# Induction of the Apolipoprotein AI Promoter by Sp1 Is Repressed by Saturated Fatty Acids

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**Insulin induces transcription of the hepatic apolipoprotein AI (apo AI) gene by increasing Sp1 binding to the promoter. To determine the effect of fatty acids on this process, HepG2 cells cotransfected with the plasmid pAI.474.CAT containing the full-length apo AI promoter and the Sp1-expressing plasmid, pCMV-Sp1, were studied. Chloramphenicol acetyl transferase (CAT) activity (% acetylation) increased 1.98-fold in cells receiving the Sp1 expression construct relative to control cells (46.4% ± 0.6% *v* 23.4% ± 1.3%, *P* < .05). Treatment of cells with 3 saturated fatty acids, stearic, myristic, and palmitic acid, repressed the ability of exogenous Sp1 to induce apo AI reporter gene expression (15.2% ± 1.7%, 22.5% ± 0.3%, 22.9% ± 0.1%, 23.5% ± 0.8%, respectively, *P* < .05). Unsaturated fatty acids, oleic, linoleic, or linolenic acid had no effect on Sp1-mediated induction of the apo AI promoter. In the presence of the *trans* fatty acids, CAT activity in the Sp1-transfected cells was similar to control cells (16.7% ± 3.3%, 19.3% ± 0.5%, and 21.0% ± 2.1% acetylation in cells exposed to elaidic acid, linolelaidic, or linolenelaidic acid, respectively). In cells treated with an equimolar mixture of oleic acid and stearic acid, apo AI promoter activity was suppressed in a manner similar to that observed in stearic acid-treated cells. Insulin (100 μU/mL) induced apo AI promoter activity 2.9-fold (22.4% ± 1.7% *v* 7.8% ± 2.4%, *P* < .05). However, in the presence of stearic acid, insulin was unable to induce apo AI promoter (6.3% ± 1.6%). Stearic acid treatment did not alter Sp1-DNA binding as measured by gel shift analysis. Therefore, saturated fatty acids blunt Sp1 induction of apo AI promoter probably at a step beyond DNA binding.**
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INSULIN INDUCES transcription of the apolipoprotein AI (apo AI) gene in hepatocytes through an insulin-responsive core element (IRCE) located between–390 and–410 bp (relative to transcription start site)[1] The IRCE contains an Sp1 binding site, and insulin treatment is associated with serine/threonine phosphorylation of Sp1 resulting in increased Sp1 binding to the IRCE.[2,3] Despite the fact that the apo AI gene is insulin responsive, plasma apo AI levels are often reduced in states of insulin resistance and hyperinsulinemia.[4] The reduced plasma concentration of high-density lipoprotein (HDL) cholesterol and its major apoprotein, namely apo AI, is primarily attributed to increased fractional clearance of HDL.[5,6] This change has been related to the increase in HDL content of triglycerides relative to cholesteryl ester and protein.[5,6] However, it is also possible that reduced expression of apo AI gene due to reduced responsiveness to insulin may contribute to the decreased plasma HDL concentrations.[7]

Because increased plasma free fatty acids (FFA) levels have been implicated as one of the causes of insulin resistance,[8] we studied the effect of FFA on Sp1-mediated and insulin-stimulated apo AI promoter activity in the cultured human hepatoma cell line, HepG2. The results indicate that saturated fatty acids, unlike the unsaturated fatty acids, prevent Sp1-stimulated induction of apo AI promoter activity.

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*Submitted January 10, 2004; accepted May 21, 2004.*

*Supported in part by the Harold Braun Memorial Fund.*

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*0026-0495/04/5310-0015$30.00/0*

*doi:10.1016/j.metabol.2004.05.011*

## MATERIALS AND METHODS

### *Materials*

Acetyl-coenzyme A and fatty acids (sodium salts) were from Sigma Chemical (St Louis, MO). Lipofectamine was purchased from Life Technologies, (Gaithersburg, MD), and $^{14}$C-chloramphenicol was from New England Nuclear (Boston, MA). Tissue culture media and fetal calf serum were purchased from BioWittaker (Walkersville, MD). All other chemicals were of reagent grade and were purchased from either Sigma Chemical or Fisher Scientific (Pittsburgh, PA).

### *Cell Culture*

HepG2 cells were maintained in Dulbecco's modified essential medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin and streptomycin (100 U/mL and 100 μg/mL, respectively). Cells were housed in a humidified incubator at 37°C with 5% $CO_2$ and 95% air. Cell viability following treatment with various fatty acids was measured by trypan blue exclusion.

