Transgenic mouse model for estrogen-regulated lipoprotein metabolism: studies on apoVLDL-II expression in transgenic mice

Eva Zsigmond, Melinda K. Nakanishi, Franca E. Ghiselli, and Lawrence Chan

Departments of Cell Biology and Medicine, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030

Abstract We have produced transgenic mice that express an estrogen-responsive avian apolipoprotein, apoVLDL-II. An apoVLDL-II natural gene construct containing 4.7 kb of 5′ flanking and 19 bp of 3′ flanking sequences together with the 4 exon/3 intron structural gene was expressed in a liver-specific manner in transgenic mice. A single injection of estrogen caused a 5.9- to 7.5-fold stimulation of apoVLDL-II mRNA in the liver. The transgene mRNA had the same initiation sites of transcription as the native mRNA isolated from laying hen liver, and the same sites were used before and after estrogen treatment. The number of hepatocytes that stain positive for immunoreactive apoVLDL-II increased from < 1% to 40–60% in 24 h after estrogen treatment. Thus, in transgenic mice as in the cockerel, hepatocytes are biochemically heterogeneous and induction of apoVLDL-II synthesis occurs by recruitment of hepatocytes. In the plasma compartment, compared to controls, transgenic mice have a 3- to 5-fold higher basal total plasma triglyceride which was accounted for by a 5.4-fold high basal VLDL triglyceride. Estrogen treatment results in a ~ 2-fold increase in the VLDL triglycerides over basal levels and 8.5-fold increase over nontransgenic mice, which did not show any change in VLDL in response to estrogen. Transgenic mice with the integrated apoVLDL-II gene provide a useful model for the study of the regulation of lipoprotein metabolism by estrogen.—**Zsigmond, E., M. K. Nakanishi, F. E. Ghiselli, and L. Chan.** Transgenic mouse model for estrogen-regulated lipoprotein metabolism: studies on apoVLDL-II expression in transgenic mice. *J. Lipid Res.* 1995. **36:** 1453–1462.

Supplementary key words triglycerides · hepatocytes · mRNA · VLDL

ApoVLDL-II is a major apolipoprotein (apo) in plasma very low density lipoprotein (VLDL) in laying hens and in estrogen-treated cockerels and roosters (1). It is produced exclusively in the liver and, in response to estrogen treatment, the mRNA for this protein increases markedly (2, 3). The estrogen effects were shown to be mediated at both transcriptional and posttranscriptional levels (2, 4, 5).

ApoVLDL-II was the first vertebrate apolipoprotein studied by modern recombinant DNA techniques (6–10). It contains 82 amino acid residues with a single cysteine at residue number 75 (11). The protein normally exists as a homodimer by disulfide linkage at this residue. ApoVLDL-II is an avian apolipoprotein with no mammalian counterpart. However, the structure of apoVLDL-II indicates that it is evolutionarily related to the mammalian apolipoproteins. The apoVLDL-II gene has the same 4 exon/3 intron structure and the protein contains the typical 11- and 22-residue internal repeats characteristic of the mammalian apolipoproteins (12). Like mammalian apolipoproteins, apoVLDL-II binds to phospholipid vesicles spontaneously (13, 14).

Estrogen treatment in the cockerel or rooster produces marked hyperlipidemia concomitant with the induction of apoVLDL-II (1, 15, 16). In contrast, the effects of estrogen in mice appear to be strain-specific and even in those strains that develop hyperlipidemia, the response seems to be of much lesser magnitude than in the avian species (17, 18). We have produced transgenic mice with the integrated apoVLDL-II gene controlled by its own promoter, and have used these animals to examine the tissue-specific expression and hormone responsiveness of apoVLDL-II. We were particularly interested in the effect of estrogen both on apoVLDL-II expression and on the plasma lipoproteins of these transgenic animals. We found that estrogen did not induce hyperlipidemia in control animals but did so in transgenic mice in which the production of apoVLDL-II was also markedly stimulated. The hyperlipidemia thus appeared to be a direct consequence of apoVLDL-II overexpression. These transgenic mice can be used to dissect the regulatory mechanisms involved in the ex-

pression of an avian estrogen-responsive gene, apoVLDL-II, integrated into the mouse genome.

MATERIALS AND METHODS

Reconstruction of the avian apoVLDL-II gene

The genomic sequences of the apoVLDL-II were isolated from a Charon 4A phage library. Two EcoRI-bounded fragments were subcloned in pBR322. The 5′ fragment covers ~ 4.7 kb of the 5′ flanking DNA, the first exon, and part of the first intron. The 3′ fragment includes the rest of the first intron, the other three exons and two introns, and ~ 1.5 kb of the 3′ flanking DNA. The cloned 3′ fragment was trimmed at a Taq I site 19 bp downstream to the end of the last exon, and ligated back to the 5′ EcoRI-RI fragment. This 7.7 kb EcoRI-Taq I fragment was subcloned in pBR322 by using Hind III linkers (Fig. 1). The amplified apoVLDL-II gene fragment was removed from the vector by Hind III digestion and purified by agarose gel electrophoresis before microinjection.

