Regulation of apoA-I gene expression: mechanism of action of estrogen and genistein

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Abstract **We have previously shown that 17-β-estradiol (E_2) and genistein increase the expression of apolipoprotein A-I (apoA-I), the major protein component of HDL, in Hep G2 cells. To elucidate the mechanism mediating the increase in apoA-I gene expression by these compounds, plasmid constructs containing serial deletions of the apoA-I promoter region were generated. The smallest region maintaining response to E_2 and genistein spanned the −220 to −148 sequence, and the estrogen antagonist ICI182,780 completely inhibited the E_2 and genistein effect. Nuclear extracts from cells treated with E_2 and genistein showed increased binding to site B oligonucleotide (−169 to −146), and nuclear extracts from genistein-treated cells showed increased binding to an early growth response factor 1 (Egr-1) oligonucleotide compared to control cells. An increase in the concentrations of Egr-1 and hepatocyte nuclear factor-3β was observed in nuclear extracts of cells treated with both compounds compared to control cells. Treatment with a specific inhibitor of mitogen-activated protein (MAP) kinase, but not with other inhibitors, abolished the stimulation of apoA-I gene expression by E_2 and genistein. These results indicate that the MAP kinase pathway is involved in the regulation of apoA-I gene expression by genistein and E_2, possibly through downstream regulation of transcription factors binding to the promoter region.**—Lamon-Fava, S., and D. Micherone. **Regulation of apoA-I gene expression: mechanism of action of estrogen and genistein.** ***J. Lipid Res.*** **2004.** 45: **106–112.**

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Apolipoprotein A-I (apoA-I), the major protein component of HDL, is synthesized by the liver and intestine and is both necessary and sufficient for the formation of HDL (1, 2). ApoA-I plays an important role in the reverse cholesterol transport pathway by *a*) interacting with the ATP binding cassette transporter A1 cell membrane receptor and promoting cell cholesterol efflux via this receptor, *b*) activating LCAT, the enzyme involved in cholesterol esterification, and *c*) carrying the excess esterified cholesterol back to the liver for excretion (3). Several studies have indicated that plasma levels of apoA-I and HDL cholesterol are significant predictors of coronary heart disease (4–6). Treatment with estrogen is associated with an increase in plasma apoA-I levels (7), and metabolic studies conducted in women using either radiolabeled or endogenously labeled apoA-I have indicated that an increased production rate of apoA-I is responsible for the increase in its plasma levels during estrogen treatment (8–10). Only oral administration of estrogen has been shown to increase both apoA-I concentration and production rate, because postmenopausal transdermal estrogen delivery, while effective in restoring serum estradiol levels to premenopausal levels, is not associated with changes in protein levels or production rates (9). The differential effect of oral and transdermal estradiol implicates a first pass through the liver as a necessary step for the increased production of apoA-I.

Genistein, an isoflavone phytoestrogen present in high concentration in soy, has partial estrogen agonist activity (11). Phytoestrogens from soy have been shown to increase plasma HDL cholesterol levels in some (12, 13) but not all (14, 15) studies.

We (16) and others (17) have previously shown that estrogen increases apoA-I gene transcription in liver cells in accordance with the results of clinical metabolic studies. We have also shown that genistein increases apoA-I transcription in liver cells (18). The increase in apoA-I gene expression by estrogen and genistein is mediated by the −256 to −41 region of the apoA-I promoter (16, 18). This region acts as a liver-specific enhancer and contains three transcription factor binding sites, site A (−214 to −192), site B (−169 to −146), and site C (−134 to −119) (19). Site A is involved in apoA-I gene expression regulation by members of the steroid/thyroid nuclear receptor superfamily, such as hepatocyte nuclear factor 4 (HNF-4) and retinoid X receptor α (20). Site B has been shown to bind HNF-3β (21). Site C is structurally similar to site A and

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binds transcription factors similar to those recognizing site A. Two binding sites for the transcription factor early growth response factor 1 (Egr-1) (−221 to −213 and −189 to −181) are also found in this region (22). An antioxidant response element (ARE) has been described in the apoA-I promoter (23). The apoA-I promoter region does not contain a classic estrogen response element (ERE). However, two ERE half-palidromic sites are located within site A, but previous work has shown that the estrogen receptor (ER) does not bind to this sequence, suggesting that other transcription factors may be involved in the estrogen-mediated increase in apoA-I expression (24).

