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Unsaturated Fatty Acids Increase Plasminogen Activator Inhibitor-1 Expression in Endothelial Cells

Lennart Nilsson, Cristina Banfi, Ulf Diczfalusy, Elena Tremoli, Anders Hamsten, Per Eriksson

Abstract—In vivo studies have demonstrated a strong positive correlation between plasma very low density lipoprotein (VLDL) triglyceride and plasma plasminogen activator inhibitor-1 (PAI-1) activity levels. Furthermore, VLDL has been shown to induce PAI-1 secretion from cultured endothelial cells. In contrast, no or variable effects on PAI-1 secretion have been reported for native low density lipoprotein. It could be speculated that fatty acids derived from VLDL triglycerides are the actual mediators, resulting in an enhanced secretion of PAI-1. In the present study, we have analyzed the effects of both saturated and unsaturated fatty acids on PAI-1 expression and secretion by endothelial cells. Addition of 0 to 50 $\mu\text{mol/L}$ of either palmitic acid or stearic acid had no effect on PAI-1 secretion from human umbilical vein endothelial cells or EA.hy926 cells. In contrast, addition of oleic acid, linoleic acid, linolenic acid, and eicosapentaenoic acid resulted in a significant increase in PAI-1 secretion from both cell types. Northern blot analysis of PAI-1 mRNA levels was in agreement with these findings. Transfection experiments demonstrated that addition of linolenic acid and eicosapentaenoic acid significantly increased PAI-1 transcription. The fatty acid response region was localized to a previously described VLDL-inducible region of the PAI-1 promoter. Electromobility shift assays demonstrated that unsaturated fatty acids induced the same complex as did VLDL, whereas saturated fatty acids had no effect. Furthermore, it was demonstrated that the activation procedure did not involve fatty acid oxidation to any significant extent. In conclusion, the present study demonstrates that unsaturated fatty acids increase PAI-1 transcription and secretion by endothelial cells in vitro. The effect appears to be mediated by a previously described VLDL-inducible transcription factor. (*Arterioscler Thromb Vasc Biol.* 1998;18:1679-1685.)

Key Words: PAI-1 ■ fatty acids ■ promoter ■ endothelial cells ■ VLDL

Plasminogen activator inhibitor-1 (PAI-1), the fast-acting inhibitor of plasminogen activators, is the principal regulator of the endogenous fibrinolytic enzyme system. Low fibrinolytic capacity has been associated with manifest coronary heart disease and increased risk of recurrent major cardiovascular events in patients with a history of cardiovascular disorders.¹

Both environmental and genetic factors contribute to determine plasma PAI-1 activity. Among PAI-1 associations with established risk indicators for coronary heart disease, the relation with VLDL has been analyzed extensively. In vivo studies consistently have demonstrated a strong positive correlation between the plasma VLDL triglyceride and PAI-1 activity levels.²⁻⁵ In vitro, VLDL has been shown to induce a concentration-dependent increase in the PAI-1 secretion from cultured human umbilical vein endothelial cells (HUVECs)⁶⁻⁸ and HepG2 cells.^{7,9} Addition of a triglyceride-rich emulsion also resulted in an enhanced secretion of PAI-1 by HepG2 cells.¹⁰ In contrast, no or variable effects on PAI-1 secretion by cultured cells have been reported for native LDL.^{6,8,11-13} Thus, the effects of lipoproteins could be influenced by their

triglyceride content. Furthermore, it could be speculated that fatty acids derived from VLDL triglycerides are the actual mediator, resulting in an enhanced release of PAI-1. Indeed, in vitro experiments have demonstrated that docosahexaenoic acid and dihomo- γ -linolenic acid induce PAI-1 mRNA in HUVECs¹⁴ and that linoleic acid enhances PAI-1 secretion from HepG2 cells.¹⁰ In agreement with the in vitro data, administration of n-3 fatty acids in vivo has resulted in increased plasma PAI-1 activity.¹⁵⁻¹⁸

Recently, a VLDL response element was identified in the promoter region of the PAI-1 gene locus that mediates VLDL-induced PAI-1 transcription in endothelial cells.¹⁹ A VLDL-inducible transcription factor binds directly downstream of the common 4G/5G polymorphic site in the PAI-1 promoter. Competitive binding between the VLDL-inducible transcription factor and the 5G allele-specific transcriptional repressor may explain the allele-specific differences in the association between plasma triglycerides and PAI-1 activity observed in non-insulin-dependent diabetic patients and in patients with coronary artery disease.²⁰⁻²²

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In the present study, we have analyzed the effects of both saturated and unsaturated fatty acids on PAI-1 expression and secretion by endothelial cells. Furthermore, the molecular mechanism whereby fatty acids stimulate PAI-1 secretion has been studied and linked to the VLDL activation pathway.