### *Plasmids and Transient Transfections*

HepG2 cells were transfected with the plasmids pAI.474.CAT and pCMV.SPORT-β-gal (Life Technologies) The plasmid pAI.474.CAT contains the full-length apo AI promoter driving the chloramphenicol acetyl transferase (CAT) reporter gene. This promoter fragment contains the apo AI IRCE[1] as well as other *cis*-elements necessary for regulating expression in both hepatocytes and intestinal cells.[9,10] The latter plasmid expresses β-galactosidase under the control of the cytomegalovirus (CMV) immediate-early region promoter and was used to normalize for transfection efficiency. A total of 1 μg of each plasmid was transfected into HepG2 cells using 5 μL Lipofectamine (Life Technologies) as described by the manufacturer, and after 24 hours, treated with various 250 μmol/L saturated (stearic acid [$C_{18}$], myristic acid [$C_{14}$], and palmitic acid [$C_{16}$]), unsaturated fatty acids (oleic [$C_{18:1}$], linoleic [$C_{18:2}$], or linolenic acid [$C_{18:3}$]) and 250 μmol/L elaidic acid (*trans*-9-octa-decenoic acid), linolelaidic acid (*trans*-9,*trans*-12-octadecadienoic acid) or linolenelaidic acid (*trans*, *trans*, *trans*-9,12,15-octadecatrienoic acid). Each fatty acid was dissolved in 0.06% fatty acid–free bovine serum albumin (BSA) in phosphate buffered saline (PBS) (50 mmol/L sodium phosphate, pH 7.4, 150 mmol/L NaCl) and added to the cell cultures at a final concentration of 250 μmol/L. The molar ratio of fatty acids to albumin was 25:1. Control cells were treated with an equal volume of 0.06% BSA in PBS.

In some experiments, cells were also treated with 100 μU/mL insulin. After 24 hours, the cells were harvested and assayed for CAT[11] and β-galactosidase activity,[12] as previously described. All experiments were performed in cell cultures that had confluence of the cells over 90%.

In experiments designed to test the effect of exogenous transcription factor expression on apo AI promoter activity, the HepG2 cells were transfected with 1 μg pAI.474.CAT, 1 μg pCMV.SPORT.β-gal, 1 μg pCMV-Sp1, or 1 μg pLEN4S. The plasmid pCMV-Sp1, kindly provided by Dr Robert Tjian (University of California, Berkeley, Berkeley, CA), constitutively expresses Sp1 under the control of the CMV long terminal repeat region (LTR). The plasmid pLEN4S (kindly provided by Dr Francis Sladek, University of California, Riverside, Riverside, CA), expresses hepatic nuclear factor 4 (HNF-4) under the control of the metallothionein promoter.[13]

### *Nuclear Extract Preparation and Gel Shift Analysis*

Nuclear protein extracts were prepared as described previously.[14] HepG2 cells (90% confluent) were treated in the presence or absence of 100 μU/mL insulin with either 0.06% BSA or 250 μmol/L stearic acid dissolved in 0.06% BSA for 24 hours. Cells were washed 3 times in ice-cold PBS and suspended in 10 mL nuclear wash buffer (10 mmol/L N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid [HEPES] [pH 8.0], 15% sucrose, 1 mmol/L ethylene diaminetetra-acetic acid [EDTA], 0.5% Triton X-100, 1 mmol/L dithiothreitol [DTT], 5 mmol/L $MgCl_2$, and 1 mmol/L phenylmethylsulfonyl fluoride [PMSF]) and incubated on ice for 10 minutes. The mixture was underlaid with nuclear wash buffer containing 30% sucrose, but no Triton-X-100, and centrifuged at 3,000 × *g* for 30 minutes at 5°C. The pellet was suspended in 1 mL of a buffer containing 10 mmol/L HEPES (pH 8.0), 500 mmol/L NaCl, 10 mmol/L $MgCl_2$, 0.1 mmol/L EDTA, 1 mmol/L DTT, 1 mmol/L PMSF, and 5 mmol/L spermidine and placed on ice for 1 hour. The supernatant fraction was obtained by centrifugation at 10,000 × *g* for 10 minutes and was dialyzed extensively against 20 mmol/L HEPES (pH 7.9), 100 mmol/L KCl, 0.2 mmol/L EDTA, 0.5 mmol/L DTT, 0.5 mmol/L PMSF, and 20% glycerol. Protein concentration was determined using the Bradford assay[15] with BSA as the standard.

Electrophoretic mobility shift assays included 10 to 15 μg nuclear protein extract and 15,000 cpm of a $^{32}$P-labeled oligonucleotide probe containing a Sp1 binding site (5′-ATTCGATCGGGGCGGGGC-GAGC-3′). The probes 5′ hydroxyl group was labeled with [γ-$^{32}$P]-adenosine triphosphate (ATP) and T4 polynucleotide kinase. The protein extract was incubated with the probe in a solution containing 12% glycerol, 12 mmol/L HEPES, (pH 7.9), 60 mmol/L KCl, 5 mmol/L $MgCl_2$, 4 mmol/L Tris-Cl (pH 7.9), 0.6 mmol/L EDTA, 0.6 mmol/L DTT, and 2 μg poly (dI-dC) · poly(dI-dC) and placed on ice for 30 minutes. The mixture was loaded onto a 5% polyacrylamide gel and fractionated by electrophoresis in 0.5 × TBE (1× TBE is 45 mmol/L Tris base, 32.3 mmol/L boric acid, and 1.25 mmol/L EDTA [pH 8.3]) at room temperature, 200 V, for 90 minutes. The gel was dried and exposed to film for autoradiography.