Microinjection of fertilized mouse eggs

The purified apoVLDL-II gene DNA was injected into the male pronucleus of fertilized mouse eggs obtained from ICR females mated to C3h males. Eggs that survived microinjection were transferred into the oviducts of pseudo-pregnant ICR foster females as previously described (19).

Transgenic mice

Transgenic mice were identified by Southern blot analysis. Heterozygous transgenic mice were produced by crossing C3h/ICR transgenic and nontransgenic littermates. Only male heterozygous mice were used for all subsequent experiments and each experimental group was comprised of mice with the same genetic background. Mice were fed ad libidum, with free access to water and were not fasted prior to experiments. Animals were maintained at 12 h light and dark cycles.

Mice were injected intramuscularly with 17β-estradiol (5 μg/g body weight at a concentration of 5 mg/ml) in dimethylsulfate (DMSO). As a control, the carrier alone was injected into some animals. Blood was obtained from the tail vein of transgenic and nontransgenic animals at various times after injection. Mice were bled at 8 AM, 2 PM, 6 PM, and 10 AM corresponding to 0 h, 6 h, 10 h, and 26 h time points respectively. Transgenic and nontransgenic mice were bled at the same time of day.

ApoVLDL-II DNA probe labeling and hybridization

A cDNA probe, pVL29, cloned in pBR322, was used as a hybridization probe. This clone includes sequences in the mature peptide region of apoVLDL-II, covering the last three exons but missing sequences from the first exon. It was labeled with [^{32}P]dCTP by nick-translation (20). Typically, a specific activity of ~ 30×10^7 cpm/μg was obtained. Southern and Northern blot hybridizations were performed at 65°C at a concentration of 3.8×10^6 cpm of probe per milliliter in 10% dextran sulfate, 500 mM Na_2PO_4, 0.7% SDS, and 5 g milk powder/L for 18 h. After hybridization, the filters were washed in 40 mM Na_2HPO_4, 1% SDS at 65°C for 75 min with several changes. The filter was air-dried and exposed to X-Omat XAR-5 film at −80°C with a Lightning Plus intensifying screen.

Isolation and analysis of nucleic acids

Total nucleic acids were isolated from tail samples by phenol–chloroform extraction and ethanol precipitation. DNA concentration was measured by the fluorescence assay described by Labarca and Paigen (21) with a Perkin-Elmer Model 650-10M fluorescence spectrophotometer.

Restriction enzyme digestion and agarose gel electrophoresis were done as described (22). For Southern transfer, the gel was washed in 0.2 N HCl for 10 min, washed briefly in water, and then denatured in 0.2 NaOH, 0.6 M NaCl for 30 min. Transfer of the gel was done as described (23).

Total RNA was isolated from various mouse tissues by using the acid quanidinium thiocyanate-phenol-chloroform extraction method of Chomczynski and Sacchi (24). For Northern blot analysis, samples (10 μg RNA) were denatured in a 6 mM sodium acetate, 60% formamide, 24 mM HEPES, 1.2 mM Na_2 EDTA, 7.2% formaldehyde solution, and were run on a 6.7% formaldehyde, 1.5% agarose gel. The gel was soaked in water for 30 min and then in 20 × SSC for 30 min. The gel was then transferred in the same manner that Southern gels were transferred (23).

Primer extension

Total RNA was prepared from the livers of transgenic mice and nontransgenic littermates as previously described by Chirgwin et al. (25). Primer extension was performed by a standard procedure (26), using 30 μg of RNA and a chicken apoVLDL-II primer (5′-TTTGTTGGTACCAGTTCAGCC-3′). The primer was 5′-end labeled with [γ-^{32}P]ATP and T4 polynucleotide kinase, and gel-purified on a 10% denaturing polyacrylamide gel prior to use for primer extension.

Immunoblot analysis

VLDL samples (25 μl) were precipitated with TCA and extracted twice with acetone. Pellets were resuspended in 15 μl of 0.062 M Tris-HCl, pH 6.8, 10%

glycerol, 2% (w/v) SDS, 5% β-mercaptoethanol, and 0.025% bromophenol blue. Samples were electrophoresed on 4–20% SDS-polyacrylamide gels and transferred onto nitrocellulose membranes (BA 85, Schleicher and Schuell).

