Glucocorticoid increases rat apolipoprotein A-I promoter activity

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Abstract The observation that glucocorticoids increase the abundance of apolipoprotein A-I led us to a search for potential underlying mechanism(s). In this report, we show that the synthetic glucocorticoid, dexamethasone, injected into rats increases serum levels of apoA-I protein, hepatic mRNA and 'run-on' transcription of the gene by 3-, 5-, and 2-fold, respectively. Results of transient transfection studies of the rat apoA-I promoter reveal that effects of dexamethasone are mediated by a *cis*-acting site B (-170 to -145). Dexamethasone treatment of hepatoma cells enhances the DNA binding activity of nuclear factors that bind this site. Unexpectedly, site B does not contain a consensus glucocorticoid receptor recognition motif nor binds to bacterially expressed glucocorticoid receptor. These results indicate that the actions of glucocorticoids on site B involve indirect mechanisms. Site B is comprised of a direct repeat of a nonanucleotide and mutation of either one abolishes the effect of glucocorticoid. Additionally, the transcriptional activity of site B in response to dexamethasone is amplified by a 5′ sequence called site S (-186 to -171). Dexamethasone has no effect on site S in the absence of site B. In summary, our data show that dexamethasone increases rat apoA-I gene expression by an indirect mechanism.–**Taylor, A. H., J. Raymond, J. M. Dionne, J. Romney, J. Chan, D. E. Lawless, I. E. Wanke, and N. C. W. Wong.** Glucocorticoid increases rat apolipoprotein A-I promoter activity. *J. Lipid Res.* 1996. **37:** 2232–2243.

Supplementary key words dexamethasone • atherosclerosis • HDL • coronary heart disease

Apolipoprotein A-I (apoA-I) is the major protein component of serum high density lipoprotein particles (HDL). Epidemiological studies have established an inverse correlation between serum levels of HDL and the incidence of coronary arterial disease (1). HDL particles promote the efflux of cholesterol from peripheral tissues and 'shuttle' it to the liver for further oxidation into bile acids (2), a physiologic process often referred to as reverse cholesterol transport. Enhanced reverse cholesterol transport lowers total body cholesterol and is thus of clinical interest. As the abundance of HDL particles is dictated by the levels of apoA-I, knowing the factors that regulate apoA-I gene expression should be useful for designing new therapeutic regimens against hypercholesterolemia.

The expression of apoA-I is regulated by tissue-specific and hormonal factors (3–5). The glucocorticoids are one class of hormones that increase apoA-I gene expression (5, 6). Patients treated with glucocorticoids have levels of HDL and apoA-I that are significantly higher than age- and sex-matched controls (7). Although the increased HDL levels may have a beneficial effect on coronary arterial disease, the benefit is limited to patients on short-term glucocorticoid therapy. Patients on prolonged glucocorticoid therapy also have increased HDL, but unfortunately, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels are also increased (8), which counteracts the beneficial HDL effect. Similarly, patients with Cushing's syndrome have increased HDL and LDL levels (9), however, they also have other complications, such as hypertension and insulin intolerance that may lead to an accelerated development of atherosclerosis (10, 11). In agreement with the clinical observations, a small number of animal studies have shown that glucocorticoids enhance hepatic expression of numerous apolipoprotein genes including apoA-I (5, 12–15). Glucocorticoids also increase the expression of apoA-I mRNA and synthesis of the protein in hepatoma cell lines, supporting the in vivo data (16).

Abbreviations: HDL, high density lipoprotein particles; LDL, low density lipoprotein particles; VLDL, very low density lipoprotein particles; HuH-7, human hepatoma cells; apoA-I, apolipoprotein A-I; GRE, glucocorticoid response element; GR-DBD, glucocorticoid receptor DNA binding protein; α1-AGP, α-1 acid glycoprotein; EMSA, electrophoretic mobility shift assay; Dex, dexamethasone.

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Thus, glucocorticoid clearly increases the synthesis and secretion of hepatic apoA-I, although the exact mechanism is unclear. As glucocorticoids have a major effect within the hepatocyte nucleus, we wondered whether the activity of the rat apoA-I promoter could be modulated by dexamethasone. The results presented here show that the rat apoA-I gene is modulated by glucocorticoid at the transcriptional level and identify the region of the rat apoA-I promoter responsible for this effect.

MATERIALS AND METHODS

Materials

The unstained and pre-stained molecular weight markers, electrophoresis grade acrylamide, N,N′-methylene-bis-acrylamide and sodium dodecyl sulfate (SDS) were all from Bio-Rad Laboratories (Mississauga, Ontario). The rat apoA-I cDNA has been described previously (17). Alpha-1 acid glycoprotein (α1-AGP) cDNA (18) was from Dr. J. M. Taylor (Pennsylvania State University, Hershey, PA) and human ribosomal RNA (19) was a kind gift from Dr. C. K. Krekoski, University of Calgary. The pAI.CAT and pRSV-β-gal plasmids used in transient transfection assays have been described previously (3). Mutated B-site DNA was synthesized at the University of Calgary and used as primers in the polymerase chain reaction (3) using pAI.BCD.CAT as a template to produce the two B site mutant constructs pAI.Bm1.CAT and pAI.Bm2.CAT. The upper and lower strands of DNA from the apoA-I promoter that we have called site S (gatccGCAGCCCCCGCAGCTTCCTGTTg; the sequence in uppercase is the fragment from -188 to -166) were synthesized with 5′ *EcoRI* sites by Gibco BRL (Burlington, Ontario) and cloned into the *SmaI* site of pGEM-CAT.5′ (17) as either monomer, dimer, or trimer (p5′S.CAT, p5′S2.CAT, p5′S3.CAT, respectively). All other reagents were of analytical grade and purchased from either Sigma or BDH. The rabbit anti-rat apoA-I antibody was generated in house (see below).

Animals

Male Sprague-Dawley rats, weighing between 150 and 200 g were purchased from the Charles River Breeding Company, St. Constance, Quebec. Glucocorticoid (dexamethasone, Dex, Sabex, Quebec) was administered by daily i.p. injection (0.5 mg/day) for 1 week. Blood was collected from anesthetized animals (17) and serum was produced by centrifugation at 800 *g* for 10 min at 4°C.

RNA isolation and analysis

Total RNA was prepared from fresh or frozen rat livers (circa 140 mg) by the guanadinium isothiocyanate-CsTFA centrifugation method (Pharmacia LKB, Piscataway, NJ) and the RNA (10 μg) was analyzed by northern blot analysis (20). The relative quantities of apoA-I mRNA were determined using video-assisted densitometry and corrected for the densities of the 18S band determined by video-assisted densitometry of a photographic negative.

Nuclei preparation and nuclear run-on transcription assay

Six male Sprague-Dawley rats were treated with Dex (0.5 mg/day i.p.) for 1 week. Six untreated control rats received sterile saline (0.9%). Freshly isolated livers of two rats were pooled and hepatic nuclei were prepared as described (21), with 100 μM phenylmethylsulfonylfluoride (PMSF) added to buffers I, II, and III. The nuclei were stored at -80°C in buffer III. Transcription assays were performed as described by Skettering, Gjernes, and Prydz (22) with minor modifications. Briefly, 3.7×10^7 nuclei were incubated in the presence of 25 mM Tris (pH 8.0), 2.5 mM $MgCl_2$, 150 mM KCl, 0.5 mM ATP, 0.5 mM GTP, 0.5 mM CTP, 10 mM creatinine phosphate, 100 μg creatinine phosphokinase, 1 unit/μl RNasin, 1 mM dithiothreitol, 0.5 mM PMSF, 10% glycerol, and 100 μCi ^{32}P-UTP at 30°C for 30 min. The reaction was stopped with the addition of DNAse I at 37°C for 10 min. The labeled RNA was purified using proteinase K, phenol–chloroform extraction in the presence of yeast tRNA, and ammonium acetate–isopropanol precipitation. The RNA-labeled product was hybridized to nylon membranes containing immobilized cDNAs for apoA-I and α1-AGP, with human rRNA (19) as control. Hybridizations were performed with 5 to 15×10^6 cpm of labeled RNA, at 65°C in 375 mM phosphate–1.5 mM EDTA buffer containing 7.5% SDS, 1% BSA, 5% dextran sulfate, and 20 μg/μl yeast tRNA, for 48 h. Membranes were subjected to high-stringency washing and exposed to X-ray film for 2–4 days at -80°C in the presence of intensifying screens. The intensity of the signal was assessed by video-assisted densitometry. These values were expressed relative to the rRNA standards, and normalized for the amount of labeled RNA added.