Specificity of binding was assessed by adding a 100-fold molar excess of the unlabeled Sp1 or site A-containing oligonucleotide (sense strand 5′-TGAACCCTTGATCCCA-3′).[16] For supershift analysis, an antibody specific either to Sp1 or nonimmune serum was added to the binding mixture. Gel electrophoresis and autoradiography were performed as described above.

### *Western Blot Analysis*

Whole cell protein extracts were prepared from HepG2 cells transfected either with no DNA or with the Sp1 expression construct, pCMV-Sp1. Cells were washed twice with PBS and suspended in Laemmli sample buffer.[17] Protein concentrations were measured using the Bradford assay. Protein samples (35 μg) were fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose,[18] and incubated with the primary anti-Sp1 antiserum (1:500) for 16 hours. Primary antibody binding was detected with a secondary goat antirabbit antiserum conjugated to horseradish peroxidase (HRP). HRP activity was visualized by enhanced chemiluminescence (ECL) followed by autoradiography.

### *Statistical Analysis*

Changes in reporter gene activity were evaluated with the 2-tailed Student's *t* test. A *P* value less than .05 was considered the limit for statistical significance. The results are presented as the mean ± SEM.

## RESULTS

### *Effect of Fatty Acids on Sp1-Mediated Induction of the apo AI Promoter*

Transfection with pCMV-Sp1 induced apo AI promoter activity 1.98-fold from 23.4% ± 1.3% to 46.4% ± 0.6% ($P < .05$) (Table 1). However, treatment of cells with 3 saturated fatty acids, stearic acid ($C_{18}$), myristic acid ($C_{14}$), and palmitic acid ($C_{16}$), repressed the ability of exogenous Sp1 to induce apo AI reporter gene expression (22.5% ± 0.3%, 22.9% ± 0.1%, 23.5% ± 0.8% in myristic, palmitic, and stearic acid-treated cells, respectively, $P < .05$) (Table 1). However, the 3 unsaturated fatty acids, oleic ($C_{18:1}$), linoleic ($C_{18:2}$), or linolenic acid ($C_{18:3}$), had no effect on Sp1-mediated induction of the apo AI promoter (Table 1). Apo AI promoter activity in HepG2 cells with oleic, linoleic, and linolenic acid was indistinguishable from pCMV-Sp1-transfected cells (46.4% ± 0.6% in pCMV-Sp1 transfected cells *v* 47.6% ± 0.3%, 44.9% ± 1.3%, 47.2% ± 0.6% in oleic, linoleic, and linolenic acid-treated cells, respectively).

### *Effect of* Trans *Fatty Acids on Sp1-Mediated Induction of apo AI Promoter Activity*

To determine if the *trans* isomers of the unsaturated fatty acids tested effect the ability of Sp1 to induce apo AI promoter

**Table 1. Effect of Various Fatty Acids on apo AI Promoter Activity**

| Treatment | CAT Activity (%) | Fold Change | *P* (Student's *t* test) |
|---|---|---|---|
| Control | 23.4 ± 1.3 | — | — |
| Sp1 | 46.4 ± 0.6 | 1.98 | .0009* |
| Sp1 + myristic acid | 22.5 ± 0.3 | 0.96 | .0004† |
| Sp1 + palmitic acid | 22.9 ± 0.1 | 0.98 | .0003† |
| Sp1 + stearic acid | 23.5 ± 0.8 | 1.00 | .0004† |
| Sp1 + oleic acid | 47.6 ± 0.3 | 2.03 | .75† |
| Sp1 + linoleic acid | 44.9 ± 1.3 | 1.91 | .64† |
| Sp1 + linolenic acid | 47.1 ± 0.6 | 2.01 | .77† |

NOTE. HepG2 cells were transfected with the plasmids pAI.474.CAT and pCMV.SPORT-β-gal, ±the Sp1 expression vector pCMV-Sp1. The cells were treated with the indicated fatty acids (final concentration of 250μmol/L) for 24 hours. Only the saturated fatty acids were able to suppress Sp1-mediated induction of the apo AI promoter. Promoter activity in cells treated with the 3 unsaturated fatty acids was similar to pCMV-Sp1-transfected cells.

*V untreated control cells not transfected with the Sp1 expression construct.