Prior to incubation with goat anti-chicken apoVLDL-II polyclonal antibody, membranes were blocked overnight in 50 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA, and 10% bovine serum albumin. Biotinylated anti-goat IgG was used as the secondary antibody and the detection was performed using the Vectastain ABC immunoperoxidase method (Vector Laboratories).

Peroxidase staining of liver slices

Paraffin-embedded liver slices were preincubated with diluted normal serum (1:66 dilution) for 20 min. Tissues were exposed to the primary antibody (1:200 dilution) for 30 min, washed in 50 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA (TBS) for 10 min and then incubated with the secondary antibody (1:200 dilution) for 30 min. After a 10-min wash in the TBS buffer, the samples were incubated with the Vectastain ABC reagent for 60 min (Vectastain, Vector Laboratories).

Liver slices were washed for 10 min in TBS buffer and exposed to the peroxidase substrate solution. The reaction was stopped by incubating in an excess of distilled H_2O for 5 min. Slides were mounted and examined under a light microscope.

Lipoprotein and lipid analysis

Anesthetized mice were bled at different time points after the injection of estrogen or DMSO. For the isolation of various lipoprotein fractions, FPLC chromatography was performed using a Beckman System Gold HPLC/FPLC with two Superose-6 columns (Pharmacia LKB Biotechnologies, Inc.) connected in series (27). To separate VLDL, LDL, and HDL classes, 250 µl of plasma was injected into the FPLC and 45 fractions of 0.5 ml were eluted with 1 mM EDTA, 154 mM NaCl, and 0.02% NaN_3 (pH 8.2). A 150-µl aliquot from each fraction was assayed for total cholesterol and triglyceride content using an enzymatic assay (Sigma Diagnostics).

For immunoblot analysis of apoVLDL-II, VLDL fractions were isolated by sequential ultracentrifugal flotation (28). Blood samples were collected from four transgenic mice injected with estrogen or DMSO and four nontransgenic mice injected with estrogen. The pooled plasma samples were overlayered with 0.9% NaCl ($d < 1.006$ g/ml) and ultracentrifuged at 4°C, 40,000 rpm in a Beckman 40.3 Ti rotor for 20 h.

The apolipoprotein composition of isolated VLDL fractions was determined by gradient (4–20%) SDS-polyacrylamide gel electrophoresis and Coomassie R250 staining.

RESULTS

Generation of transgenic mice

We produced two independent lines of transgenic mice with the integrated apoVLDL-II gene. The 7.7 kb gene construct used for microinjection contained the entire apoVLDL-II gene together with 4.7 kb of 5′ flanking DNA and 19 bp of the 3′ flanking DNA (**Fig. 1**). Transgenic animals were identified by Southern blot analysis of tail DNA using an apoVLDL-II cDNA probe. The two transgenic lines both contained 50–75 copies of the apoVLDL-II transgene.

Tissue-specific expression of the apoVLDL-II transgene

By Northern blot analysis, apoVLDL-II mRNA was relatively low in RNA samples isolated from the liver of untreated transgenic animals and it was not present in RNA from untreated control mice (data not shown). To examine the tissue specificity at high level expression, transgenic and control mice were treated with a single injection of 17β-estradiol (5 µg/g body weight) and Northern blot analysis was performed on RNA samples isolated from multiple tissues (**Fig. 2A**). ApoVLDL-II mRNA of the appropriate size was detected in samples in the liver in all transgenic lines. After overexposure of the blot, the message was also detected at very low concentrations in the kidney in one of the lines. The mRNA was undetectable in samples isolated from the spleen, thymus, small intestine, large intestine, brain, lung, or testis. The appropriate size of the mRNA indicates that the transgene transcript was correctly spliced in the transgenic mice. ApoVLDL-II transcripts were undetectable in RNA samples from estrogen-treated nontransgenic animals (data not shown).

Estrogen stimulation of apoVLDL-II mRNA accumulation

We examined the effect of estrogen on apoVLDL-II mRNA expression in transgenic mouse liver by North-

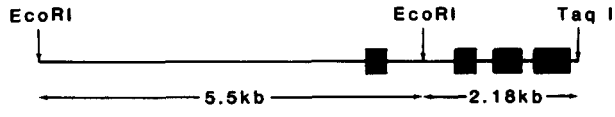

Fig. 1. ApoVLDL-II transgene construct. The apoVLDL-II transgene was constructed from two EcoRI fragments. The 5′ fragment comprised of 4.7 kb of the 5′ flanking DNA, the first exon and part of the first intron. The 3′ fragment covered the remainder of the first intron, three other exons, and two introns, as well as ~ 1.5 kb of the 3′ flanking region. Prior to purification for microinjection, the 3′ end of the gene construct was cut at a Taq I site 19 bp downstream from the end of exon 4. Hind III linkers were used to subclone the 7.7 kb EcoRI-Taq I fragment in pBR322. The heavy bars represent the exons, and the thin lines represent the 5′ and 3′ flank regions and introns.