The current report shows that the mechanism responsible for the transcription activation of the apoA-I gene by estrogen and genistein in liver cells is mediated by a short sequence in the promoter region that binds the Egr-1 and HNF-3β transcription factors. Nuclear concentration of these two transcription factors is increased by treatment of cells with these estrogenic compounds. Activation of the mitogen-activated protein (MAP) kinase pathway mediates, at least in part, the increase in apoA-I gene expression by estrogen and genistein.

MATERIALS AND METHODS

Materials

Genistein and 17-β-estradiol (E_2) were purchased from Sigma (St. Louis, MO). The MAP kinase inhibitor PD 98059 was purchased from Cell Signaling (Beverly, MA). The protein kinase A (PKA) inhibitor myristoylated 14-22 amide and the protein kinase C (PKC) inhibitor bisindolylmaleimide I were obtained from Calbiochem (San Diego, CA). ICI182,780 was purchased from Tocris (Balwin, MO). The Egr-1 (rabbit polyclonal) and HNF-3β (goat polyclonal) antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). γ-^{32}P-ATP was obtained from Perkin-Elmer Life Sciences (Boston, MA).

Cell culture experiments

Hep G2 cells were maintained in DMEM (BioWhittaker, Walkesville, MD) supplemented with 10% FBS (Hyclone, Logan, UT) and 1% Glutamax, 100 U/ml of penicillin, and 100 μg/ml of streptomycin (Invitrogen, Chicago, IL), as previously reported (16, 18). All experiments were carried out in phenol red-free DMEM/F12 medium containing 5% charcoal/dextran-treated FBS. An enzyme-linked immunosorbent assay was used for the determination of apoA-I in the culture media, as previously described (16). Transfection experiments were carried out in six-well dishes with 1.5 μg of the test plasmid and 0.25 μg of *Renilla* luciferase plasmid (Promega, Madison, WI) as an internal control reporter and using the Lipofectamine reagent (Invitrogen). Six hours after transfection, genistein and E_2 were added at the concentration of 10 μM. When indicated, ICI182,780 was added to the culture medium 1 h before genistein and E_2 at the concentration of 10 μM. Similarly, inhibitors of MAP kinase (10 μM), PKA (10 μM), and PKC (1 μM) were added to the culture medium 30 min before genistein and E_2. Cells were collected for luciferase measurements after 36 h.

Results are presented as the mean ± SD of three to six independent experiments conducted in duplicate.