Methods

Cell Culture

HUVECs were isolated from umbilical cords obtained at normal deliveries. The umbilical vein was cannulated and perfused with 50 mL PBS to remove any blood, whereafter the vein was filled with 20 mL 0.1% collagenase dissolved in PBS and incubated for 15 minutes at 37°C. The collagenase solution was drained from the cord and collected, and the cord was flushed gently with 20 mL PBS, which was added to the collagenase solution. The cells in these pooled solutions were recovered by centrifugation at 200g for 5 minutes and seeded out on 9-cm culture dishes in M199 medium with 20% FCS, antibiotic/antimycotic (Sigma Chemical Co), and 25 µg/mL endothelial cell growth supplement (Sigma Chemical Co). The cells were subcultured onto 0.2% gelatin (in PBS)-coated dishes when confluent. Cells from pooled multiple cords were used for experiments until the fourth passage. The endothelium-derived cell line EA.hy926 (a kind gift from Dr C.-J.S. Edgell, University of North Carolina, Chapel Hill, NC) was cultured in DMEM with high glucose supplemented with 10% FCS, HAT (100 µmol/L hypoxanthine, 0.4 µmol/L aminopterin, and 16 µmol/L thymidine), penicillin, and streptomycin as described.²³

VLDL Preparation

VLDL for incubation with HUVECs was prepared by density gradient ultracentrifugation.²⁴ The endotoxin content in the VLDL preparations was tested using a *Limulus* amoebocyte lysate assay (COATEST Endotoxin, Endosafe Inc). Endotoxin levels were shown to be <0.1 ng/mg protein.

Preparation of Fatty Acid–BSA Complexes

Fatty acid–BSA complexes were prepared essentially according to the method of Spector and Hoak.²⁵ In brief, 25 mg of fatty acids (16:0, 18:0, 18:1, 18:2, 18:3, and 20:5; Sigma Chemical Co) was dissolved in 7.5 mL hexane, and 800 mg Celite was added. The solvent was removed under N₂ by continuous magnetic stirring. When the solvent had evaporated completely, fatty acid–free BSA (25 mL of 0.25 mmol/L; Sigma Chemical Co) was added. The mixture was stirred for 1 hour at room temperature with N₂ constantly passing over the surface. After centrifugation at 800g for 5 minutes, the supernatants were decanted carefully. Samples containing fatty acid–BSA complexes were filtered and stored in aliquots under N₂ at –20°C.

13-Hydroperoxy-9,11-octadecadienoic acid (13-OOH-18:2) was synthesized as described.²⁶ In brief, linoleic acid was incubated with soybean lipoxygenase at 0°C in borate buffer at pH 9.0. The product was purified by silicic acid column chromatography, and the purity was determined by high-performance liquid chromatography.

Determination of PAI-1 Protein Secretion

Semiconfluent cultures of HUVECs or EA.hy926 cells were incubated for 8 to 10 hours in M199 or DMEM medium, respectively, containing 1% charcoal-treated FCS. This incubation was followed by a 14-hour incubation with fatty acids added in the same type of medium. After collecting the conditioned medium and centrifugation at 9000g for 5 minutes, the PAI-1 protein concentration in the medium was quantified using an ELISA (TintELIZE PAI-1, Biopool) that detects active and inactive (latent) forms of PAI-1, as well as tissue plasminogen activator/PAI-1 complexes. The cells were either trypsinized and counted or lysed with 0.01 M NaOH followed by measurement of total protein.²⁷ PAI-1 secretion was expressed as percentage of control (vehicle containing the same amount of BSA solution added). Trolox (Fluka), a peroxyl radical

scavenger, was used in some experiments to prevent fatty acid oxidation in the medium. The EA.hy926 cells were incubated with 20 µmol/L of Trolox for 30 minutes before addition of fatty acid–BSA complexes and subsequent incubation for 14 hours before collecting the medium.