†*V* untreated cells transfected with the Sp1 expression construct (N = 6).

activity, HepG2 cells were transfected with pAI.474.CAT and pCMV-Sp1, then treated with 250 μmol/L elaidic acid (*trans*-9-octa-decenoic acid), linolelaidic acid (*trans*-9,*trans*-12-octadecadienoic acid) or linolenelaidic acid (*trans*, *trans*, *trans*-9,12,15-octadecatrienoic acid). Control cells were transfected with pAI.474.CAT and the empty CMV expression construct. As expected, CAT activity increased 2.55-fold, from 23.9% ± 1.3% acetylation in control cells to 61.0% ± 3.4% acetylation in cells expressing Sp1. In the presence of the *trans* fatty acids however, CAT activity in the Sp1-transfected cells was similar to control cells (16.7% ± 3.3%, 19.3% ± 0.5%, and 21.0% ± 2.1% acetylation in cells exposed to elaidic acid, linolelaidic, or linolenelaidic acid, respectively) (Table 2).

### *Effect of a Mixture of Stearic and Oleic Acid on Sp1-Mediated Induction of apo AI Promoter Activity*

To determine if saturated or unsaturated fatty acids are dominant in their ability to regulate Sp1 activity with regard to apo AI promoter activity, HepG2 cells were transfected with pAI.474.CAT ± pCMV-Sp1, then treated with 250 μmol/L stearic acid, oleic acid, or stearic + oleic acid. Control cells were transfected with pAI.474.CAT and the empty CMV expression construct. CAT activity increased 1.71-fold, from 17.1% ± 0.8% acetylation in control cells to 29.3% ± 1.2% acetylation in cells expressing Sp1 (Table 3). As expected, CAT activity in Sp1-transfected cells exposed to stearic acid was repressed (from 17.1% ± 0.8% to 13.8% ± 0.6% acetylation, respectively). Oleic acid had no effect on the ability of Sp1 to induce apo AI promoter activity (28.1% ± 1.1% acetylation in Sp1-transfected cells exposed to oleic acid). In cells exposed to both fatty acids, apo AI promoter activity was suppressed in a manner similar to that observed in stearic acid-treated cells (15.7% ± 1.0% acetylation).

### *Effect of Stearic Acid on Insulin Induction of the apo AI Promoter*

To determine whether or not fatty acids can interfere with insulin induction of the apo AI promoter, HepG2 cells transfected with the full-length apo AI-CAT reporter plasmid along with the control plasmid pCMVC.SPORT.β-gal were treated with stearic acid (250 μmol/L in 0.06% BSA in PBS) or solvent (0.06% BSA in PBS), with and without insulin (100 μU/mL). CAT activity (Fig 1A) in cells treated with insulin increased 2.9-fold (Fig 1B), from 7.8% ± 2.4% to 22.4% ± 1.7% ($P < .05$). CAT activity from cells treated with stearic acid was not statistically different from the controls (8.1% ± 3.1% acetylation). However, insulin in the presence of stearic acid was unable to induce the apo AI promoter (6.3% ± 1.6% acetylation, $P < .05$ relative to insulin-induced state). These results suggest that in the presence of stearic acid, insulin is ineffective in inducing apo AI promoter activity.

**Table 2. Effect of *trans* Fatty Acids on Sp1-Mediated Induction of apo AI Promoter Activity**

| Treatment | CAT Activity (%) | Fold Change | *P* (Student's *t* test) |
|---|---|---|---|
| Control | 23.9 ± 1.3 | — | — |
| Sp1 | 61.0 ± 3.4 | 2.55 | .0005* |
| Sp1 + elaidic acid | 16.7 ± 3.3 | 0.70 | .003† |
| Sp1 + linolelaidic acid | 19.3 ± 0.5 | 0.81 | .0003† |
| Sp1 + linolenelaidic acid | 21.0 ± 2.1 | 0.88 | .0006† |

NOTE. HepG2 cells were transfected with the plasmids pAI.474.CAT and pCMV.SPORT-β-gal, ± the Sp1 expression vector pCMV-Sp1. The cells were treated with the indicated fatty acids (final concentration of 250μmol/L) for 24 hours. All the *trans* fatty acids tested inhibited Sp1-mediated induction of apo AI promoter.

**V* untreated control cells not transfected with the Sp1 expression construct.

†*V* untreated cells transfected with the Sp1 expression construct (N = 6).