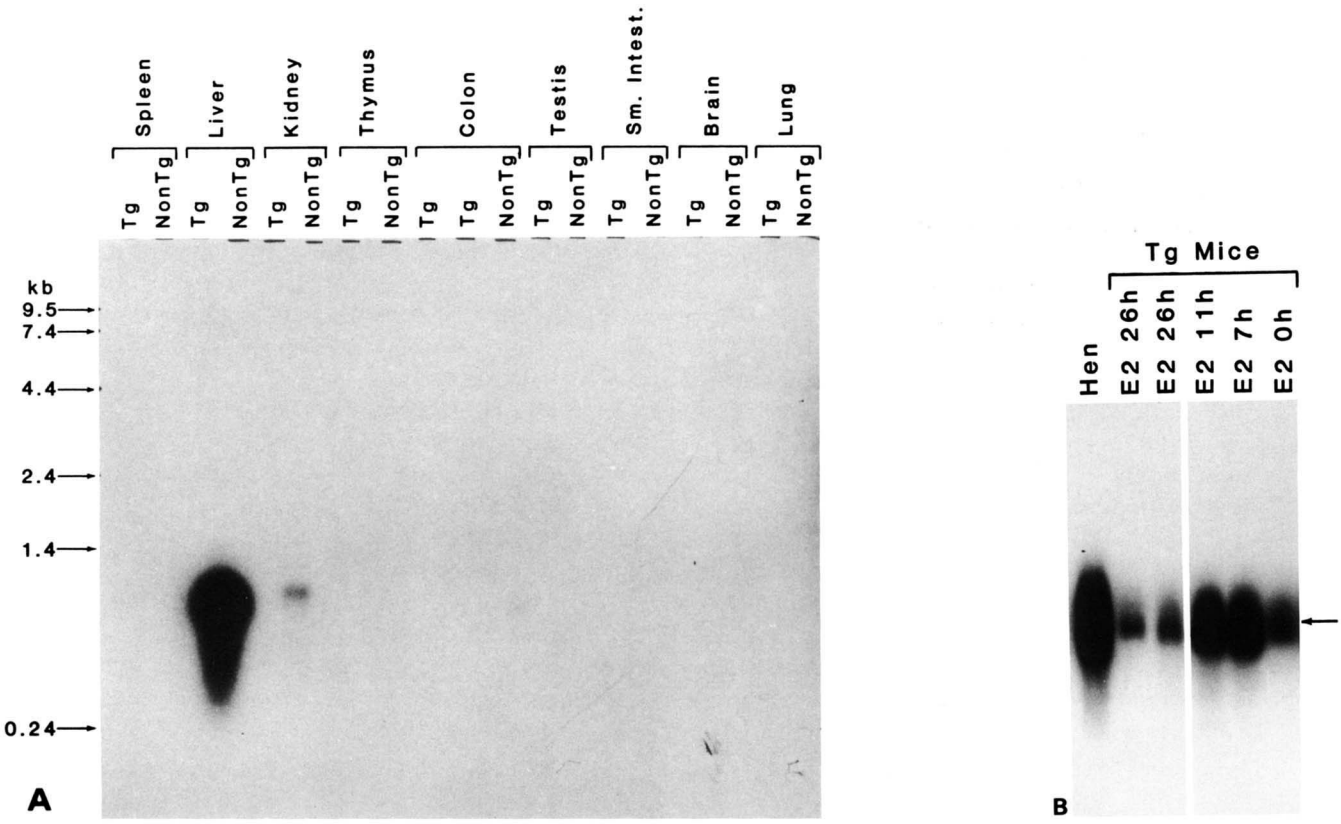

Fig. 2. A: Northern blot analysis of tissue-specific expression of apoVLDL-II in transgenic mice. Male transgenic and non-transgenic control mice were injected with 17β-estradiol (5 μg/g body weight at a concentration of 5 mg/ml) in DMSO intramuscularly. Twenty four hours post-injection, tissues were excised and snap frozen in liquid N_2. Total RNA was isolated from spleen, liver, kidney, thymus, colon, testis, small intestines, brain, and lung, as previously reported by Chomczynski and Sacchi (24). Northern blot analysis was performed as described in Materials and Methods. B: Time course of apoVLDL-II expression in the liver of transgenic mice. Northern blot analysis was performed on total RNA isolated from the livers of transgenic mice at 0, 7, 11, and 26 h after an intramuscular injection of 17β-estradiol (5 μg/g). Compared to basal levels (0 h), apoVLDL-II expression increased 5.5-fold after 7 h and 7.5-fold after 11 h of hormone injection. Twenty six hours post-estrogen injection, apoVLDL-II RNA expression decreased to basal levels. RNA from hen liver was used as a positive control.