Northern Blot Analysis

Semiconfluent cultures of EA.hy926 cells were preincubated for 8 to 10 hours in DMEM containing 1% charcoal-treated FCS before incubation with the fatty acids. Total RNA from the EA.hy926 cells was isolated according to the Rneasy handbook (Qiagen). Northern blotting and hybridization on DuPont GeneScreen Plus nylon membranes (NEN Research Products) were performed according to the manufacturer's protocol. Blots were hybridized with 10⁶ cpm/mL [³²P]dCTP-labeled *Sfi*I and *Bgl*III fragment (1255 bp) of the cDNA for PAI-1 (courtesy of Dr T. Ny, Department of Medical Biochemistry and Biophysics, University of Umeå, Umeå, Sweden).

Transfection Assay

EA.hy926 cells were transfected using a calcium phosphate precipitation method as described by Sambrook et al.²⁸ pRSV-galactosidase control vector (Promega) was cotransfected as an internal control. The construction of the PAI-1 CAT plasmids has been described elsewhere.¹⁹ The 4G-PAI-pCAT construct comprises the human PAI-1 sequences –804 to 17. The truncated promoter constructs, –708-PAI-pCAT and –609-PAI-pCAT, were constructed from the 4G-PAI-pCAT as described.¹⁹ The 4G-9DEL-PAI-pCAT plasmid was constructed using the Altered sites II in vitro mutagenesis system (Promega). A 9-bp deletion was introduced just downstream of the 4G/5G polymorphic site of the 4G-PAI-pCAT construct.¹⁹ The cells were transfected at 80% to 90% confluence. One to 3 hours before transfection, the dishes received fresh complete medium. Cells were incubated for 4 hours with calcium phosphate–precipitated DNA (15 µg plasmid per 90-mm dish). After a 2-minute 15% (vol/vol) glycerol shock, fresh medium containing 1% charcoal-treated FCS and fatty acids was added, and the cells were harvested for transient expression 16 to 18 hours later. CAT activity was analyzed subsequently according to Sambrook et al.²⁸

Electromobility Shift Assay (EMSA)

Nuclear extracts were prepared according to Alksnis et al.²⁹ All buffers were supplemented freshly with 0.7 µg/mL leupeptin, 16.7 µg/mL aprotinin, 0.5 mmol/L PMSF, and 0.33 µL/mL 2-mercaptoethanol. The protein concentration in the extracts was estimated by the method of Kalb and Bernlohr.³⁰ For EMSA, a double-stranded oligonucleotide comprising the –675 to –653 region of the PAI-1 promoter was designed. Semiconfluent cultures of HUVECs were incubated for 8 to 10 hours in M199 medium containing 1% charcoal-treated FCS. This was followed by an 8-hour incubation with fatty acids before the preparation of the cell extracts. Incubation conditions for EMSA were as described.¹⁹ To test for specific interaction of the VLDL- and fatty acid–induced factor, nonlabeled specific and nonspecific probes were used as competitors¹⁹ (data not shown).

Statistical Methods

Differences in continuous variables between 2 groups were tested by an unpaired Student *t* test. Data are mean ± SD.

Results

Effects of Fatty Acids on PAI-1 Secretion and mRNA Levels in Endothelial Cells

Fatty acids were incubated with HUVECs or with the HUVEC-derived cell line EA.hy926, and the PAI-1 secreted into the medium was measured using ELISA. Palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3), and eicosapentaenoic (EPA) (20:5) acids were complexed with BSA and incubated for 14 hours with the cells before

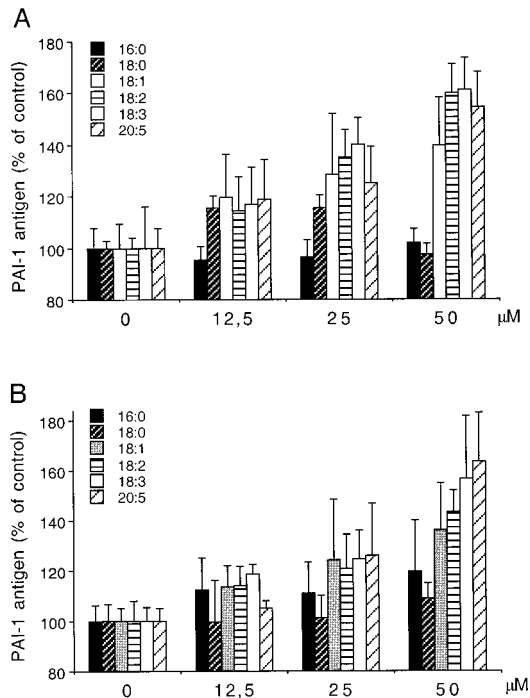


Figure 1. Fatty acid induction of PAI-1 secretion from HUVECs (A) and EA.hy926 cells (B). Fatty acids were incubated with the cells for 14 hours, whereafter PAI-1 contents of culture medium were determined by ELISA. Results (mean±SD) are given as percentage of control. Results were derived from 4 to 8 experiments, all performed in triplicate.