Plasmid constructs

The −256A-I.Luc and −41A-I.Luc plasmids, containing the −256 to +396 and the −41 to +396 regions of the apoA-I gene, respectively, have been previously described (16). To generate the −192A-I.Luc plasmid, the −192 to −42 *Pst*I-*Pst*I fragment was isolated from the −256A-I.Luc plasmid and inserted into the *Pst*I site of the −41A-I.Luc plasmid, and correct orientation was documented by sequencing. The −133A-I.Luc construct was created by cloning the *Sau*3A-*Hind*III fragment of −256A-I.Luc into the *Bgl*II-*Hind*III site of the pGL2 basic vector (Promega). The −220[Δ−110/−42]A-I.Luc plasmid, which contains the −220 to −110 region of the apoA-I promoter inserted in front of nucleotide −41 of the −41A-I.Luc plasmid, was obtained by cloning the *Hind*III-*Hind*III fragment of plasmid −220[[Δ−110/−42]A-I.CAT, previously described and a gift from Dr. S. Karathanasis (19), into the *Hind*III site of the pGL2 basic vector. To generate the −256[Δ−132/−42]A-I.Luc construct, the −256 to −133 region of the apoA-I gene was amplified by PCR, with the forward primer containing an *Sst*I overhanging site and the reverse primer containing an *Xho*I overhanging site; the PCR product was then cut with *Sst*I and *Xho*I and cloned into the *Sst*I-*Xho*I site of −41A-I.Luc. A similar approach was used for the generation of both the −256[Δ−148/−42]A-I.Luc and the −256[Δ−185/−42]A-I.Luc plasmid constructs. A plasmid containing two sites A (2siteAA-I.Luc) was constructed by cloning a double-stranded synthetic oligonucleotide into the *Sst*I-*Xho*I site of the −41A-I.Luc plasmid. The 2siteBA-I.Luc and 2AREA-I.Luc plasmids were generated following the same procedure. The −256A-I.Luc plasmid was used as template for site-directed mutagenesis of the two ERE half-palindromic sequences in site A. Mutagenesis was performed by the QuickChange method (Stratagene, La Jolla, CA) using the following primers (and their complementary oligonucleotides): 5′-CCGCCCCCACTGGGCCCTTGACCCCTGCCCTGCAG-3′ and 5′-CCGCCCCCACTGAACCCTTGGGCCCTGCCCTGCAG-3′ (mutated sequences are underlined). Correct mutations were confirmed by plasmid sequencing.

Electrophoresis mobility shift assay

Double-stranded oligonucleotides were labeled with ^{32}P using T4 polynucleotide kinase and γ^{32}P-ATP. Nuclear extracts from Hep G2 cells that had been treated with 10 μM E_2 or 10 μM genistein, or control cells, were prepared as previously described (25). Electrophoresis mobility shift assays (EMSAs) were performed with 7 μg of nuclear protein extracts in a 20 μl reaction mixture containing 10 mM Tris HCl (pH 7.5), 4% glycerol, 1 mM $MgCl_2$, 0.5 mM EDTA, 0.5 mM DTT, 50 mM NaCl, and 5 μg of poly(dI-dC). When indicated, specific nonlabeled oligonucleotide competitors were used at 100× molar excess concentration. The binding reactions were incubated at 4°C for 10 min, and then with 2 pmol of a specific double-stranded ^{32}P-labeled oligonucleotide for 20 min. For supershift assays, 1 μg of specific antibody was added to the reaction mixture at the end of the binding reaction and incubated for an additional 40 min at room temperature before electrophoresis. Reactions were subjected to electrophoresis in a 5% nondenaturing polyacrylamide gel in 0.5% Tris-borate-EDTA buffer. The labeled oligonucleotides were: site B (5′-CTTGCTGTTTGCCCACTCTATTTGCCCAGCCC-3′), and Egr-1 (5′-GGATCCAGCGGGGGCGAGCGGGGCGA-3′).

Western blots

Twenty micrograms of nuclear extracts were electrophoresed in a 10% polyacrylamide gel and transferred to Hybond ECL nitrocellulose membranes. Nonspecific protein binding sites were blocked using blocking solution B (1× TBS, 1% nonfat dry milk, 1% BSA, 0.05% Tween 20) for 1 h at room temperature. Incubation with primary antibody (1:200 dilution) was performed at room temperature for 1 h in blocking solution B. The membrane was then rinsed three times with the washing solution (1×

TBS, 0.05% Tween 20), incubated with horseradish peroxidase-conjugated secondary antibody in blocking solution for 1 h at room temperature, and rinsed again three times with washing solution. Finally, membranes were incubated with developing reagent (WesternBreeze, Invitrogen) and exposed to X-ray films.