**Table 3. Effect of Mixing Stearic and Oleic Acid on Sp1-Mediated Induction of apo AI Promoter Activity**

| Treatment | CAT Activity (% acetylation) | Fold Change | *P* (Student's *t* test) |
|---|---|---|---|
| Control | 17.1 ± 0.8 | — | — |
| Sp1 | 29.3 ± 1.2 | 1.71 | .001* |
| Sp1 + stearic acid | 13.8 ± 0.6 | 0.81 | NS |
| Sp1 + oleic acid | 28.1 ± 1.1 | 1.64 | .001* |
| Sp1 + stearic/oleic acid | 15.7 ± 1.0 | 0.92 | NS |

NOTE. HepG2 cells were transfected with the plasmids pAI.474.CAT and pCMV.SPORT-β-gal, ± the Sp1 expression vector pCMV-Sp1. The cells were treated with the indicated fatty acids at a final concentration of 250μmol/L for 24 hours. Oleic acid had no effect on the ability of Sp1 to induce apo AI promoter activity, while stearic acid suppressed this response. However, in the presence of both fatty acids, apo AI promoter activity was suppressed suggesting that the effect of saturated fatty acids is dominant.

Abbreviation: NS, not significant.

**V* untreated control cells not transfected with the Sp1 expression construct (N = 6).

### *Effect of Stearic Acid on HNF-4–Related Changes in apo AI Promoter*

To determine the specificity of the effects on Sp1 activity, we examined the effect of stearic acid on HNF-4 activity with regard to the apo AI promoter. Exogenous HNF-4 expression is known to suppress apo AI promoter activity in hepatocytes.[19] In the present experiments, CAT activity in HNF-4 transfected cells was significantly suppressed in both control and stearic acid–treated cells relative to cells receiving empty vector (27.8% ± 2.0% *v* 19.3% ± 1.7% in control HNF-4 nonexpressing and expressing cells, respectively, $P < .05$; 32.6% ± 2.5% *v* 21.6% ± 0.5% in stearic acid–treated HNF-4 nonexpressing and expressing cells, respectively, $P < .02$) (Table 4). Thus, stearic acid treatment had no effect on HNF-4–related suppression of apo AI promoter.

### *Effect of Stearic Acid on Sp1 Expression in HepG2 Cells*

To determine if stearic acid suppresses exogenous Sp1 synthesis that may account for the findings described above, Sp1 levels were measured in control and stearic acid–treated cells. Steady-state Sp1 levels were undetectable in whole cell extracts prepared from mock-transfected control and stearic acid–treated cells (Fig 2, lanes 1 and 2, respectively). However, cells transfected with pCMV-Sp1 synthesize more Sp1 than mock-transfected cells (Fig 2, lanes 3), the level of which did not

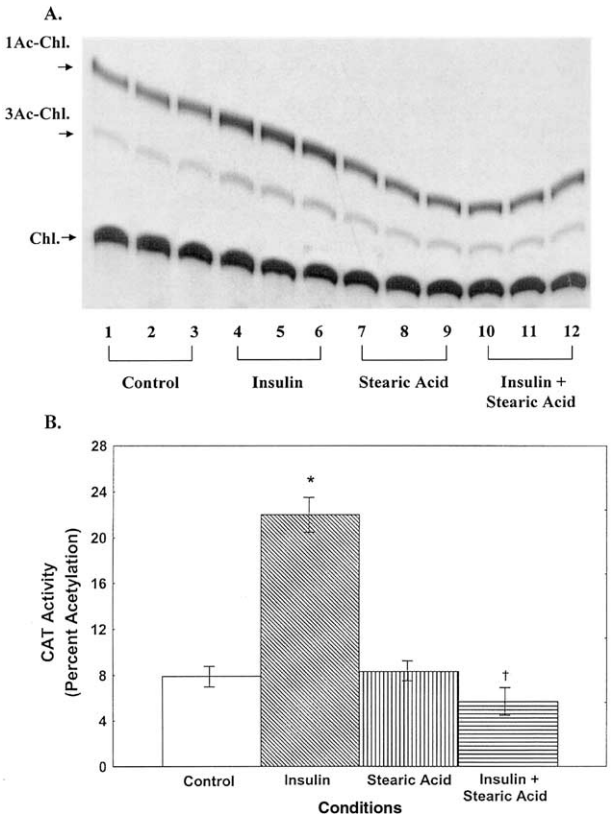


**Fig 1. Effect of stearic acid on insulin-induction of the apo AI promoter. (A) HepG2 cells were transfected with the plasmids pAI.474.CAT and pCMV.SPORT-$\beta$-gal and treated with insulin (100 $\mu$U/mL), stearic acid (250 $\mu$mol/L) or insulin + stearic acid for 24 hours or vehicle (0.06% BSA in PBS, control). (B) CAT activity (chromatogram in A) (percent acetylation) was normalized to $\beta$-galactosidase activity and is shown for each treatment group. As expected, insulin treatment induced apo AI promoter activity. However, in the presence of stearic acid, induction by insulin was suppressed. *$P <$ .05 relative to control cells; †$P <$ .05, relative to insulin-treated cells.**

change significantly with stearic acid treatment (Fig 2, lane 4). Although endogenous Sp1 levels were undetectable in whole-cell extracts, it can be detected by immunoprecipitation (data not shown) or by measuring Sp1-DNA binding activity as observed in the gel shift experiments described below.