Cell culture and CAT assay

Male human fetal hepatoma cells (HuH-7 cells) were cultured in RPMI1640-ISE medium, as previously described (23). These cells are known to express endogenous glucocorticoid receptor (24). For transient transfection, cells were sub-cultured to 25 cm^2 flasks for 72 h. After transfection (3) with test plasmids cells were exposed to Dex (3.3×10^{-7} to 3.3×10^{-5} M) for a further 24 h. Cells were harvested, protein was produced, and assays for β-galactosidase and chloramphenicol acetyl

transferase (CAT) activities were performed as described (23). All assays were adjusted so that results were in the linear range for each assay. CAT enzyme activities were corrected for protein concentration, β-galactosidase activity, and assay duration (17).

Preparation of purified rat apoA-I

The purification of rat apoA-I was performed so that suitable anti-apoA-I antibodies could be generated for western blot analysis. First, HDL was purified from rat serum using an adaptation of the shallow KBr gradient ultracentrifugation method of Jansen, Schoonderwoerd, and Dallinga-Thie (25) with the following minor modifications. All KBr solution densities were as described but each volume was 2.3 ml instead of 2.4 ml. The final overlay 1.05 g/ml solution was 2.2 ml. Centrifugation and collection of apoA-I was as described except the pump was operated at 1 ml per min. Fraction numbers 9 to 16 were pooled and dialyzed against NaCl (150 mM) overnight. ApoA-I was precipitated with 10 volumes of acetone at -20°C for 30 min (26) and collected at 17,400 *g* for 15 min. An aliquot was analyzed by SDS-PAGE analysis (27) and the gel was stained with Coomassie Brilliant Blue R250. The protein appeared as a single band with an apparent molecular mass of 28,200 Daltons (**Fig. 1A**). This is very close to the predicted molecular mass (28,016 Daltons). Amino acid analysis of the purified protein, excised directly from the gel, showed 93% homology with the predicted amino acid composition (data not shown). This protein was then used to produce anti-apoA-I antibody.

Generation of rabbit anti-apoA-I antibody

Two New Zealand White rabbits were inoculated with 0.5 mg of purified rat apoA-I protein solubilized in 1 ml of Freund's adjuvant. After 6 weeks the animals were bled and the serum was produced by differential centrifugation. The rabbits were rechallenged 6 weeks later and rebled. Finally, 1 year later the rabbits were rechallenged with antigen and the serum was collected again. The antibodies from the second and third collections were used in dot-blot and western blot studies (28).

Dot-blot analysis of purified apoA-I and rat serum

Purified apoA-I, partially purified bovine serum albumin, and rat serum were dot-blotted to polyvinylidene difluoride (PVDF) membrane (Millipore Corporation, Nepean, Ontario). Rabbit anti-rat apoA-I antibody (1/100 dilution; second bleed) interacted only with the purified rat apoA-I and rat serum (Fig. 1B).

Western blot analysis of purified apoA-I protein and rat serum

One-microgram samples of total serum proteins from control and Dex-treated rats were separated by SDS-PAGE (12% gel) as described (29). Proteins were transferred to PVDF membranes and blocked in blocking buffer (5% [w/v] skim milk powder in TBS [10 mM Tris-HCl, pH 7.5, 150 mM NaCl]) for 1 hour or overnight. The membrane was washed copiously with TBS and exposed to rabbit anti-apoA-I antibody in blocking buffer (dot-blot: 1/100 dilution; second bleeding in 5% blocking buffer: western blot 1/5000 dilution; third bleeding in 0.5% blocking buffer) for 1 h at room temperature. After further washing and exposure to donkey anti-rabbit IgG conjugated to horseradish peroxidase (Amersham, Oakville, Ontario) for 1 h, immunoreactive proteins were detected by chemiluminescent detection according to the manufacturer's instructions (ECL, Amersham). Visualization of rat

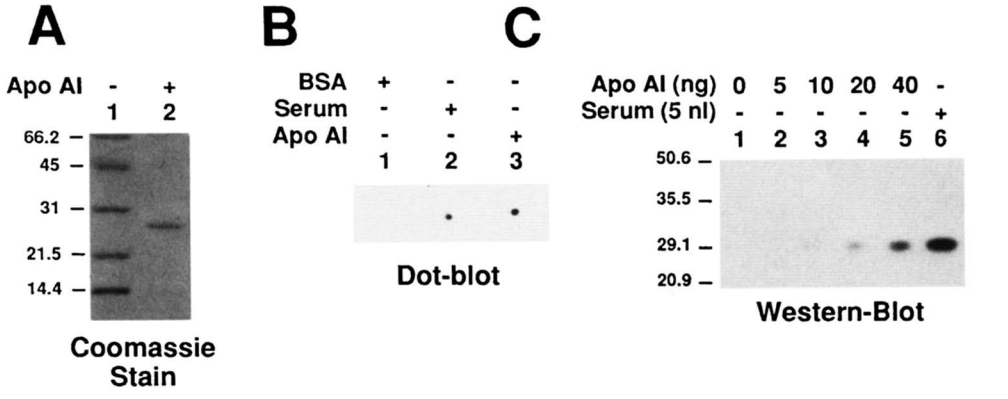

Fig. 1. Purification and characterization of rat apoA-I protein. (A) Coomassie Brilliant Blue-stained SDS-PAGE gel of molecular weight markers (lane 1) and KBr column-purified rat apoA-I protein (lane 2). (B) Dot blot analysis of partially purified bovine serum albumin (10 μg; lane 1) or rat serum (10 μg; lane 2) or purified apoA-I protein (100 ng; lane 3). Rabbit anti-rat apoA-I antibody was used at 1/100 dilution (second bleed). (C) Western blot analysis of increasing quantities of purified rat apoA-I (lanes 2–5) or rat serum diluted to be equivalent to 5 nl (lane 6). Pre-stained molecular weight markers (lane 1) were used both to identify rat apoA-I signal and to act as a negative control. The positions of the molecular weight markers are shown to the left of panels A and C. All experiments were performed at least twice.

apoA-I protein was performed within 10 min (dot-blot) or 1 min (western blot). The relative concentration of apoA-I was assessed by video-assisted densitometry. The lower and upper limits of the assay were approximately 5 ng of purified protein (Fig. 1C) and 150 ng of rat serum, respectively. The inter- and intra-assay coefficients of variation were 12.8% and 6.5%, respectively.

Electrophoretic mobility shift assays (EMSA)

The glucocorticoid response element (GRE; TC-TC) containing plasmid (30) and the glucocorticoid receptor DNA-binding domain (GR-DBD) protein were gifts from Dr. R. Hache, University of Ottawa, Canada. The *BamHI-PvuII* fragment of TC-TC plasmid (30) was labeled with ^{32}P-αCTP in the presence of the Klenow fragment of DNA polymerase I and purified by size-exclusion chromatography (31). Synthetic strands for site S and for site B were annealed and labeled with either ^{32}P-αCTP or ^{32}P-αATP and purified in the same manner. Glucocorticoid receptor (GR; pT7x556) in the expression vector pAR3040 (32) was expressed in *E. coli* strain BL21 (DE3) and protein extracts were produced using an alkaline lysis method (33). Nuclear proteins from untreated and Dex-treated (3.3×10^{-5} M; 24 h) HuH-7 cells (~10^9 cells) were prepared using a low-salt, high-salt dialysis method (34). The binding reactions, loading of sample, and electrophoresis were essentially as described (35), with the exception that oligomer counts, protein concentrations, poly dI.dC concentrations, and time of electrophoresis were optimized for each probe. Radioactivity for the GRE and site B probes was visualized by exposure to Kodak Ektascan EMC-1 X-ray film and highly sensitive X-ray film (Kodak BIOMAX MR) was used for site S probe, all at -80°C in the presence of intensifying screens.