RESULTS

Characterization of the promoter region of the apoA-I gene mediating the increased expression by estrogen and genistein

We have previously shown that treatment of Hep G2 cells with E_2 or genistein is associated with a dose-dependent increase in apoA-I concentration in the culture media (16, 18). The maximum effect (4- to 5-fold) is observed at 10 μM concentration of both E_2 and genistein (16, 18). The need for superphysiologic concentrations of E_2 is consistent with the clinical observations of increased production of apoA-I during oral, but not transdermal, administration of estrogen (9). Therefore, all experiments were performed with 10 μM E_2 and genistein.

Transfection experiments previously conducted in our laboratory have indicated that the −256 to −41 region of the apoA-I gene is responsive to treatment with either E_2 or genistein (16, 18). To further characterize the region of the apoA-I promoter involved in the E_2- and genistein-mediated activation of transcription, plasmids containing serial deletions of the −256 to −41 region of the apoA-I gene promoter were constructed. As indicated in **Fig. 1A**, two plasmid constructs, the plasmid containing the −220 to −110 sequence and the plasmid containing the −256 to −148 sequence, maintained full estrogenic response when transfected into Hep G2 cells. Further deletion of this region, as in plasmids containing the −192 to −41 sequence or the −256 to −185 region, partially abolished the estrogenic response. These results indicate that the −220 to −148 region is sufficient for estrogenic response. This region contains the following well-characterized transcription binding sites: site A (−214 to −192), site B (−169 to −146), and two Egr-1 sites (−221 to −213 and −189 to −181) (Fig. 1B). To explore the individual roles of site A and site B on the estrogenic response of the apoA-I gene, plasmid constructs containing two sites A or two sites B were transfected into Hep G2 cells; however, neither of these two plasmids showed an increased expression following treatment with E_2 or genistein (Fig. 1A). In addition, mutations of each of the two ERE half-sites in site A did not affect estrogen responsiveness, indicating that these sequences are not relevant for the response to estrogen. A plasmid containing two apoA-I ARE sites did not respond to treatment (Fig. 1A), and a plasmid containing the −133 to −41 region failed to respond to treatment (data not shown).

The effect of ICI182,780 on the modulation of apoA-I gene expression by E_2 and genistein was tested in transient transfection experiments conducted with the plasmid con-