collecting the conditioned medium. As shown in Figure 1A and 1B, the effects of the fatty acids on PAI-1 secretion from HUVECs and EA.hy926 cells were similar. Palmitic acid or stearic acid (0 to 50 μmol/L) had no major effect on PAI-1 secretion from either HUVECs or EA.hy926 cells. A small increase in PAI-1 release from HUVECs was obtained with 10 to 25 μmol/L stearic acid (Figure 1A). In contrast, oleic acid, linoleic acid, linolenic acid, and EPA showed a dose-dependent effect on PAI-1 secretion from both cell types. Addition of 50 μmol/L of either oleic acid, linoleic acid, linolenic acid, or EPA resulted in a 40% ($P<0.001$), 59% ($P<0.001$), 60% ($P<0.001$), and 54% ($P<0.001$) increase in PAI-1 secretion from HUVECs, respectively, and in a 35% ($P<0.001$), 42% ($P<0.001$), 55% ($P<0.001$), and 62% ($P<0.001$) increase in PAI-1 secretion from EA.hy926 cells, respectively (Figure 1A and 1B). The basal secretion of PAI-1 from HUVECs and EA.hy926 cells was 100 to 120 ng/10⁵ cells and 20 to 30 ng/10⁵ cells, respectively.

To test whether oxidation of the unsaturated fatty acids was implicated in their effect on PAI-1 secretion, the peroxy radical scavenger Trolox (20 μmol/L) was incubated with EA.hy926 cells before addition of 50 μmol/L linolenic acid. No effect of the antioxidant was demonstrated on the linolenic acid-mediated induction of PAI-1 secretion (data not shown). As a positive control for the activity of Trolox, it was demonstrated that Trolox decreased the UV-induced mobility change of LDL on agarose gel electrophoresis. We also studied the effect of 13-OOH-18:2 on PAI-1 secretion from EA.hy926 cells. Assuming that a maximum of 10% auto-oxidation of the fatty acid, 0 to 5 μmol/L of 13-OOH-18:2,

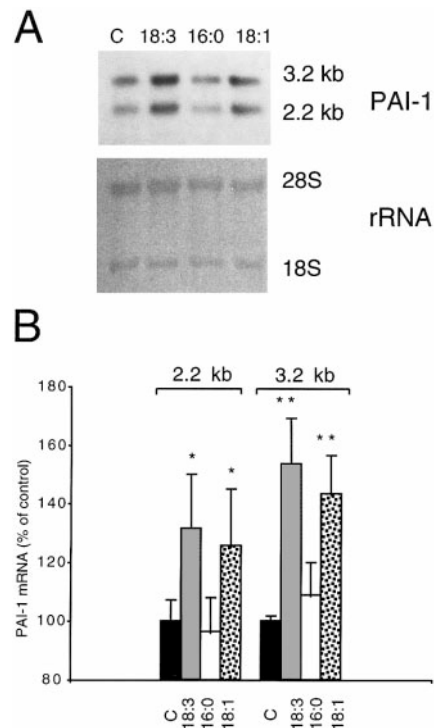


Figure 2. A, Northern blot analysis of PAI-1 mRNA recovered from EA.hy926 cells after an 8-hour incubation with 50 μmol/L of linolenic (18:3), palmitic (16:0), or oleic (18:1) acid. Total RNA (5 μg) was hybridized with labeled cDNA probe for PAI-1. C indicates vehicle containing same amount of BSA solution added as control. The corresponding blotting filters stained with methylene blue showing the 28S and 18S ribosomal RNAs demonstrate that approximately equal amounts of RNA were loaded. B, Quantification of 3 experiments. Results (mean±SD) are given as percentage of control. * $P<0.05$; ** $P<0.01$.

was incubated with EA.hy926 for 14 hours. No effect on PAI-1 secretion was detected with any of the 13-OOH-18:2 concentrations used. Addition of 0.1, 0.5, 1.0, or 5.0 μmol/L of the peroxidized linoleic acid resulted in 96±12%, 97±6%, 99±9%, and 99±12% of the control PAI-1 antigen secretion, respectively (mean±SD of 3 experiments performed in triplicate).