**Table 4. Effect of Stearic Acid on HNF-4–Mediated Suppression of the apo AI Promoter**

| Treatment | CAT Activity (%) | Fold Change | *P* (Student's *t* test) |
|---|---|---|---|
| Control | 27.8 ± 2.0 | — | — |
| Stearic acid | 32.6 ± 2.5 | 1.17 | .82 |
| HNF-4 | 19.3 ± 1.7 | — | — |
| HNF-4 + stearic acid | 21.6 ± 0.5 | 1.12 | .74 |

NOTE. HepG2 cells were transfected with the plasmids pAI.474.CAT and pCMV.SPORT-$\beta$-gal, ± the HNF-4 expression vector pLEN4S. The cells were treated with stearic acid (final concentration of 250$\mu$mol/L) for 24 hours. As expected, HNF-4 suppressed apo AI promoter activity significantly. Stearic acid had no effect on apo AI promoter activity either in the presence or absence of exogenous HNF-4, suggesting that the effect is specific to Sp1 (N = 6).

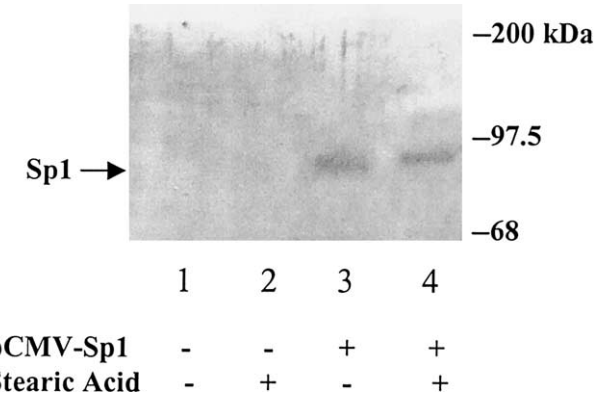


**Fig 2. Exogenous Sp1 expression in HepG2 cells. Sp1 levels were assessed in mock-transfected HepG2 cells (lanes 1 and 2) or cells transfected with the plasmid pCMV-Sp1 (lanes 3 and 4). After 24 hours, whole cell protein extracts were prepared and used for Western blot analysis. Sp1 protein is detectable only in cells transfected with the expression construct and does not change with stearic acid treatment.**

*Effect of Stearic Acid on Sp1-DNA Binding*

Because stearic acid repressed Sp1 induction of the apo AI promoter, the effect of insulin ± stearic acid on Sp1-DNA binding was examined by gel shift. Only 1 protein-DNA complex was observed with the Sp1 oligonucleotide probe (Fig 3A). Insulin exposure increased Sp1-DNA binding both in the presence or absence of stearic acid. Competition with the unlabeled Sp1-binding site-containing oligonucleotide (Fig 3B) displaced Sp1 from the labeled DNA (compare lanes 2 and 3), while an unrelated oligonucleotide (site A) had no effect (compare lanes 4 and 5). Finally, the authenticity of Sp1-DNA binding was assessed by supershift analysis. Addition of the anti-Sp1 antiserum inhibited Sp1 binding to the probe (Fig 3B, lane 7), while nonimmune antiserum had no effect on Sp1 DNA binding (Fig 3B, lane 8). These results suggest that stearic acid must affect Sp1 activity, not through inhibition of DNA binding or through changes in steady-state levels of Sp1, but through posttranslational means.

## DISCUSSION

The results of this study show that saturated fatty acids, such as stearic acid, have an important modulating role in insulin-mediated induction of apo AI gene expression. Because the effect of insulin on the apo AI promoter is mediated by Sp1, the effect of select saturated and unsaturated fatty acids on Sp1-mediated activation of the apo AI promoter was examined. Treatment of cells with 3 saturated fatty acids, stearic acid ($C_{18}$), myristic acid ($C_{14}$), and palmitic acid ($C_{16}$), repressed the ability of exogenous Sp1 to induce apo AI reporter gene expression (Table 1). However, the 3 unsaturated fatty acids, oleic ($C_{18:1}$), linoleic ($C_{18:2}$), or linolenic acid ($C_{18:3}$), had no effect on Sp1-mediated induction of the apo AI promoter (Table 1). These results suggest that the ability of FFA to