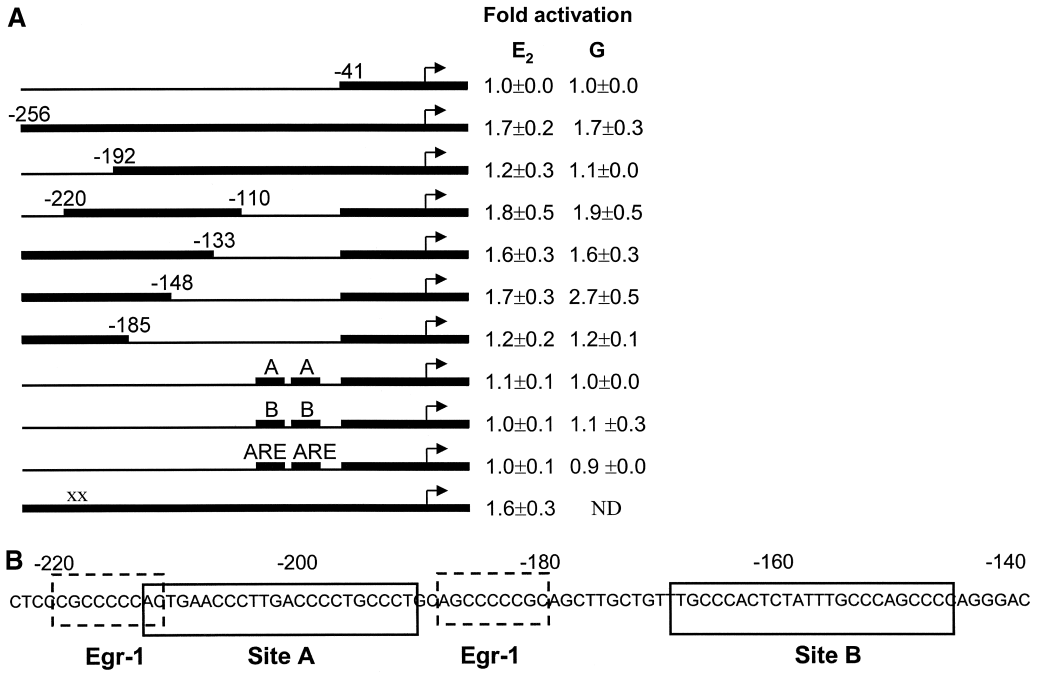

Fig. 1. Characterization of the estrogenic response sequence in the apolipoprotein A-I (apoA-I) gene promoter. A: Hep G2 cells were transiently transfected with serial deletion constructs of the apoA-I promoter (1.5 μg) and a *Renilla* luciferase plasmid (0.25 μg) for 6 h, followed by treatment with vehicle, 17-β-estradiol (E_2) (10 μM), or genistein (10 μM) for 36 h. The last plasmid construct contains a mutation on the half estrogen response element (ERE) on site A, as indicated in Materials and Methods (only one mutant is shown, but similar results were obtained with both mutants of the two half EREs). Fold activation represents the mean ± SD of three or more independent transfection experiments conducted in duplicate. B: Shows the nucleotide sequence of the −220 to −148 region of the apoA-I promoter and indicates the transcription factor binding regions.

taining the −256 to −148 region of the apoA-I promoter (−256[Δ−148/−42]A-I.Luc). While E_2 and genistein significantly increased expression of this plasmid construct compared with control ($P < 0.02$), both compounds failed to increase expression when cells were pretreated for 1 h with ICI182,780 (**Fig. 2**). ICI182,780 alone had no effect on the expression of the −256[Δ−148/−42]A-I.Luc plasmid.

Effect of estrogen and genistein on nuclear Egr-1 and HNF-3β concentrations

EMSA experiments were conducted with site B and Egr-1 oligonucleotides. As shown in **Fig. 3A**, an increase in the DNA-protein complex was observed when nuclear extracts of cells treated with genistein were incubated with the Egr-1 probe, as compared with control cells. The binding was specific and abolished by 100-molar excess of cold Egr-1 competitor. Incubation with the Egr-1 antibody resulted in a weakening of the band (Fig. 3A). However, a supershift band is not visible, probably due to the light signal. When the site B oligonucleotide was used, nuclear extracts of cells treated with E_2 and genistein showed greater intensity of two bands compared with control cells (two upper bands on Fig. 3B). Incubation of DNA-protein complexes with the antibody against HNF-3β resulted in the disappearance of one of these two bands (second band from the top), with resulting supershift clearly evident with extracts from cells treated with E_2 and genistein.

To assess the effect of these estrogenic compounds on the nuclear concentration of Egr-1 and HNF-3β, Western blotting experiments were performed. Egr-1 levels were increased 2-fold in E_2-treated cells, and 4-fold in genistein-treated cells, as assessed by densitometry scanning (**Fig. 4**). A 4-fold increase in HNF-3β protein concentrations in cells treated with E_2 and genistein, as compared with control cells, was observed as well.

Role of MAP kinase in the activation of apoA-I gene expression by estrogen and genistein

Because Egr-1 is induced via the MAP kinase pathway in other cells (26), we tested whether this second messenger pathway is important in apoA-I induction by E_2 and genistein in liver cells. After transfection, cells were pretreated for 30 min with the MAP kinase inhibitor PD 98059 (10 μM) and then with E_2 or genistein. PD 98059 abolished the estrogen- and genistein-mediated increase in expression of the −256[Δ−148/−42]A-I.Luc plasmid (**Fig. 5**). Inhibitors of PKA (10 μM) and PKC (1 μM) did