Because fatty acids increased the secretion of PAI-1 from HUVECs and EA.hy926 cells in a similar fashion, RNA and transfection analyses (shown below), experiments that require many cells, were performed only in EA.hy926 cells. Northern blot analysis of mRNA levels was in agreement with the finding that unsaturated fatty acids increase the secretion of PAI-1 by EA.hy926 cells. Figure 2 shows representative Northern blot analyses of the mRNA recovered from EA.hy926 cells after stimulation with 50 μmol/L of either palmitic, oleic, or linolenic acid. Linolenic and oleic acid had a significant effect on PAI-1 mRNA levels. Both the 3.2-kb ($P<0.01$) and the 2.2-kb ($P<0.05$) PAI-1 transcripts were increased. In contrast, 50 μmol/L of palmitic acid did not have an effect on PAI-1 mRNA levels (Figure 2). The stimulatory effect on PAI-1 mRNA by linolenic acid was detected after 2 hours (Figure 3).

Fatty Acid Activation of PAI-1 Transcription

A transfection assay was performed using an 804-bp fragment of the PAI-1 promoter coupled to a CAT gene. As

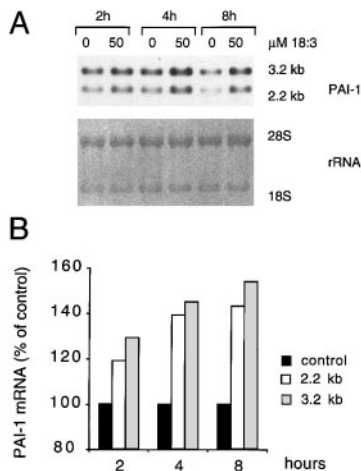


Figure 3. A, PAI-1 mRNA recovered from EA.hy926 cells after 2- to 8-hour incubation with 0 to 50 $\mu\text{mol/L}$ linolenic (18:3) acid. Total RNA (5 μg) was hybridized with a labeled cDNA probe for PAI-1. Vehicle containing the same amount of BSA solution was added as control. Corresponding blotting filters stained with methylene blue showing the 28S and 18S ribosomal RNAs demonstrate that approximately equal amounts of RNA were loaded. B, Quantification of the above autoradiogram. PAI-1 mRNA is given as percentage of control.

demonstrated in Figure 4, addition of palmitic (Figure 4A) or stearic (Figure 4B) acid did not have any effect on PAI-1 transcription. In contrast, both linolenic acid (Figure 4C) and EPA (Figure 4D) significantly increased PAI-1 transcription ($P < 0.01$ and $P < 0.01$, respectively). To localize the fatty acid-responsive region(s) in the PAI-1 promoter, we used several truncations of the promoter. As demonstrated in Figure 5, both the -804 -PAI-pCAT (Figure 5A) and the -708 -PAI-pCAT (Figure 5B) promoter constructs responded significantly to addition of 50 $\mu\text{mol/L}$ EPA, whereas the -609 -PAI-pCAT (Figure 5C) promoter construct did not. This implies that the response element is located between positions -609 and -708 of the PAI-1 promoter. This region contains the previously identified VLDL response element located between residues -672 and -657 . To determine whether the same response element in the PAI-1 promoter is involved in both VLDL- and fatty acid-mediated induction of PAI-1 transcription, we performed a transfection assay using a promoter construct with a 9-bp deletion (residues -670 to -662) of the VLDL response element (Figure 6). This deletion previously has been shown to eliminate the VLDL responsiveness of the PAI-1 promoter. As shown in Figure 6B and 6C, use of this promoter construct completely abolished the EPA-mediated induction of PAI-1 transcription.

Unsaturated Fatty Acids Increase the Binding of a VLDL-Inducible Transcription Factor to the PAI-1 Promoter

Because the transfection assays indicated that the recently characterized VLDL-inducible transcription factor could be involved in the fatty acid-mediated activation of PAI-1 transcription, EMSAs were performed. Nuclear extracts derived from HUVECs treated with fatty acids for 8 hours were incubated with a probe containing the -675 to -653 region of the PAI-1 promoter. As shown in Figure 7, the unsaturated