ern blot analysis (Fig. 2B). After an intramuscular injection of 17β-estradiol (5 μg/g body weight), apoVLDL-II mRNA expression increased about 5.5-fold at 7 h and 7.5-fold at 11 h. It returned to basal level at 26 h. The mRNA bands were relatively broad whether the RNA was isolated from transgenic mice or from laying hen liver, the tissue that normally produces apoVLDL-II.

Transcription initiation of apoVLDL-II transgene

The apoVLDL-II transgene contained ~ 4.7 kb of 5′ flanking sequence. To determine whether transcription initiation took place at the authentic initiation site before and 11 h after estrogen administration, we performed primer extension assay on the total liver RNA using a primer specific for apoVLDL-II (**Fig. 3B**). Laying hen liver RNA was included as a control for this experiment. It is evident that the major transgene transcription product was initiated at the identical site as hen apoVLDL-II mRNA. It maps to 76 bases upstream of the

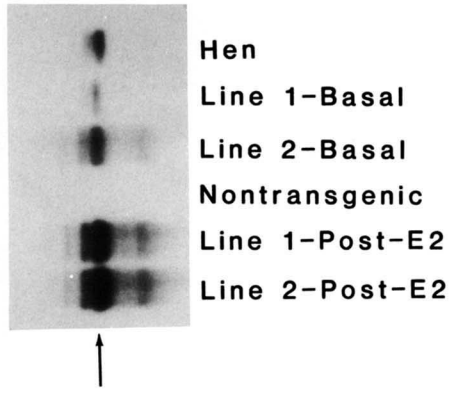

Fig. 3. Primer extension assay. Transgenic and nontransgenic mice were injected with 17β-estradiol (5 mg/ml) i.m. Eleven hours after the hormone treatment, livers were excised, frozen in liquid N_2 and total RNA was prepared as previously described (25). Primer extension assay was performed on RNA obtained from two different lines of transgenic mice according to the protocol reported in Materials and Methods. Hen liver RNA was used as a control.

translation initiation codon. Minor products initiated at neighboring sites were also observed in the transgenic mRNA which are also observed in the hen on overexposure. Furthermore, the primer extension assay also shows that the same transcription initiation sites were used before and following estrogen treatment.

Immunoreactive apoVLDL-II in plasma VLDL of transgenic mice

Immunoblot analysis was performed on isolated VLDL fractions from the plasma of transgenic and control mice (**Fig. 4**). Ten hours after the injection of 17β-estradiol, two immunoreactive apoVLDL-II bands were identified in VLDL fractionated from the plasma of transgenic mice. The ~ 9.5 and ~ 19 kDa bands corresponded to the reduced (monomeric) and non-reduced (dimer) forms of apoVLDL-II, which typically was relatively resistant to reduction by β-mercaptoethanol (1). Plasma VLDL from transgenic mice treated with DMSO also contained the two immunoreactive apoVLDL-II bands, but this basal level of expression was 8-fold lower than the estrogen-induced levels. No apoVLDL-II was identified in LDL or HDL fractions and no immunoreactive bands were observed in VLDL isolated from the plasma of 17β-estradiol-treated control mice. Coomassie blue staining of an identical gel showed that apoVLDL-II, apoB-48, and apoB-100 are the major apoproteins in VLDL fractions isolated from the plasma of transgenic mice.

Immunolocalization of apoVLDL-II in the transgenic mouse liver

We previously showed that the estrogen induction of apoVLDL-II in the cockerel liver occurs by recruitment of hepatocytes (29). We have examined the liver of transgenic and control mice by immunoperoxidase staining. Representative photomicrographs are shown in **Fig. 5.** Before estrogen treatment, or after treatment with DMSO (as control, Fig. 5A), <1% of the hepatocytes stained positively for apoVLDL-II. In some fields, no immunostaining was discerned. In Fig. 5B (4 h after estrogen injection), only two positive cells were found among 384 cells in the photomicrograph. Twenty four hours after a single injection of estrogen, the proportion of positive cells went up to 51.1% (experiment 1, mouse line 1, Fig. 5C), 57.8% (experiment 2, mouse line 1, data not shown), and 44.3% (experiment 1, mouse line 2, data not shown), respectively. No immunostaining of cells from nontransgenic mice was observed (Fig. 5D, 5E, 5F).