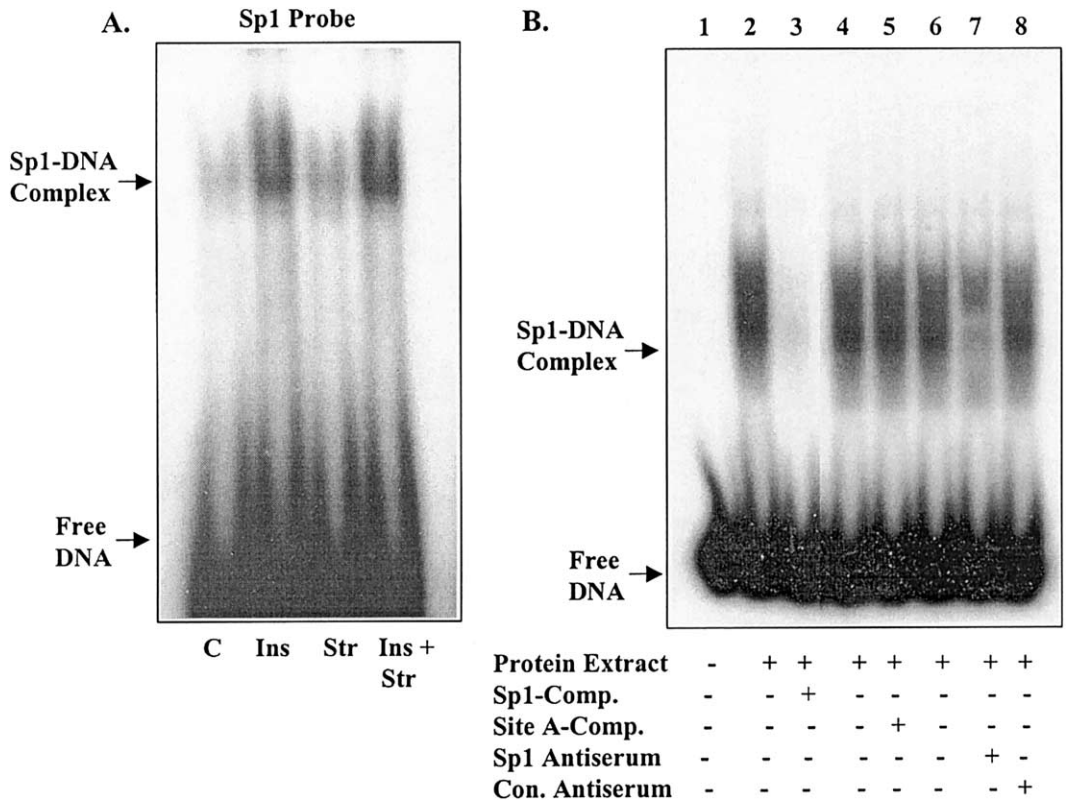


**Fig 3. Effect of stearic acid on Sp1-DNA binding. (A) Nuclear protein extracts prepared from HepG2 cells treated with insulin (Ins), stearic acid (Str), both insulin and stearic acid (Ins + Str), or solvent-treated (C) HepG2 cells were incubated with an oligonucleotide probe containing a consensus Sp1 binding site and fractionated by nondenaturing polyacrylamide gel electrophoresis. Free DNA and the Sp1-DNA complex are indicated (arrows). Insulin increased Sp1/DNA binding in either the presence or absence of stearic acid. (B) Authenticity of Sp1 binding to the oligonucleotide probe was assessed by competition experiments and supershift analysis. Free DNA and the Sp1-DNA complex are indicated (arrows). Addition of a 100-fold molar excess of an unlabeled Sp1 competitor oligonucleotide displaced binding activity, while addition of an unlabeled site A oligonucleotide had no effect on factor binding. Addition of antiserum to Sp1 displaced factor binding, while addition of nonimmune serum did not.**

repress Sp1-mediated induction of apo AI promoter activity is dependent on degree of saturation, but not necessarily chain length. This is in contrast to previously published observations on the importance of the length of the fatty acid chains as a determinant of its effect on the expression of plasminogen activator inhibitor-1 (PAI-1) gene.[20]

*Trans* fatty acids, like saturated fatty acids, blunt Sp1 upregulation of apo AI promoter activity (Table 2). The reduced apo AI promoter activity in the presence of *trans* fatty acids is consistent with the literature on the effect of dietary *trans* fatty acids on lowering HDL cholesterol levels.[21,22]

The results of studies on the effect of mixing monounsaturated or polyunsaturated FFA with saturated FFA may vary depending on the precise combinations and proportions. Nevertheless, the limited set of data presented here suggests that within the concentration ranges tested, monounsaturated FFA cannot prevent the downregulation of apo AI transcription by saturated FFA (Table 3).

In the presence of stearic acid, insulin or its key downsteam transcription factor Sp1, was ineffective in stimulating apo AI promoter activity. Thus, stearic acid blunts insulin induction of apo AI promoter activity at least partly through inhibiting Sp1 activity. This appears to be a selective effect, because stearic acid had no effect on the activity of the transcription factor HNF-4 (Table 4), another transcription factor that modulates the expression of the apo AI gene and has a binding site within site A of the apo AI promoter.[13,23,24]

Sp1-DNA binding was equally stimulated by insulin in the presence or absence of stearic acid. Thus, it is likely that Sp1 activity is altered at a posttranslational level causing impairment in the biologic effects of this transcription factor, but not its affinity for binding DNA. Alternatively, the changes in binding may have been modest, such that it was not detectable by the gel shift analysis used.