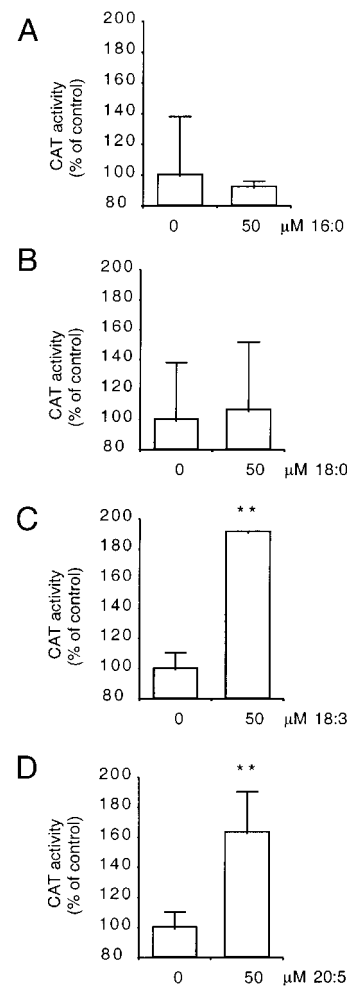


Figure 4. Unsaturated fatty acids activate transcription from the PAI-1 promoter in EA.hy926 cells. A promoter construct containing 804 residues of the PAI-1 promoter coupled to a CAT gene was transfected transiently into EA.hy926 cells. Palmitic acid (16:0) (A), stearic acid (18:0) (B), linolenic acid (18:3) (C), or EPA (20:5) (D) (50 $\mu\text{mol/L}$) was incubated with the cells for 16 to 18 hours. Bars indicate mean \pm SD, and PAI-1 transcription rate is given as percentage of control after correction for β -galactosidase activity. Results are based on 3 experiments performed in duplicate. ** $P < 0.01$.

fatty acids induced the same complex as did 75 $\mu\text{g/mL}$ of VLDL. Neither stearic acid (Figure 7) nor palmitic acid (Figure 8) had any effect on the binding of the VLDL-inducible factor. In contrast, oleic acid, linoleic acid, and EPA (Figures 7 and 8) increased the binding of the VLDL-inducible factor.

Discussion

In the present study, we demonstrated that unsaturated fatty acids increase PAI-1 transcription and secretion by endothelial cells in vitro. The effect appears to be mediated by a previously described VLDL-inducible transcription factor. To the best of our knowledge, this study is the first to demonstrate a mechanism by which fatty acids can modulate PAI-1 transcription positively.

Both VLDL and unsaturated fatty acids induced the binding of the same transcription factor to the PAI-1 promoter in

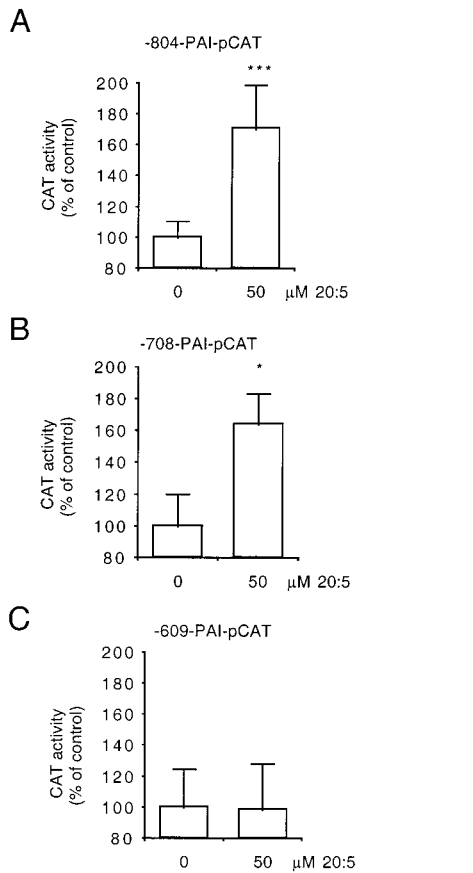


Figure 5. Fatty acid response element is located within the -609 to -708 region of the PAI-1 promoter. Promoter constructs containing 804 (A), 708 (B), or 609 (C) residues of the PAI-1 promoter coupled to a CAT gene were transfected transiently into EA.hy926 cells and induced by 50 μmol/L EPA (20:5). Bars indicate mean ± SD, and PAI-1 transcription rate is given as percentage of control after correction for β-galactosidase activity. Results are based on 3 experiments performed in duplicate. **P* < 0.05; ****P* < 0.001.

vitro. Fatty acids derived from VLDL triglycerides also may function as activators of the factor in vivo. The fatty acid composition of the VLDL used in this study, unfortunately, is not available. In a previous study, the weight percentages of 16:0, 18:0, 18:1, 18:2, and 20:5 in VLDL from fasting subjects were 32.5%, 3.8%, 38.6%, 16.9%, and 0.2%, respectively (E.T. et al, unpublished data, 1995). The concentrations of fatty acids used in the present study are in accordance with the concentration of nonesterified fatty acids found in serum. As demonstrated by Crofts et al,³¹ the concentrations of nonesterified 16:0, 18:0, 18:1, and 18:2 were 74, 47, 68, and 36 μmol/L, respectively, in serum of fasting control subjects.