Plasma lipids in transgenic and control mice

Plasma lipids were measured in control and transgenic animals (**Fig. 6**). Basal plasma cholesterol was only

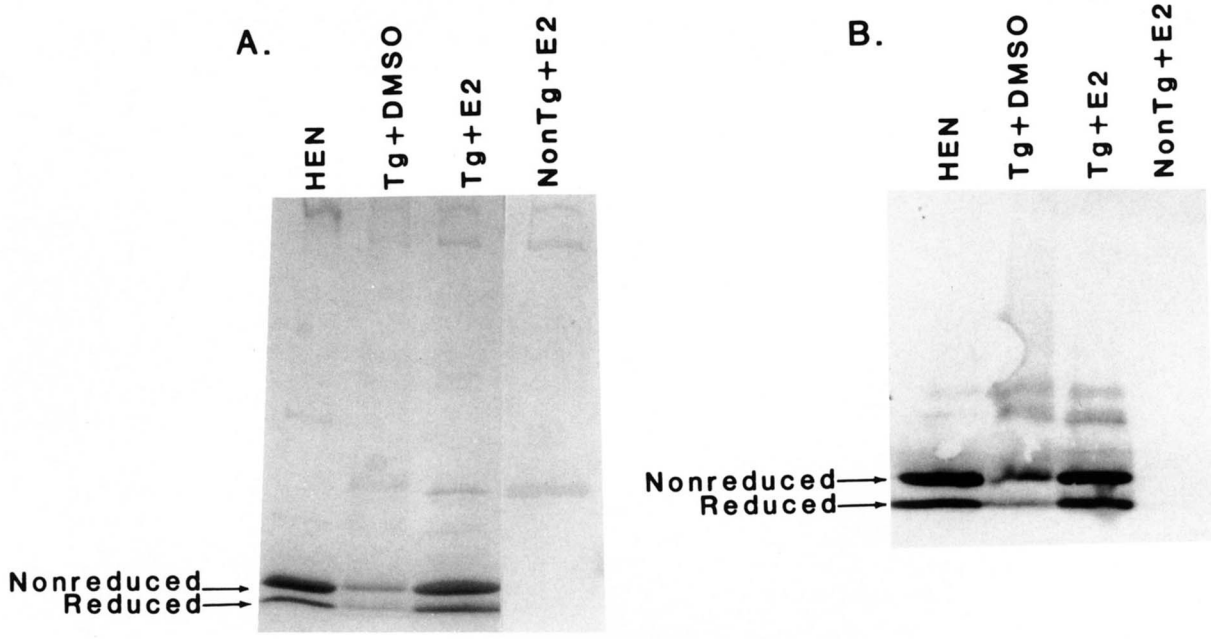

Fig. 4. Immunoblot analysis. Immunoblot was performed on VLDL samples (25 μl) isolated by sequential flotation ultracentrifugation from the plasma of transgenic mice injected 10 h previously with DMSO or estrogen, and from the plasma of estrogen-treated control animals. Samples were purified and electrophoresed as described in Materials and Methods. Panel A shows the Coomassie blue-stained gel and panel B represents the autoradiogram of the immunoblot. VLDL isolated from hen plasma was included as a control. The non-reduced ~ 19 kDa dimeric and reduced ~ 9.5 kDa monomeric forms of the apoVLDL-II proteins are marked by arrows. In panel A, the major bands in the top of the gel represent apoB-100 (for hen plasma) and apoB-100 and apoB-48 (for mouse plasma).