RESULTS

Dexamethasone increases abundance of apoA-I protein and mRNA

Western blot analysis of rat serum probed with antiserum to rat apoA-I identified a single protein band with an apparent molecular mass of 28,000 Daltons (Fig. 1C, lane 6). Rats treated with Dex showed a significant 3- to 4-fold higher serum apoA-I protein level than control rats (**Fig. 2A and 2B**). The abundance of hepatic apoA-I mRNA in the same rats correlated with the protein levels and were 5- to 6-fold higher in the glucocorticoid-treated animals (Fig. 2C and 2D).

Dexamethasone enhances rat apoA-I promoter activity

Dex increased the relative run-on transcription rate of apoA-I mRNA by 2- to 3-fold (**Fig. 3B**), when compared to untreated controls. Furthermore, a positive control (36), α1-AGP (Fig. 3A) was also significantly increased by Dex (1.2 ± 0.08-fold; n = 3).

The promoter activity of the construct, pAI.474.CAT (**Fig. 4**) transfected into HuH-7 cells showed a dose-dependent increase in activity with a maximal 3.1-fold response at 3.3×10^{-6} M Dex (**Fig. 5**). Deletional constructs pAI.235.CAT and pAI.186.CAT that contain the *cis*-acting sites S and B (Fig. 4) showed similar dose-dependent increases in activity with maximal responses at 3.3×10^{-6} M Dex of 3.3- and 3.1-fold, respectively. The deletional constructs that lack site B, pAI.144.CAT and pAI.46.CAT, were not responsive to Dex (Fig. 5). The two constructs that contain site B, pAI.170.CAT and p5′B.CAT, showed reduced responses to graded doses of Dex in comparison to the wild-type construct pAI.474.CAT (Fig. 5). The promoter activities of these two constructs were significantly different from pAI.144.CAT and pAI.46.CAT at 3.3×10^{-6} M and 3.3×10^{-5} M Dex (Fig. 5).

Glucocorticoid responsive element in rat apoA-I DNA

Transient transfection assays of apoA-I deletional CAT constructs into HuH-7 cells subsequently treated with 3.3×10^{-6} M Dex showed the glucocorticoid responsive element to be located to site B (**Fig. 6**). The constructs pAI.474.CAT, pAI.235.CAT, and pAI.186.CAT all showed increased promoter activities of just above 3 times their untreated controls (Fig. 6). The activity of pAI.170.CAT (Fig. 4), a construct that lacks site S but retains a single site B, was significantly induced by Dex to 30 ± 17% (mean ± SD; n = 4) over its untreated control. Similarly, Dex increased the promoter activity of p5′B.CAT, an artificial dual site B construct, by 59 ± 10% (n = 3). In contrast, the site B-deficient deletional constructs pAI.144.CAT and pAI.46.CAT and the artificial constructs p5′A.CAT, p5′S.CAT, p5′S2.CAT and p5′S3.CAT showed no increases in promoter activities in response to 3.3×10^{-6} M Dex (Fig. 6).

Functional importance of site B

Transient transfection studies using the deletional construct pAI.BCD.CAT (containing *cis*-acting sites B, C, and D, **Fig. 7A**) in untreated HuH-7 cells indicated 0.037% CAT conversion/μg protein per h. This value was arbitrarily set to 1.0 (Fig. 7). Similar cultures treated with 3.3×10^{-5} M Dex showed a 2.4-fold increase in promoter activity to 0.09% CAT conversion/μg protein per h. The magnitude of the Dex response is comparable

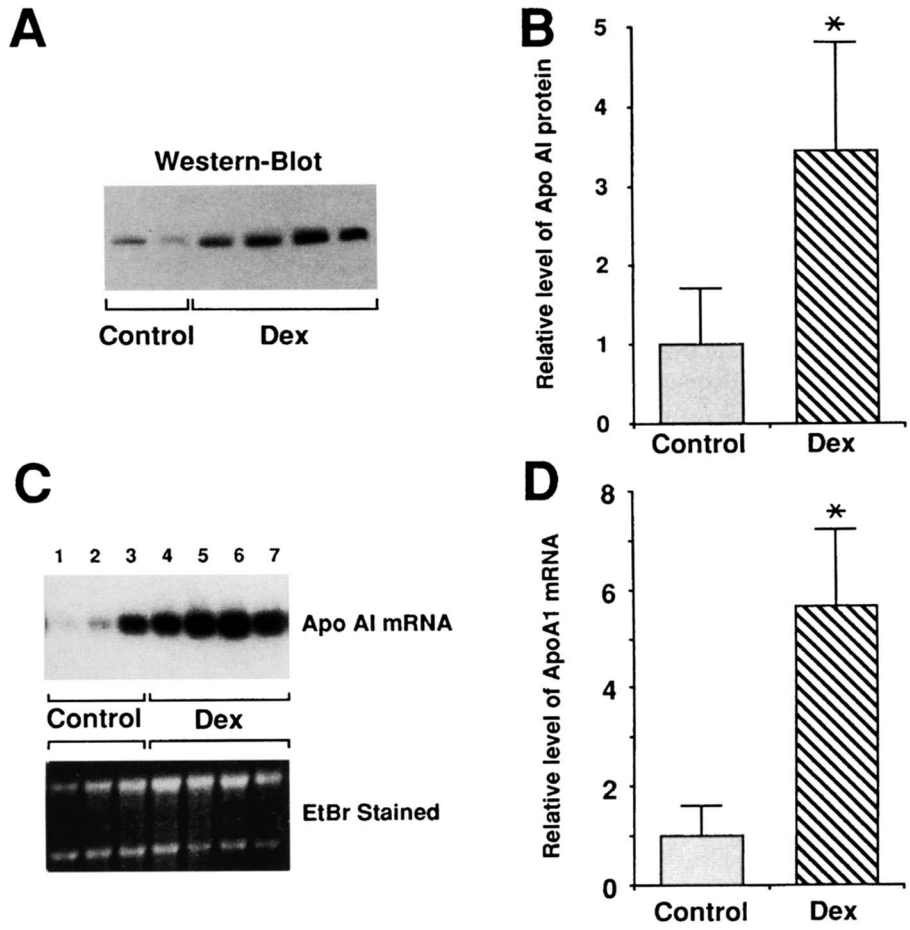

Fig. 2. Effect of dexamethasone on rat apoA-I protein and hepatic mRNA production. (A) Western blot analysis of 1 μg of total serum proteins from control (n = 9) and Dex-treated (n = 8) rats. (B) Quantitation of immunoreactive chemiluminescent signal from the data shown in 2A. Data are means ± SD analyzed twice. (C) Northern blot analysis of total hepatic RNA (10 μg) from control and Dex-treated animals (upper panel) and 28S and 18S visualization with ethidium bromide staining of the gel (lower panel). (D) Quantitation of hybridized ^{32}P-labeled apoA-I cDNA signal from the data shown in 3C. Data are means ± SD from 9 control and 7 Dex-treated animals analyzed twice. *$P < 0.05$; Student's unpaired *t*-test.

to that of the B, C, D site-containing construct, pAI.170.CAT, of 0.082% CAT conversion/μg protein per h. The 5′ B-site mutant construct pAI.Bm1.CAT and the 3′ mutant construct pAI.Bm2.CAT (Fig. 7A) showed significantly reduced relative CAT activities of 15% and 14%, respectively. Similarly, the relative CAT activity of the deletional construct pAI.158.CAT (Fig. 7A) was also significantly reduced to 5% of pAI.BCD.CAT. Furthermore, the CAT activities of these templates were no longer inducible by Dex (Fig. 7B).