Unlike saturated fatty acids, unsaturated fatty acids were unable to suppress Sp1-related induction of apo AI promoter activity. This finding suggests that intracellular signaling pathways may be altered in saturated fatty acid–treated cells. Future studies should explore the potential differences between saturated and unsaturated fatty acids in altering insulin signaling and activation of Sp1 activity in HepG2 cells. The diversity of the effects of saturated and unsaturated fatty acids on modulating transcriptional process have been previously recognized.[25] It is noteworthy that oxidized fatty acids enhance

intestinal apo AI gene expression through peroxisome proliferator-activated receptors (PPARs).[26] The current study in hepatocytes shows that the nonoxidized fatty acids, especially the saturated fatty acids, do not stimulate, but may indeed blunt apo AI expression.

Because insulin is a positive modulator of apo AI gene transcription,[1,2] the lack of increased apo AI production in the face of hyperinsulinemia in obese individuals may be regarded as yet another aspect of insulin resistance. There are multiple potential mediators of insulin resistance in obesity. At the present time the increase in plasma FFA and increased levels of some adipokines, such as tumor necrosis factor $\alpha$ (TNF $\alpha$) are believed to be key mediators of insulin resistance.[27] Indeed, it has been shown that TNF $\alpha$ downregulates apo AI gene expression.[28] The increased serum levels of FFA may have additional effects on apo AI gene expression. It is also possible that the inhibitory effect of FFA on insulin-mediated signaling is also inhibitory to the positive effect of insulin on apo AI gene expression.

These observations are in agreement with previously published studies showing that in HepG2 cells, lipid supply is an important determinant of apolipoprotein synthesis.[29-34] The increased plasma FFA concentrations in insulin-resistant states may contribute to the reduced plasma apo AI levels by blunting insulin-mediated apo AI gene transcription. It is possible that the apo AI–inducing effect of fibrates is, at least in part, indirectly related to reduced plasma FFA levels in addition to the direct effect of fibrates on apo AI gene expression.[35-37] Similarly, the increased plasma apo AI levels following treatment with nicotinic acid[38,39] or thiazolidenediones[40,41] may also partly be related to decreased circulating FFA levels allowing for derepression of the apo AI gene. However, other changes in lipoprotein metabolism including alterations in fractional clearance of apo AI, changes in apoAI mRNA stability, or transcriptional modulation with PPARs appear to have a more important role in the increased apo AI levels following treatment with these agents.[35-41]

It is noteworthy that measurements of apo AI secretion and apo AI mRNA following treatment of nontransfected HepG2 cells with fatty acids showed no significant change in our laboratory (data not shown). It appears that the main effect of fatty acids on apo AI synthesis occurs only during increased Sp1 activation, while the effects during basal conditions of Hep G2 cells that are not overexpressing Sp1, are modest at best.

The effect of the fatty acids on apo AI promoter activity could not be attributed to nonspecific toxicity, because cell viability was documented by trypan blue exclusion to be over 95% in each experiment. Furthermore, $\beta$-galactosidase activity in the transfected cells was not altered following treatment of cells with any of the fatty acids used.

Previously published studies have shown that unsaturated fatty acids decrease apo AI synthesis in HepG2 cells.[30] In addition, clinical studies have found that dietary intake of saturated fatty acids increase plasma HDL cholesterol level.[31] Dietary fatty acids produce diverse effects at transcriptional, as well as posttranscriptional levels, and the changes differ according to species and strain.[32] Compared with saturated fatty acids, dietary polyunsaturated fatty acids upregulate hepatic SR-B1 expression, increase HDL cholesterol ester transport to the liver, and as a consequence, plasma HDL cholesterol level is reduced.[33] It is noteworthy that diet enriched in saturated fatty acids, unlike diets enriched in unsaturated fatty acids, is associated with increased HDL cholesterol and in apo AII levels, but not in apo AI levels.[34] These latter observations are consistent with the current study highlighting potential adverse effects of saturated fatty acids on apo AI gene transcription.

Overall, the present study indicates that saturated FFA represses the induction of apo AI promoter in HepG2 cells by insulin or by exogenous expression of the transcription factor, Sp1. Unlike saturated fatty acids, the unsaturated fatty acids do not appear to have this potential deleterious effect on apo AI gene expression. These observations provide an additional rationale for the current dietary recommendations of limiting intake of saturated fat in the diet. In addition, it is tempting to speculate that the increased plasma FFA concentrations commonly observed in obesity may contribute, at least partly, to the reduction in plasma apo AI and HDL levels. Nevertheless, caution should be exercised when extrapolating findings in cell cultures to intact organisms.

## ACKNOWLEDGMENT

We thank Brian Plummer for excellent technical help.

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