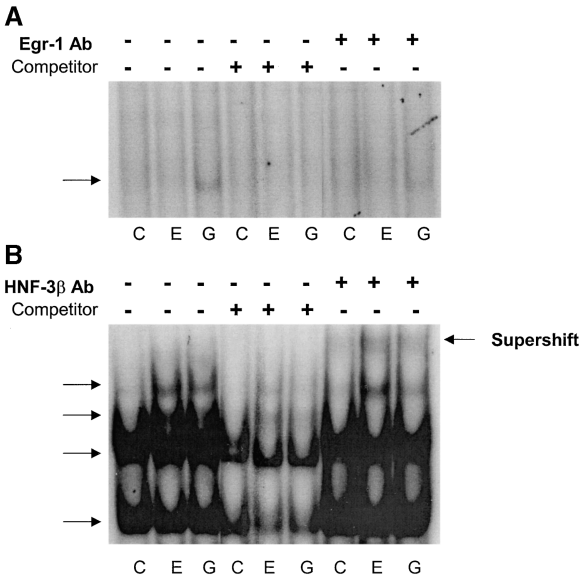

Fig. 3. Electrophoresis mobility shift assay. Seven micrograms of nuclear protein extracts from control cells (C) and from cells treated with 10 μM E_2 (E) or with 10 μM genistein (G) were incubated with ^{32}P-labeled early growth response factor 1 (Egr-1) (A) and site B (B) double-stranded oligonucleotides as described in Materials and Methods. Specific Egr-1 and hepatocyte nuclear factor (HNF)-3β antibodies were added to the reaction mixture where indicated. The protein-DNA complexes were run on a 5% nondenaturing polyacrylamide gel. The specific protein-DNA complexes are indicated by arrows at left.

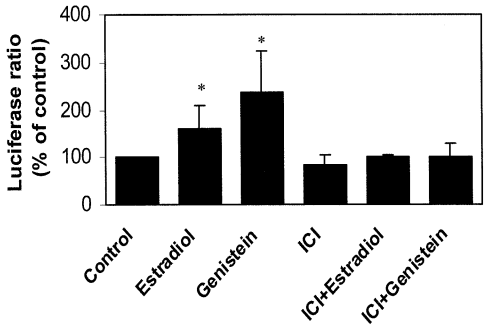

Fig. 2. Effect of ICI182,780 on the activation of apoA-I promoter by estrogen and genistein. Cells were transiently transfected with the −256 to −148 apoA-I promoter construct and then grown for 36 h in vehicle, E_2 (10 μM), or genistein (10 μM), with or without pretreatment for 1 h with ICI182,780 (10 μM). Bars represent the mean (±SD) of six independent experiments. * $P < 0.02$ compared with control.

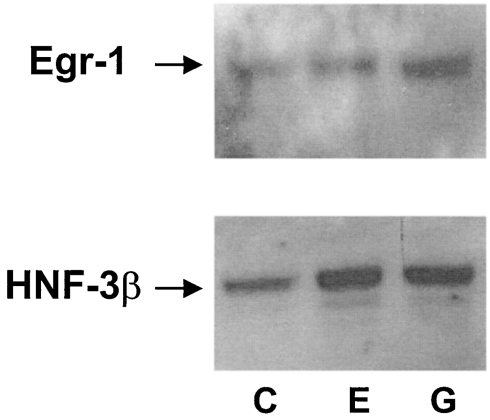

Fig. 4. Modulation of Egr-1 and HNF-3β nuclear concentrations by estrogen and genistein. Twenty micrograms of nuclear protein extracts from control (C) cells and from cells treated with E_2 (E) and genistein (G) were electrophoresed in a 10% polyacrylamide gel and then transferred to a nitrocellulose membrane, followed by blotting with specific antibodies against Egr-1 and HNF-3β.