Dexamethasone induces site B binding activity that is not glucocorticoid receptor

In EMSA studies, bacterially expressed GR-DBD bound to the consensus GRE sequence (**Fig. 8A**), but not to either site B or site S oligomer sequences (Fig. 8A). However, lysate from bacteria with or without GR contained DNA binding activities that were not GR and bound to site B and site S (Fig. 8A). Nuclear extracts from HuH-7 cells contained DNA binding activities that formed a doublet with site B, and the intensity of this pattern was induced with 3.3×10^{-5} M Dex (Fig. 8B). Competition studies with unlabeled site B, site S, and TRE_{pal} oligomers (35) indicated that this binding was specific because only excess unlabeled site B oligomer inhibited the formation of the retarded complexes (data not shown). HuH-7 cells also produced a doublet of DNA binding activities that bound weakly to site S DNA (Fig. 8B). The upper slower migrating protein-DNA complex was inducible by Dex, but was not easily discernible (Fig. 8B). Only the upper complex appears to be caused by a specific protein-DNA interaction because competition studies with excess unlabeled site S DNA inhibited the formation of the upper and not the lower complex (data not shown).

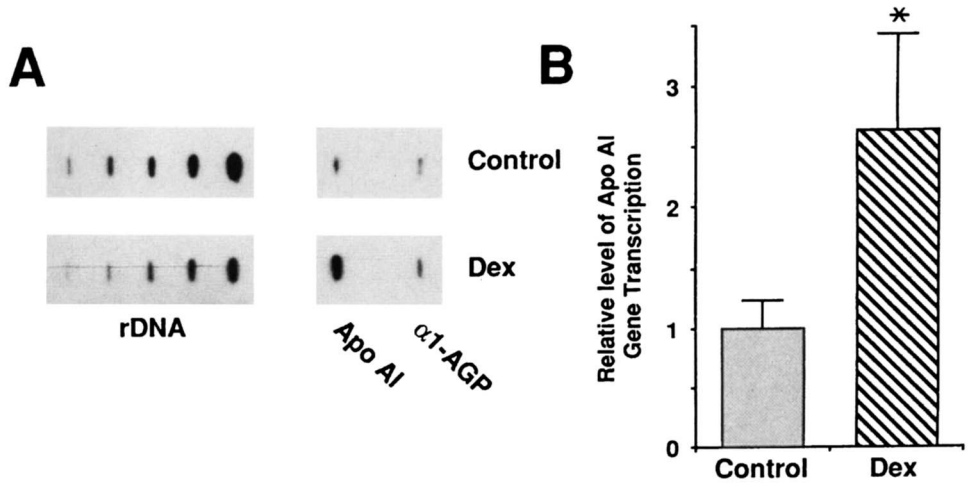

Fig. 3. Dexamethasone increases the in vivo transcription of rat apoA-I. (A) Nuclei from two male rat livers treated with saline (upper panel) or Dex (lower panel) were allowed to chain-elongate nascent RNA in the presence of ^{32}P-UTP. The RNA was hybridized to cDNAs from rRNA (1–32 ng), rat apoA-I (1 μg) and α1-AGP (1 μg). Hybridized RNA detected by autoradiography are representative of experiments performed 3 times. (B) The relative rates of transcription for apoA-I and α1-AGP were determined by video-assisted densitometry and normalized for rRNA signals. The data were further corrected for total RNA counts and are means ± SD for 3 data points per group. *$P < 0.05$ Student's paired *t*-test.

DISCUSSION

In this report we have examined the glucocorticoid induction of rat apoA-I gene in vivo and in vitro. Given the importance of apoA-I protein in regulating serum levels of HDL and the inverse correlation between these particles and coronary arterial disease, increased knowledge of factors that up-regulate apoA-I gene expression may help in developing new treatments for the consequences of hypercholesterolemia.

Our animal studies showed that treatment with Dex increased serum apoA-I concentrations by 3 to 4 times that of control and that this protein increase was matched by a 5- to 6-fold increase of the hepatic mRNA abundance. These findings are in keeping with the studies of Elshourbagy et al. (6) and Staels et al. (15) who found that the glucocorticoids Dex, hydrocortisone, and triamcinolone increased rat serum and hepatic mRNA levels by 2- to 3-fold. Other studies, however, have shown less or no effect of Dex in rats (14–16). In humans, Dex causes a 25% increase in both serum apoA-I and HDL concentrations (37) suggesting that similar mechanisms operate in humans and rats to augment apoA-I gene expression.

These in vivo data show the rat apoA-I gene is responsive to Dex. This prompted us to ask whether the effect

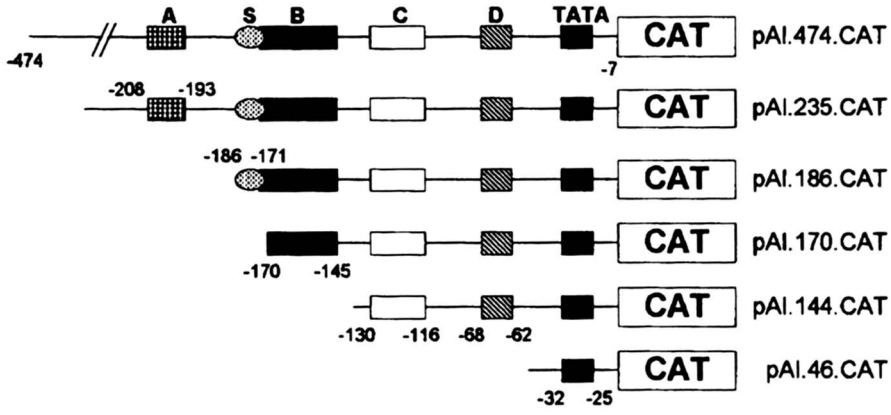

Fig. 4. Rat apolipoprotein A-I promoter-CAT constructs. The *cis*-acting elements of the rat apoA-I promoter are represented by the boxes. The numbering is relative to the transcription start site, according to the rat apoA-I numbering system of Widom et al. (40). The oval indicates the position of the steroid amplification site designated site S.

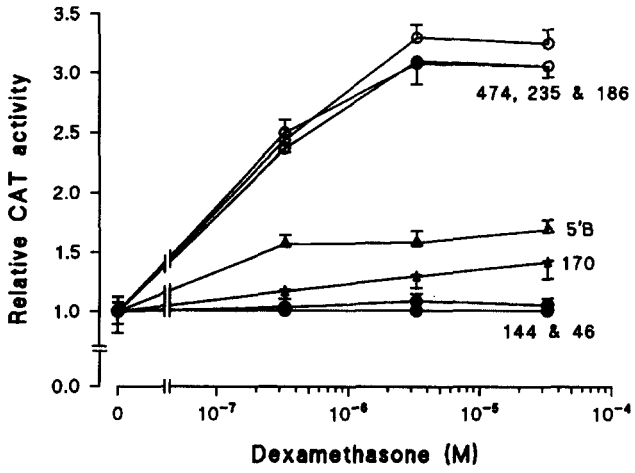

Fig. 5. Site B contains the glucocorticoid-responsive sequence. HuH-7 cells were transfected with the indicated deletional-CAT constructs (e.g., 474 = pAI.474.CAT) and treated with the indicated concentrations of Dex for 24 h. Cellular proteins were assayed as described (23), corrected for DNA uptake by β-galactosidase expression, and the data are expressed as CAT activity relative to untreated cells containing the same deletional construct. Values are shown as means ± SD for at least two experiments performed in triplicate. Error bars are not shown where they are encompassed by the symbol. Statistical indicators have been omitted for reasons of clarity.

of Dex is at the transcriptional level, as glucocorticoids are known to interact with nuclear receptors to modulate the expression of other hepatic genes (38).

To address this question two approaches were taken. First, nuclear transcription run-on assays (Fig. 3) showed that Dex increased apoA-I transcription by 2 to 3 times that of control animals. These data indicate clearly that Dex acts at the transcriptional level. In agreement with this observation, Dex increased apoA-I transcription rates in HepG2 cells (22). Second, the activity of a rat apoA-I promoter-CAT reporter construct, pAI.474.CAT, was measured using human HuH-7 cells exposed to Dex (Fig. 5). Our results showed a dose-dependent increase in reporter activity with a 3.1-fold maximal stimulation at 3.3×10^{-6} M Dex. Together these observations indicate that Dex increases apoA-I transcription.