Several fatty acid-inducible transcription factors have been described. Among these, the peroxisomal proliferator activator receptor (PPAR) family has been studied extensively.³² Members of the PPAR family are ligand-dependent transcription factors that bind to their cognate ligand with high affinity and then activate gene transcription through binding to a specific hormone response element in the promoter region of the target gene (a peroxisome proliferator activator response element [PPRE]). The VLDL/fatty acid response element in the PAI-1 promoter shows some homology with a PPRE.¹⁹

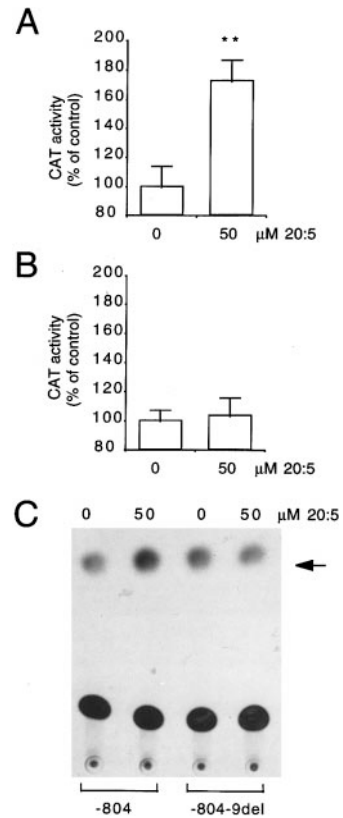


Figure 6. Fatty acid response element coincides with VLDL response element. Promoter constructs containing 804 (A) or 804 residues with a 9-bp deletion of VLDL response element (B) of PAI-1 promoter coupled to CAT gene were transfected transiently into EA.hy926 cells and induced by 50 μmol/L EPA (20:5). Bars indicate mean ± SD, and the PAI-1 transcription rate is given as percentage of control after correction for β-galactosidase activity. Results are based on 3 experiments performed in duplicate. ***P* < 0.01. C, Example of autoradiography of thin-layer chromatography analysis of CAT assay using 1-deoxychloramphenicol (Amersham) as substrate.

However, the sequence homology between the VLDL/fatty acid response element and a PPRE is only moderate, with a 67% homology with each hexamer of the site. A variety of fatty acids, both saturated and unsaturated, activate PPAR in vitro,³³ and it has been proposed that fatty acids are the natural ligands of PPARs.^{34,35} Furthermore, unsaturated fatty acids recently have been demonstrated to bind PPAR in vitro.³⁶ The fact that saturated fatty acids do not activate the VLDL-inducible transcription factor suggests that this factor is not identical with any of the 3 subtypes of PPAR known to date. However, PPARs belong to a rapidly growing family of “orphan” receptors, and it is likely that new members will appear. We are now in the process of cloning the VLDL/fatty acid-inducible transcription factor.

Because lipoproteins are readily oxidized when incubated with cultured cells in vitro, it can be envisaged that oxidized fatty acids are the mediators of the VLDL/fatty acid-enhancing effect on PAI-1. However, as no inhibitory effect on PAI-1 secretion was obtained with Trolox and 13-OOH-18:2 did not induce PAI-1 secretion, it seems reasonable to assume that the activation procedure did not involve fatty acid oxidation to any significant extent. We cannot exclude the

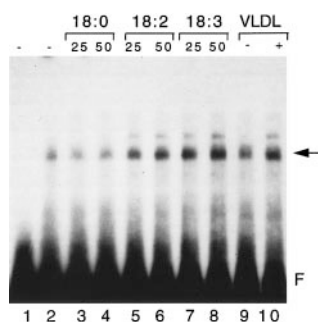


Figure 7. Fatty acid induction of the VLDL-inducible transcription factor. Representative autoradiogram of 2 EMSA experiments using protein extracts derived from HUVECs that had been incubated with BSA-vehicle (lane 2), 25 to 50 $\mu\text{mol/L}$ of either stearic (18:0) (lanes 3 and 4), linoleic (18:2) (lanes 5 and 6), or linolenic (18:3) acid (lanes 7 and 8), VLDL-vehicle (lane 9), and 75 $\mu\text{g/mL}$ VLDL (lane 10), and bound to the -675/-653 PAI-1 probe. Lane 1 shows probe in the absence of nuclear extract. F indicates free probe; arrow, VLDL-inducible factor.