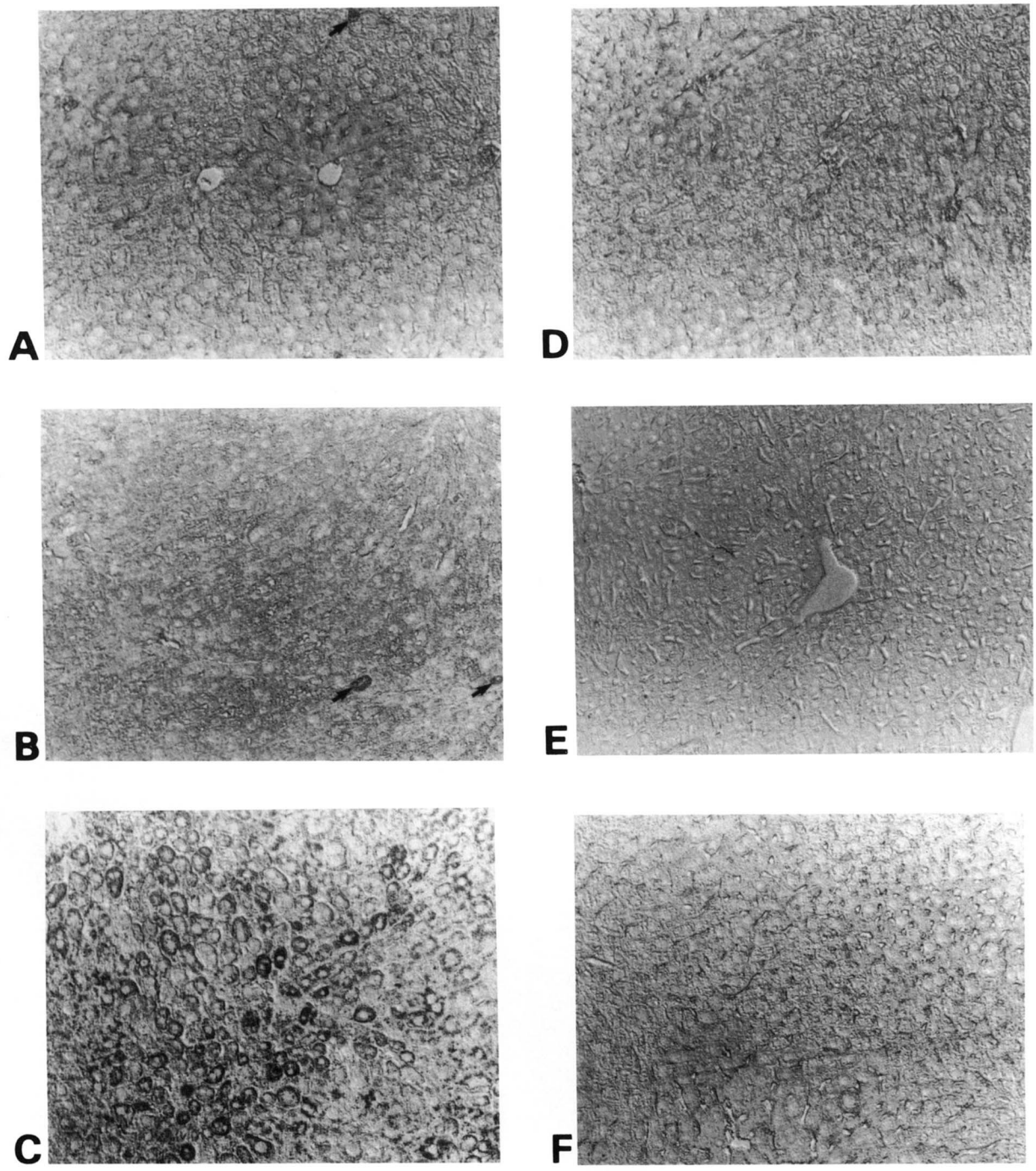

Fig. 5. Immunocytochemical localization of apoVLDL-II in liver sections. Transgenic and control mice were injected with DMSO or 17β-estradiol intramuscularly. Livers were excised 4 h or 24 h after injection and tissue sections were incubated with goat anti-chicken apoVLDL-II polyclonal antibody, 1:200 dilution for 30 min. After extensive washing in 50 mM Tris, pH 8.0, 150 mM NaCl, 1 mM EDTA (TBS), liver sections were exposed to biotinylated anti-goat IgG for 30 min. Samples were then washed in TBS and the immunostaining was performed using Vectastain ABC immunoperoxidase substitute (Vectostain, Vector Laboratories). Stained liver sections were mounted on slides and examined by microscopy (× 150). Panels A and D show peroxidase-stained liver sections obtained from transgenic and nontransgenic mice, respectively, 24 h after the injection of DMSO. The effects of estrogen injection on immunoperoxidase staining of hepatocytes from transgenic mice are shown in panels B (4 h) and C (24 h), and from nontransgenic mice in panels E (4 h) and F (24 h).