The region of the promoter mediating the effects of Dex was delineated using deletional constructs devoid of sequences from -474 to -187 tested in the transient transfection assay system. The data indicated that each construct retained the ability to respond to hormone (Fig. 5). The use of further deletional constructs showed that deletion of the site S-containing -186 to -171 fragment reduced promoter responsiveness to Dex and deletion of the site B-containing -170 to -145 fragment abolished it. These findings show that Dex induction of rat apoA-I promoter activity is mediated by the actions of a DNA fragment referred to as site B and that this activity is amplified by the actions of the adjacent site S.

To demonstrate that site B mediates the effect of Dex, the activity of an artificial construct, p5′B.CAT, was tested and compared to a similar construct p5′A.CAT, that contains an L-triiodothyronine-responsive element (17). These constructs have two B-motifs or two A-motifs linked to the SV40 promoter, respectively. A deletional construct, pAI.170.CAT, that contains only one B-motif was also compared. As expected, 3.3×10^{-6} M Dex had no effect on p5′A.CAT (Fig. 6), but caused a 59% increase in p5′B.CAT activity (Fig. 6) and a 30% increase in pAI.170.CAT activity. These observations indicate that Dex acts through site B and that the effect of the hormone is additive (a 30% increase in promoter activity with one B site and a 59% increase with two).

The B-motif is comprised of two nonanucleotide repeats arranged in a head to tail orientation separated by four bp (Fig. 7). Both of these repeats appear to be critical for the glucocorticoid response mediated by the

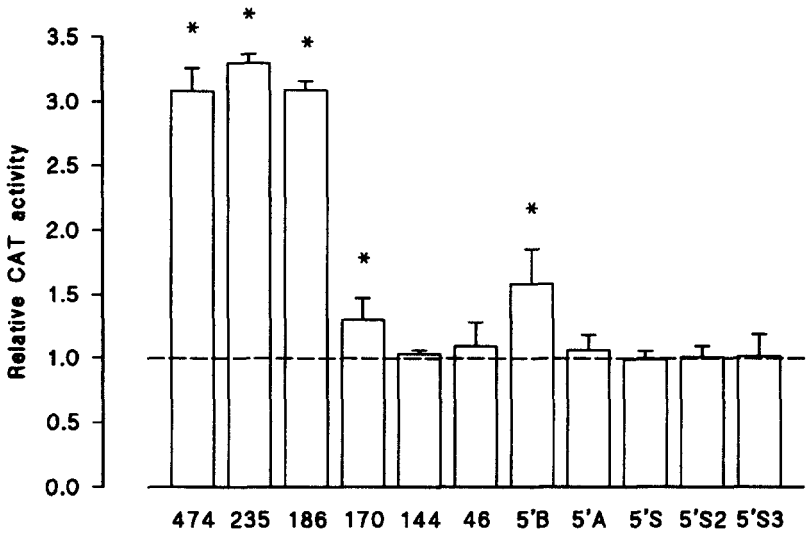

Fig. 6. Site S amplifies the glucocorticoid effect on site B. HuH-7 cells were transfected with the indicated deletional-CAT constructs and treated with 3.3×10^{-6} M Dex for 24 h. Cellular proteins were assayed and data are expressed as CAT-activity relative to untreated cells containing the same deletional construct (represented by the dotted line). Values are shown as means ± SD for at least 2 experiments performed in triplicate. (*$P < 0.05$ ANOVA with least significant difference test).

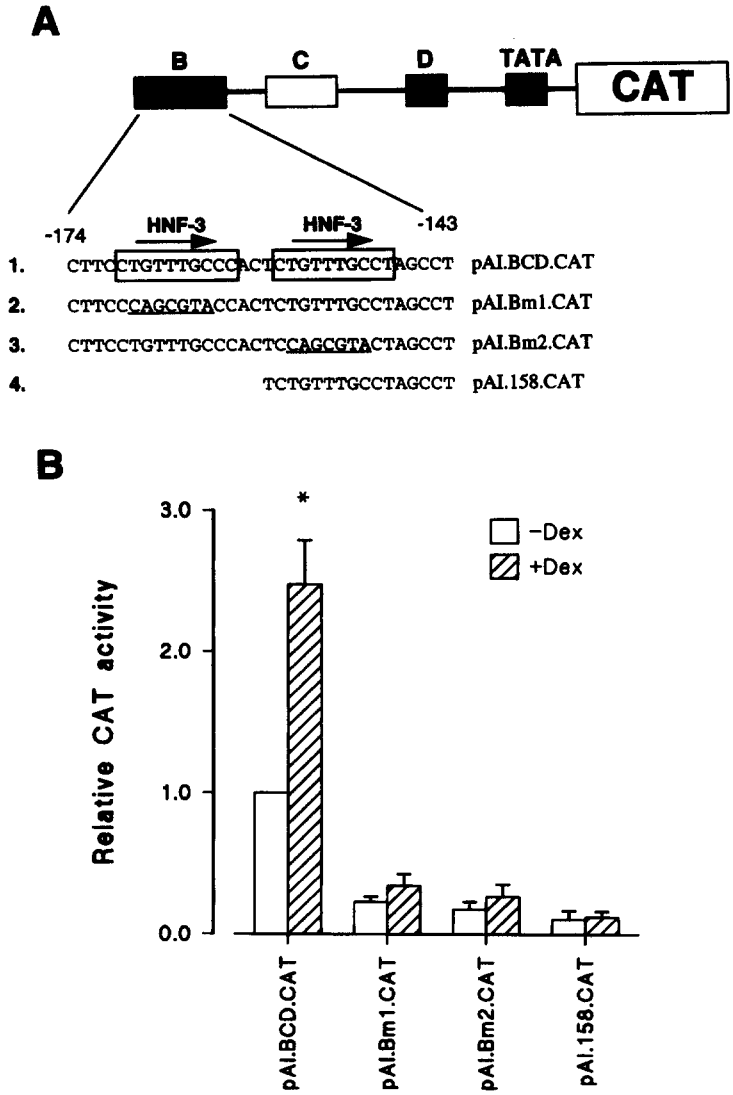

Fig. 7. Mutation or deletion of HNF-3 sites in site B abolishes glucocorticoid responsiveness. HuH-7 cells were transfected with either wild-type (pAI.BCD.CAT), mutated (pAI.Bm1.CAT, pAI.Bm2.CAT), or deleted (pAI.158.CAT) pAI-CAT constructs and treated with 3.3×10^{-5} M Dex for 24 h. Cellular proteins were assayed and data are expressed as CAT activity relative to pAI.BWT.CAT. Values are shown as means ± SD from two experiments performed in duplicate. (*$P < 0.01$; ANOVA with least significant difference test).

B-motif, because disruption of either nonanucleotide repeat inhibited the activity of the entire -174 to -143 fragment (Fig. 7). These results indicate that Dex can induce rat apoA-I expression only in the presence of an intact site B.

Although site B mediates the glucocorticoid induction of rat apoA-I promoter activity, this *cis*-acting element does not contain a consensus GRE (GGTACAnnnTGTTCT; ref 39). This unexpected finding suggests that GR is unlikely to bind to site B, but hepatonuclear proteins do bind to site B (40). The EMSA studies in the present report (Fig. 8) indicate that GR does not bind to site B nor to the adjacent site S. However, Dex clearly induces DNA binding activities to site B and possibly also to site S (Fig. 8B). Therefore, we can conclude that glucocorticoid activates apoA-I expression through the induction of other nuclear factors. Additional evidence that Dex is working indirectly, via a so-called type II mechanism (41), comes from our previous data showing that rat apoA-I mRNA induction by Dex is blocked by the protein synthesis inhibitor, cycloheximide (5). Glucocorticoids and other steroid hormones also have an indirect effect on the expression of many genes (42–48). For example, ovalbumin gene transcription is induced by estrogen, but its promoter does not contain a consensus sequence for estrogen receptor binding (46).