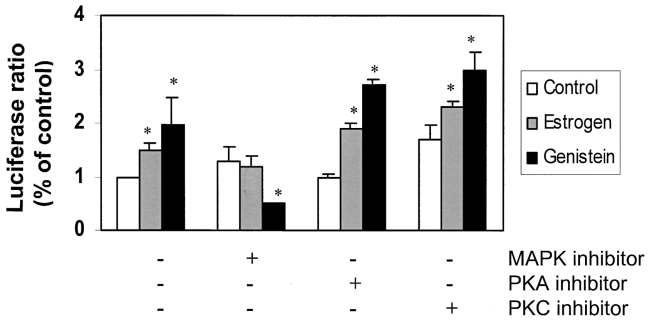

Fig. 5. Effect of mitogen-activated protein (MAP) kinase inhibition on the estrogen and genistein activation of apoA-I gene expression. Hep G2 cells were transiently transfected with the plasmid containing the −256 to −148 region of the apoA-I promoter. Following transfection, cells were pretreated for 30 min with inhibitors of the MAP kinase (PD 98059, 10 μM), protein kinase A (PKA) (myristoylated PKI 14–22 amide, 10 μM), and protein kinase C (PKC) (bisindolylmaleimide I, 1 μM) pathways. E_2 and genistein were then added, and cell extracts were collected after 36 h for the determination of luciferase activity (expressed as percent of control untreated cells). Bars represent the mean (±SD) of four independent experiments with MAP kinase inhibitors and two independent experiments (in duplicate) with PKA and PKC inhibitors. * $P <$ 0.05 compared with respective controls.

not have a significant effect on the estrogenic upregulation of this plasmid (Fig. 5).

DISCUSSION

Oral estrogen treatment has been shown to increase plasma levels of HDL cholesterol and apoA-I (7). While this effect has been known for almost 50 years (27), the underlying mechanism has never been fully elucidated. Our study shows that both estrogen and genistein activate the expression of the apoA-I gene in liver cells, leading to increased production of apoA-I protein. However, the classic genomic ER ligand-activated pathway does not directly mediate the transcription activation of the apoA-I gene by estrogen and genistein. While site A on the promoter region of the apoA-I gene contains two ERE half-palindromic sequences, these do not play a role in the estrogen regulation of the apoA-I gene expression, as indicated by a similar estrogenic response in the wild-type plasmid and the site A mutation plasmids. Instead, under control conditions, the expression of the site A mutated plasmids was reduced compared with that of the wild-type plasmid (data not shown), in agreement with previous observations (19), indicating that binding of transcription factors to this site is important for basal apoA-I expression. Our finding that reducing the binding of transcription factors to site A did not change the estrogen response indicates that site A may not be relevant in the activation of apoA-I gene expression by estrogen and genistein. This is also suggested by the lack of estrogenic response of the plasmid containing two sites A. The estrogen antagonist ICI182,780 completely abolished the estrogen- and genistein-mediated increased expression of the plasmid containing the −256 to −148 region of the apoA-I promoter, indicating that ER activation through ligand binding indirectly regulates apoA-I gene expression. Over the last few years, it has become evident that estrogen, and steroid hormones in general, may regulate gene expression via alternative pathways that do not require a classic genomic action. Nongenomic effects of estrogen, via the activation of the MAP kinase, PKC, and PKA pathways, have been described (28). In most cases, this mode of nongenomic regulation of gene expression requires a cell membrane-bound ER that, when ligand-activated, elicits a specific response via second messengers.