possibility that intracellular oxidation of fatty acids is mediating the stimulatory effect and that the negative effect of 13-OOH-18:2 compared with linoleic acid is a result of an altered uptake by the cells. The finding that oleic acid, a fatty acid that shows very limited proneness to oxidation, enhanced PAI-1 secretion to a similar extent as polyunsaturated fatty acids, further supports the interpretation that the effect of unsaturated fatty acids on PAI-1 secretion is not secondary to oxidation. The finding that the fatty acid-mediated increase of PAI-1 mRNA levels already occurs after 2 hours also supports this notion.

An abundance of studies have confirmed the positive association between plasma triglycerides and plasma PAI-1 activity.² Reduction of hypertriglyceridemia also has been indicated to improve the fibrinolytic potential.³⁷⁻³⁹ A concomitant reduction of body weight, serum triglycerides, and plasma PAI-1 activity has been reported in several studies.⁴⁰⁻⁴² Fish oils or long-chain, polyunsaturated n-3 fatty acids have been shown to lower triglyceride concentrations in hypertriglyceridemia when given in high concentrations.

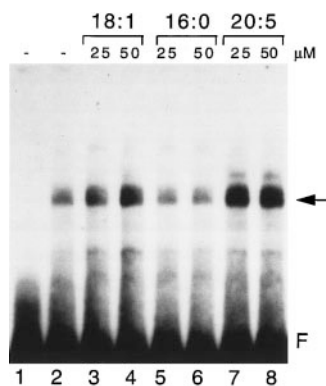


Figure 8. Fatty acid induction of a VLDL-inducible transcription factor. Representative autoradiogram of 2 EMSA experiments using protein extracts derived from HUVECs that had been incubated with BSA vehicle (lane 2), 25 to 50 $\mu\text{mol/L}$ of oleic acid (18:1) (lanes 3 to 4), palmitic acid (16:0) (lanes 5 to 6), or EPA (20:5) (lanes 7 to 8), and bound to the -675/-653 PAI-1 probe. Lane 1 shows probe in the absence of nuclear extract. F indicates free probe; arrow, VLDL-inducible factor.

However, the fatty acid intervention studies suggest that, in addition to the lowering of the triglyceride levels, there could be a positive and direct effect of the n-3 fatty acids on PAI-1 expression. For example, supplementation of the diet with n-3 fatty acids reduced the triglyceride level but increased plasma PAI-1 activity in non-insulin-dependent diabetes mellitus patients¹⁵ or patients undergoing coronary bypass surgery.¹⁷ Intake of n-3 polyunsaturated fatty acids or fish oils has also been associated with increased plasma PAI-1 activity in healthy individuals.^{16,18} Taken together, these clinical data support the notion that n-3 polyunsaturated fatty acids have a direct and positive effect on PAI-1 secretion also in vivo. The present study, along with 2 previous reports, demonstrate that this is, indeed, the case in vitro. Docosahexaenoic acid increased PAI-1 mRNA levels in HUVECs,¹⁴ and linoleic acid increased PAI-1 secretion from HepG2 cells.¹⁰ Here, we show that unsaturated fatty acids, including n-3 fatty acids, increase PAI-1 secretion, mRNA levels, and PAI-1 transcription in endothelial cells. However, it should be noted in this context that there are also some clinical studies showing an association between n-3 fatty acid intake and decreased plasma PAI-1 activity. Lopez-Segura et al⁴³ showed that consumption of a diet rich in monounsaturated fatty acids resulted in a significant decrease in both plasma PAI-1 activity and antigen in healthy individuals. Furthermore, the triglyceride levels were not affected by the dietary treatment. Similarly, n-3 polyunsaturated fatty acids recently have been shown not to affect plasma PAI-1 activity in patients with hypertension.⁴⁴ These reservations notwithstanding, the in vitro findings presented here suggest that unsaturated fatty acids have a direct enhancing effect on PAI-1 synthesis and that this could explain the apparent discrepancy between increased plasma PAI-1 activity and decreasing triglyceride levels during n-3 fatty acid supplementation in vivo.

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