A search of the sequence data bases and alignment of the site B sequence showed that this site has the potential to bind two copies of the hepatic nuclear factor-3 (HNF-3). Indeed, recent studies by Harnish et al. (49, 50) have shown that nuclear factor-3β (HNF-3β) binds to these sites in the human apoA-I promoter and increases apoA-I transcription. Whether HNF-3β can interact with the rat apoA-I B-motif is unknown, but sequence similarities with the human site B sequence

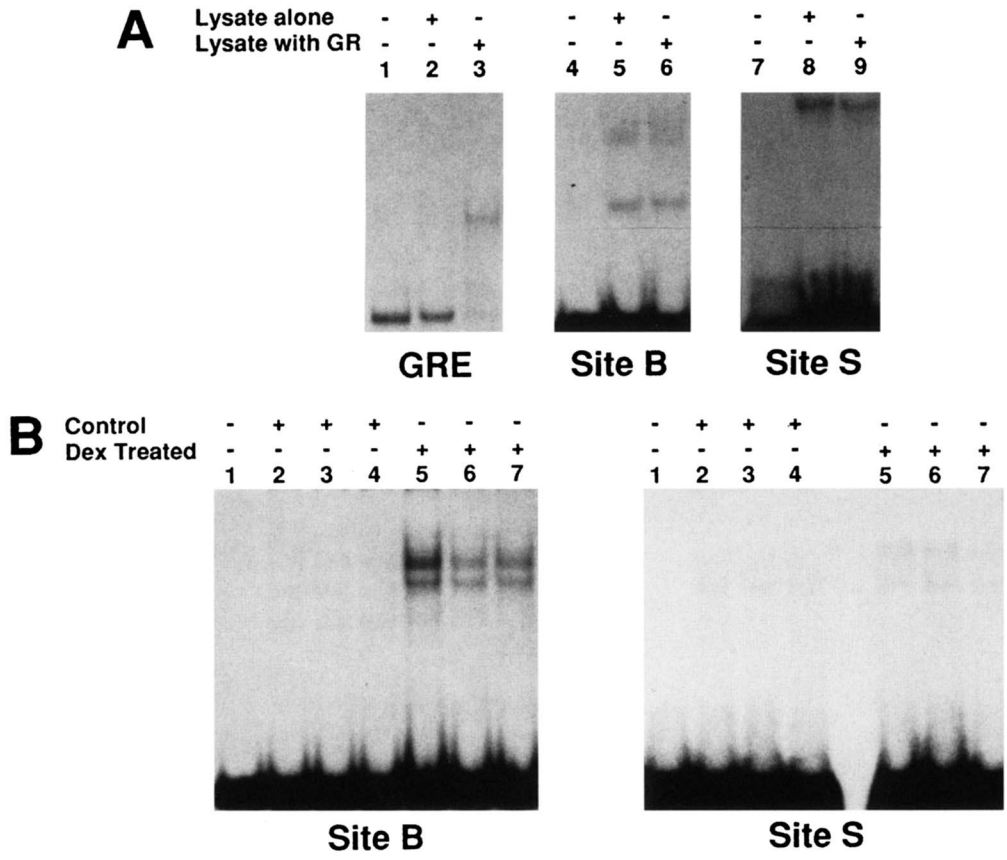

Fig. 8. Site B and site S bind nuclear proteins that are not GR (A) Bacterially expressed GR-DBD protein fragment (lanes 3, 6, and 9) and control bacterial lysate (lanes 2, 5, and 8) were incubated with radiolabeled GRE (750 cpm), site B (4000 cpm), or site S (2000 cpm) oligomers for 30 min. Optimum conditions were 2 μg protein and 2 μg poly dI.dC for GRE; 4 μg protein and 4 μg poly dI.dC for site B; 6 μg protein and 4 μg poly dI.dC for site S in a final 20 μl reaction; 9 μl loaded. Autoradiograms are representative of experiments performed at least twice. (B) Nuclear extracts (8 μg protein) from control (lanes 2–4 of each panel) and Dex-treated (lanes 5–7) HuH-7 cells were incubated with site B (4000 cpm) and site S (2000 cpm) oligomers for 30 min in the presence of 4 μg poly dI.dC. Each lane represents a separate culture and nuclear protein extraction. Each EMSA was performed at least twice.

and the HNF-3β binding site in the tyrosine aminotransferase gene (TAT; ref 51) suggest that it should. As Dex induces site B binding activities and HNF-3β is known to bind to this site in the human apoA-I gene, then Dex may increase the abundance or activity of HNF-3β.

Many glucocorticoid-inducible hepatic genes, such as human α-1 antitrypsin (52) and human transferrin (53), have potential HNF-3β sites, but unlike the rat apoA-I promoter these have recognizable GREs in their promoter/enhancer regions. Another hepatic gene, TAT, shows a more complex response to glucocorticoids. Not only does the TAT promoter/enhancer bind GR to a consensus GRE, it also recruits HNF-3β to a glucocorticoid response unit (GRU) under the influence of the steroid (54). The TAT gene appears to have an added level of control, having both GREs and GRUs allowing direct and indirect glucocorticoid action. Therefore, the apoA-I gene may differ from these glucocorticoid-inducible hepatic genes because of the absence of any recognizable GRE.

Although it is clear that Dex exerts its effect through apoA-I site B, the magnitude of the effect was amplified by an adjacent site that we have named site S (-186 to -171; Fig. 4). The activities of the constructs, pAI.474.CAT, pAI.235.CAT and pAI.186.CAT, that contain both site S and B were 3-fold higher than the pAI.170.CAT template that lacks site S but has site B (Fig. 6). The glucocorticoid effect was not mediated through site S because data from p5′S.CAT, p5′S2.CAT and p5′S3.CAT showed no effect of Dex (Fig. 6). The S-motif is found in the apoA-I promoter regions of all animal sequences stored in the sequence databases, suggesting that it may be an important control point for apoA-I gene expression.

The question that arises from these studies is what *trans*-acting factor interacts with site S? Although DNase

I footprinting (40) with hepatonuclear proteins showed that site S was not protected, Kilbourne et al. (55) have recently shown this site binds the developmental transcription factor, Egr-1. Many hepatic genes contain Egr-1 sites and are regulated during development (56), but what, if any, effect Egr-1 has on rat apoA-I gene expression is currently unknown. Additionally, site S has the potential to bind the *trans*-activating factor PEA-3 (57). However, it would appear that activation of the apoA-I gene through this site by Dex is unlikely, as other hepatic genes (58–60) that contain PEA-3 sites are not regulated by glucocorticoid. Only one PEA-3-containing gene, haptoglobin, has clearly been shown to be indirectly regulated by glucocorticoid (58). Interestingly, the promoter region of the haptoglobin gene is similar to the rat apoA-I gene, in that it also lacks a GRE (58).

The indirect model put forward here may be too simple and gene regulation by glucocorticoid may be even more complex. For example, the smooth muscle gene, glutathione S-transferase (44) is up-regulated by glucocorticoid but has neither HNF-3β, Egr-1, nor PEA-3 binding sites. The authors also attribute the glucocorticoid effect to an indirect mechanism (44) but do not identify any potential *trans*-activators. The mechanism of glucocorticoid-induction of hepatic genes lacking a recognizable GRE is clearly complex, but seems to involve an indirect type II mechanism (41).

Although, the data presented here show that the major effect of Dex on apoA-I is at the transcriptional level, post-transcriptional effects of this steroid are possible. If transcription–translation had reached an equilibrium and Dex affected transcription alone, then the fold induction at each end-point should be equal. As apoA-I transcription is raised 2- to 3-fold, mRNA levels 5- to 6-fold and secreted protein 3- to 4-fold, Dex may have additional post-transcriptional effects. Other hormones affect the apoA-I gene post-transcriptionally, for example, thyroid hormone has a major effect on transcription (5, 17, 35), and also affects translation of apoA-I mRNA (61).