Our results show that the estrogen-responsive region of the apoA-I promoter is contained in the −220 to −148 sequence. This region contains two binding sites for Egr-1, and sites A and B. The abundance of Egr-1 protein was increased in nuclear extracts of liver cells treated with estrogen and genistein, suggesting a role of Egr-1 in the activation of apoA-I expression by these compounds. Both Western blotting and EMSA experiments indicated a greater effect of genistein on nuclear Egr-1 content compared with estrogen. The molecular basis for this difference is not known. In cardiac myocytes, estrogen causes an increase in Egr-1 expression leading to increased Egr-1 protein (26). The regulation of Egr-1 by estrogen in cardiomyocytes is mediated by serum response elements located in the promoter region of Egr-1, and ICI182,780 completely abolishes this effect (26). The activation of Egr-1 expression by estrogen in cardiomyocytes is also abolished by the MAP kinase inhibitor PD 98059, implicating the MAP kinase pathway in the activation of Egr-1 expression by estrogen (26). Egr-1 is a transcription factor that plays an important role in several tissues and has also been implicated in the basal apoA-I transcription (29). Our study provides evidence that estrogen and genistein are important modulators of apoA-I expression through Egr-1. Egr-1 has been shown to be involved in the increase in apoA-I gene expression in human apoA-I transgenic mice with nephrotic syndrome (29), and also to play an important role in the reduced apoA-I expression in zinc-deficient status (30). It has been shown that MAP kinase activation also leads to increased apoA-I expression by a different pathway involving not the −220 to −148 promoter region but an upstream region (−425 to −376) and requiring the participation of the transcription factor Sp1 (31). Epidermal growth factor and insulin mediate an increase in apoA-I expression via this mechanism (31). Our analysis of the signaling pathway involved in apoA-I gene expression in liver cells by estrogen and genistein has indicated that MAP kinase is specifically involved. Transfection experiments with the −256 to −148 apoA-I plasmid construct have shown that cells grown in the presence of the MAP kinase inhibitor PD 98059 do not respond to either estrogen or genistein. These results are consistent with the concept that upregulation of apoA-I expression is effected by estrogen-mediated activation of the MAP kinase pathway. PKC has been implicated in the regulation of MAP kinase activation (32). However, in our experiments, inhibition of PKC did not have an effect on apoA-I activation by estrogen and genistein.

We also showed an increase in HNF-3β levels in nuclear extracts of liver cells treated with estrogen or genistein. EMSA analysis of site B in the apoA-I promoter, which binds this transcription factor with high affinity, indicated a specific increase in the binding of nuclear transcription factors to site B in cells treated with estrogenic compounds, and in particular, a specific increase in HNF-3β binding, as indicated by the specific supershift of a DNA-protein complex by the anti-HNF-3β antibody. The HNF-3β gene has been cloned (33) but little is known about its regulation by estrogen. Therefore, we do not know if HNF-3β levels are directly or indirectly regulated by estrogen. Our transfection experiments with serial deletion constructs of the apoA-I promoter region have shown that, as in site A, the isolated site B does not respond to estrogen, even when present in duplicate copy. These results, taken together, indicated that a synergism of different transcription factors may be necessary for the activation of apoA-I expression by estrogenic compounds.

To our knowledge, there is only one previous study in the literature that has examined the modulation of the apoA-I promoter activity by estrogenic compounds: Zhang et al. (23) showed that equilenin (a compound of conjugated equine estrogen, the most commonly prescribed hormonal replacement therapy), but not estradiol, increases apoA-I media concentration and promoter activity in Hep G2 cells via interaction with the ARE site (23). Our results show that the ARE site does not play a role in the modulation of apoA-I gene expression by estrogen and genistein, as indicated by a lack or response of the plasmid construct containing two ARE sites and the positive estrogenic response of plasmids not containing the ARE site (−256[Δ−148/−42]A-I.Luc). In addition, our results showing a significant increase in apoA-I concentration in the media and in the A-I transcription activation following treatment with estradiol, while in contrast to the findings of Zhang et al. (23), are clearly in agreement with previous studies (16, 17, 34).

In summary, we have demonstrated that *1*) estrogen and genistein increase the expression of the apoA-I gene in liver cells and that this effect is abolished by the estrogen antagonist ICI182,780; *2*) estrogen and genistein increase Egr-1 and HNF-3β concentration in nuclear extracts of liver cells; and *3*) this process requires the MAP kinase signaling pathway.

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