The present studies have shown that the synthetic glucocorticoid, Dex, stimulates transcription of the apoA-I gene by an indirect mechanism. Dex increases site B DNA binding activity that is not GR and most likely to include HNF-3β. Transactivation of the promoter through site B is augmented by an adjacent 5′ sequence we have called site S.

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REFERENCES

1. Abbott, R. D., P. W. Wilson, W. B. Kannel, and W. P. Castelli. 1988. High density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction. The Framingham Study. *Arteriosclerosis.* **8:** 207–211.
2. Eisenberg, S. 1984. High density lipoprotein metabolism. *N. Engl. J. Med.* **317:** 1237–1245.
3. Chan, J., H. Nakabayashi, and N. C. Wong. 1993. HNF-4 increases activity of the rat apoA-I gene. *Nucleic Acids Res.* **21:** 1205–1211.
4. Tam, S. P., T. K. Archer, and D. G. Deeley. 1986. Biphasic effects of estrogen on apolipoprotein synthesis in human hepatoma cells: mechanism of antagonism by testosterone. *Proc. Natl. Acad. Sci. USA.* **83:** 3111–3115.
5. Wong, N. C. W., and J. H. Oppenheimer. 1986. Multihormonal regulation and kinetics of induction of a hepatic mRNA sequence which is slowly responsive to triiodothyronine. *J. Biol. Chem.* **261:** 10387–10393.
6. Elshourbagy, N. A., M. S. Boguski, W. S. Liao, L. S. Jefferson, J. I. Gordon, and J. M. Taylor. 1985. Expression of rat apolipoprotein A-IV and A-I genes: mRNA induction during development and in response to glucocorticoids and insulin. *Proc. Natl. Acad. Sci. USA.* **82:** 8242–8246.
7. Ettinger, W. H., H. F. Klinefelter, and P. O. Kwiterovitch. 1987. Effect of short-term, low-dose corticosteroids on plasma lipoprotein lipids. *Atherosclerosis.* **63:** 167–172.
8. Nashel, D. J. 1986. Is atherosclerosis a complication of long-term corticosteroid treatment? *Am. J. Med.* **80:** 925–929.
9. Taskinen, M. R., E. A. Nikkila, R. Pelkonen, and T. Sane. 1983. Plasma lipoproteins, lipolytic enzymes, and very low density lipoprotein triglyceride turnover in Cushing's syndrome. *J. Clin. Endocrinol. Metab.* **57:** 619–626.
10. Troxler, R. G., E. A. Sprague, R. A. Albanese, R. Fuchs, and A. J. Thompson. 1977. The association of elevated plasma cortisol and early atherosclerosis as demonstrated by coronary angiography. *Atherosclerosis.* **26:** 151–162.
11. Sowers, J. R. 1992. Insulin resistance, hyperinsulinemia, dyslipidemia, hypertension, and accelerated atherosclerosis. *J. Clin. Pharmacol.* **32:** 529–535.
12. Mitamura, T. 1987. Glucocorticoid-induced elevation of serum high-density lipoprotein-cholesterol and its reversal by adrenocorticotropin in the rat. *Biochim. Biophys. Acta.* **917:** 121–130.
13. Bagdade, J. D., E. Yee, J. Albers, and O. J. Pykalisto. 1976. Glucocorticoids and triglyceride transport: effects on triglyceride secretion rates, lipoprotein lipase, and plasma lipoproteins in the rat. *Metabolism.* **25:** 533–542.
14. Masumoto, A., S. Koga, E. Uchida, and H. Ibayashi. 1988. Effects of insulin, dexamethasone and glucagon on the production of apolipoprotein A-I in cultured rat hepatocytes. *Atherosclerosis.* **70:** 217–223.
15. Staels, B., A. van Tol, L. Chan, G. Verhoeven, and J. Auwerx. 1991. Variable effects of different corticosteroids on plasma lipids, apolipoproteins, and hepatic apolipoprotein mRNA levels in rats. *Arterioscler. Thromb.* **11:** 760–769.
16. Varma, V. K., T. K. Smith, M. Sorci-Thomas, W. H. Ettinger, Jr. 1992. Dexamethasone increases apolipoprotein A-I concentrations in medium and apolipoprotein A-I

mRNA abundance from HepG2 cells. *Metabolism.* **41:** 1075–1080.
17. Romney, J. S., J. Chan, F. E. Carr, A. D. Mooradian, and N. C. Wong. 1992. Identification of the thyroid hormone-responsive messenger spot 11 as apolipoprotein-A-I messenger RNA and effects of the hormone on the promoter. *Mol. Endocrinol.* **6:** 943–950.
18. Ricca, G. A., R. W. Hamilton, J. W. McLean, A. Conn, J. E. Kalinyak, and J. M. Taylor. 1981. Rat alpha 1-acid glycoprotein mRNA. Cloning of double-stranded cDNA and kinetics of induction of mRNA levels following acute inflammation. *J. Biol. Chem.* **256:** 10362–10368.
19. Sylvester, J. E., D. A. Whiteman, R. Podolsky, J. M. Pozsgay, J. Respess, and R. D. Schmickel. 1986. The human ribosomal RNA genes: structure and organization of the complete repeating unit. *Hum. Genet.* **73:** 193–198.
20. Sambrooke, J., E. F. Fritsch, and T. Maniatis. 1989. Molecular Cloning: a Laboratory Manual. 2nd edition. Cold Spring Harbor Laboratory Press, Plainview, NY. 7.37–7.52.
21. Marzluff, W. F., and R. C. C. Huang. 1984. Transcription of RNA in isolated nuclei. *In* Transcription and Translation—a Practical Approach. B. D. Hames and S. J. Higgins, editors. IRL Press, Oxford, UK. 92–93.
22. Skettering, G., E. Gjernes, and H. Prydz. 1995. Regulation of lecithin:cholesterol acyltransferase by TGF-β and interleukin-6. *Biochim. Biophys. Acta.* **1255:** 267–272.
23. Nakabayashi, H., T. Hashimoto, Y. Miyao, K. K. Tjong, J. Chan, and T. Tamaoki. 1991. A position-dependent silencer plays a major role in repressing alpha-fetoprotein expression in human hepatoma. *Mol. Cell. Biol.* **11:** 5885–5893.
24. Nakabayashi, H., K. Watanabe, A. Saito, A. Otsuru, K. Sawadaishi, and T. Tamaoki. 1989. Transcriptional regulation of alpha-fetoprotein expression by dexamethasone in human hepatoma cells. *J. Biol. Chem.* **264:** 266–271.
25. Jansen, H., G. C. Schoonderwoerd, and G. M. Dallinga-Thie. 1983. Separation of rat plasma HDL subfractions by density gradient centrifugation and the effect of incubation on these fractions. *Biochim. Biophys. Acta.* **754:** 271–278.
26. Hager, D. A., and R. R. Burgess. 1980. Elution of proteins from sodium dodecyl sulfate-polyacrylamide gels, removal of sodium dodecyl sulfate, and renaturation of enzymatic activity: results with sigma subunit of *Escherichia coli* RNA polymerase, wheat germ DNA topoisomerase, and other enzymes. *Anal. Biochem.* **109:** 76–86.
27. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
28. Malik, V. S., and E. P. Lillehoj. 1994. Antibody Techniques. Academic Press, San Diego, CA. 190–191 (dot-blot); 273–289 (western).
29. Bollag, D. M., and S. J. Edelstein. 1990. Protein Methods. John Wiley, New York, NY. 96–128.
30. Truss, M., G. Chalepakis, E. P. Slater, S. Mader, and M. Beato. 1991. Functional interaction of hybrid response elements with wild-type and mutant steroid hormone receptors. *Mol. Cell. Biol.* **11:** 3247–3258.
31. Maniatis, T., E. F. Fritsch, and J. Sambrooke. 1983. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, New York. 464–465.
32. Studier, F. W., and B. A. Moffatt. 1991. Use of bacteriophage T7 RNA polymerase to direct selective high-level expression of cloned genes. *J. Mol. Biol.* **189:** 113–130.
33. Freedman, L. P., B. F. Luisi, Z. R. Korszun, R. Basavappa, P. B. Sigler, and K. R. Yamamoto. 1988. The function and structure of the metal coordination sites within the glucocorticoid receptor DNA binding domain. *Nature.* **334:** 543–546.
34. Unit 12.1 DNA-Protein Interactions. 1995. *In* Current Protocols in Molecular Biology. F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl, editors. John Wiley & Sons, New York, NY. 12.1.1–12.1.3.
35. Taylor, A. H., P. Wishart, D. E. Lawless, J. Raymond, and N. C. W. Wong. 1996. Identification of functional positive and negative thyroid hormone-responsive elements in the rat apolipoprotein A-I promoter. *Biochemistry.* **35:** 8281–8288.
36. Kulkarni, A. B., R. Reinke, and P. Feigelson. 1985. Acute phase mediators and glucocorticoids elevate alpha 1-acid glycoprotein gene transcription. *J. Biol. Chem.* **260:** 15386–15389.
37. Berg, A. L., and P. Nilsson-Ehle. 1994. Direct effects of corticotropin on plasma lipoprotein metabolism in man—studies in vivo and in vitro. *Metabolism.* **43:** 90–97.
38. Gustafsson, J. A., J. Carlstedt-Duke, S. Okret, A. C. Wikstrom, O. Wrange, F. Payvar, and K. Yamamoto. 1984. Structure and specific DNA binding of the rat liver glucocorticoid receptor. *J. Steroid Biochem.* **20:** 1–4.
39. Gehring, U. 1988. Glucocorticoid receptor action. *In* Hormones and Their Actions. Part 1. B. A. Cooke, R. J. B. King, and H. J. van der Molen, editors. Elsevier Science Publishers BV, Amsterdam. 217–239.
40. Widom, R. L., J. A. Ladias, S. Kouidou, and S. K. Karathanasis. 1991. Synergistic interactions between transcription factors control expression of the apolipoprotein A-I gene in liver cells. *Mol. Cell. Biol.* **11:** 677–687.
41. Bamberger, C. M., H. M. Schulte, and G. P. Chrousos. 1996. Molecular determinants of glucocorticoid receptor function and tissue sensitivity to glucocorticoids. *Endocr. Rev.* **17:** 245–261.
42. Pajovic, S., V. E. Jones, K. R. Prowse, F. G. Berger, and H. Baumann. 1994. Species-specific changes in regulatory elements of mouse haptoglobin genes. *J. Biol. Chem.* **269:** 2215–2224.
43. Hocke, G. M., D. Barry, and G. H. Fey. 1992. Synergistic action of interleukin-6 and glucocorticoids is mediated by the interleukin-6 response element of the rat α2-macroglobulin gene. *Mol. Cell. Biol.* **12:** 2282–2294.
44. Fan, W., R. Trifiletti, T. Cooper, and J. S. Norris. 1992. Cloning of a mu-class glutathione S-transferase gene and identification of the glucocorticoid regulatory domains in its 5′ flanking sequence. *Proc. Natl. Acad. Sci. USA.* **89:** 6104–6108.
45. Puissant, C., and L. M. Houdebine. 1991. Cortisol induces rapid accumulation of whey acid protein mRNA but not of asl and b-casein mRNA in rabbit mammary explants. *Cell. Biol. Int. Rep.* **15:** 121–129.
46. Nordstrom, L. A., D. M. Dean, and M. M. Sanders. 1993. A complex array of double-stranded and single-stranded DNA-binding proteins mediates induction of the ovalbumin gene by steroid hormones. *J. Biol. Chem.* **268:** 13193–13202.
47. Paliogianni, F., A. Raptis, S. S. Ahuja, S. M. Najjar, and D. T. Boumpas. 1993. Negative transcriptional regulation of human interleukin 2 (IL-2) gene by glucocorticoids through interference with nuclear transcription factors AP-1 and NF-AT. *J. Clin. Invest.* **91:** 1481–1489.

48. Caldenhoven, E., J. Linden, S. Wissink, A. Van de Stolpe, J. Raaijmakers, L. Koenderman, S. Okret, J-A. Gustafsson, and P. T. Van der Saag. 1995. Negative cross-talk between RelA and the glucocorticoid receptor: a possible mechanism for the anti-inflammatory action of glucocorticoids. *Mol. Endocrinol.* **9:** 401–412.
49. Harnish, D. C., S. Malik, and S. K. Karathanasis. 1994. Activation of apolipoprotein A-I gene transcription by the liver-enriched factor HNF-3. *J. Biol. Chem.* **269:** 28220–28226.
50. Harnish, D. C., S. Malik, E. Kilbourne, R. Costa, and S. K. Karathanasis. 1996. Control of apolipoprotein A-I gene expression through synergistic interactions between hepatocyte nuclear factors 3 and 4. *J. Biol. Chem.* **271:** 13621–13628.
51. Roux, J., R. Pictet, and T. Grange. 1995. Hepatocyte nuclear factor 3 determines the amplitude of the glucocorticoid response of the rat tyrosine aminotransferase gene. *DNA Cell Biol.* **14:** 385–396.
52. Castell, J. V., M. J. Gomez-Lechon, M. David, R. Fabra, R. Trullenque, and P. C. Heinrich. 1990. Acute-phase response of human hepatocytes: regulation of acute-phase protein synthesis by interleukin-6. *Hepatology.* **12:** 1179–1186.
53. Gelin, J. L., L. L. Moldawer, B. M. Iresjo, and K. G. Lundholm. 1993. The role of the adrenals in the acute phase response to interleukin-1 and tumor necrosis factor-alpha. *J. Surg. Res.* **54:** 70–78.
54. Espinas, M. L., J. Roux, R. Pictet, and T. Grange. 1995. Glucocorticoids and protein kinase A coordinately modulate transcription factor recruitment at a glucocorticoid-responsive unit. *Mol. Cell. Biol.* **15:** 5346–5354.
55. Kilbourne, E. J., R. Widom, D. C. Harnish, S. Malik, and S. K. Karathanasis. 1995. Involvement of early growth response factor Egr-1 in apolipoprotein A-I transcription. *J. Biol. Chem.* **270:** 7004–7010.
56. Sukhatme, V. P., X. Cao, L. C. Chang, C-H. Tsai-Morris, D. Stamenkovitch, P. C. P. Ferreira, D. R. Cohen, S. A. Edwards, T. B. Shows, T. Curran, M. M. Le Beau, and E. D. Adamson. 1988. A zinc finger-encoding gene coregulated with c-fos during growth and differentiation, and after cellular depolarization. *Cell.* **53:** 37–43.
57. Wasylyk, B., C. Wasylyk, P. Flores, A. Begue, D. Leprince, and D. Stehelin. 1990. The c-ets proto-oncogenes encode transcription factors that cooperate with c-Fos and c-Jun for transcriptional activation. *Nature.* **346:** 191–193.
58. Pajovic, S., V. E. Jones, K. R. Prowse, F. G. Berger, and H. Baumann. 1994. Species-specific changes in regulatory elements of mouse haptoglobin genes. *J. Biol. Chem.* **269:** 2215–2224.
59. Nerlov, C., D. De Cesare, and F. Pergola. 1992. A regulatory element that mediates cooperation between a PEA3-AP-1 element and an AP-1 site is required for phorbol ester induction of urokinase enhancer activity in HepG2 hepatoma cells. *EMBO J.* **11:** 4573–4582.
60. Rorth, P., C. Nerlov, F. Blasi, and M. Johnsen. 1990. Transcription factor PEA3 participates in the induction of urokinase plasminogen activator transcription in murine keratinocytes stimulated with epidermal growth factor or phorbol-ester. *Nucleic Acids Res.* **18:** 5009–5017.
61. Vandenbrouck, Y., B. Janvier, C. Loriette, G. Bereziat, and M. Mangeney-Andreani. 1995. Thyroid hormone modulates apolipoprotein A-I gene expression at the post-transcriptional level in HepG2 cells. *Eur. J. Biochem.* **231:** 126–132.