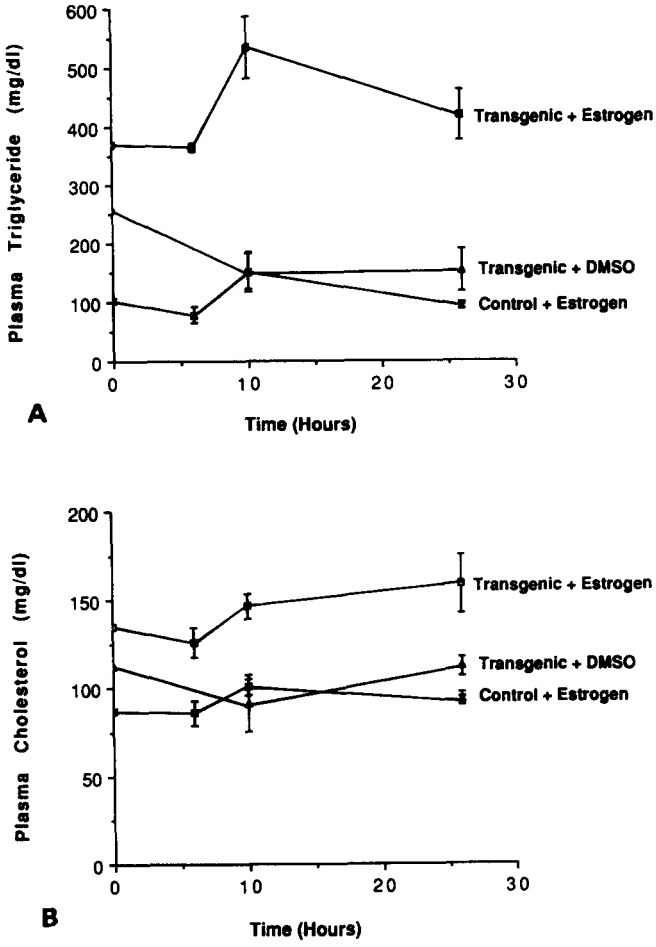

Fig. 6. Plasma triglyceride and cholesterol levels in transgenic and control mice. Transgenic and control mice were bled at 0, 6, 10, and 26 h after the injection of 17β-estradiol or DMSO. At each time point, 10 μl of plasma was used to assay total triglycerides (panel A) and cholesterol (panel B), using an enzymatic assay (Sigma Diagnostics). Points represent mean ± SEM; n = 5 for 0 h; n = 3 for 6 h, n = 6 for 10 h, and 26 h time points. At 0 h, plasma triglyceride concentrations of transgenic mice injected with estrogen or DMSO and nontransgenic mice treated with estrogen were 371.18 ± 49.55 mg/dl, 257.60 ± 76.10 mg/dl, and 102.67 ± 12.65 mg/dl, respectively. The basal plasma cholesterol concentrations were 135.00 ± 12.28 mg/dl, 112.83 ± 34.80 mg/dl, and 86.75 ± 9.98 mg/dl for estrogen-treated transgenic, DMSO-treated transgenic, and estrogen-injected nontransgenic mice, respectively. Estrogen injection resulted in significantly higher plasma triglyceride and cholesterol levels in transgenic mice compared to control mice at each time point ($P < 0.01$).

slightly higher in transgenic animals. Basal plasma triglycerides, in contrast, tended to be 3- to 5-times higher in transgenic animals than in nontransgenic littermate controls. In response to a single injection of estrogen, plasma cholesterol increased slightly at 10 h and 24 h in transgenic animals but it did not change in controls (Fig. 6B). Plasma triglycerides in transgenic mice increased at 10 h after estrogen, 1.5-fold over basal levels and 3.5-fold over control levels. The higher basal triglyceride level in transgenic mice did not respond to the administration of carrier (DMSO) alone (Fig. 6A).

Plasma lipoproteins in transgenic and control mice

To investigate which of the plasma lipoprotein fractions were affected by estrogen treatment, we fractionated the plasma lipoproteins by FPLC using a procedure reported by Cole et al. (27) that uses two Superose-6 columns connected in series. Plasma samples from individual estrogen-treated control, DMSO-treated, and estrogen-treated transgenic animals at various times after treatment were loaded on the FPLC system. Representative profiles from single animals are shown in **Fig. 7.** In the basal state, transgenic animals had an elevated VLDL fraction compared to nontransgenic controls (Fig. 7). After a single injection of estrogen, the major species of lipoproteins induced by the hormone was in the VLDL fraction. This stimulation occurred when the animals were treated with estrogen and did not happen when only carrier (DMSO) was administered to the transgenic mice. In nontransgenic littermate controls, after estrogen treatment, the VLDL peak remained unchanged. We next analyzed the lipoprotein fractions from four different animals from each group (**Fig. 8**). In the basal state, statistically significantly higher cholesterol was detected in the VLDL and LDL of transgenic mice, compared to controls (Fig. 8B). Triglyceride was higher in the VLDL fraction but the difference did not reach statistical significance in the LDL fraction (Fig. 8A). After estrogen treatment, VLDL and LDL cholesterol and triglyceride were significantly higher in transgenic mice than in controls ($P < 0.01$). HDL cholesterol, in contrast, was lower in transgenic animals than in nontransgenic controls ($P < 0.01$).

DISCUSSION

We have produced transgenic mice with the chicken apoVLDL-II gene. We showed that with an integrated construct containing the entire 3 kb structural gene together with 4.7 kb in the 5′ flanking and 19 bp in the 3′ flanking regions is expressed in a tissue-specific manner. Furthermore, this transgene also responds to estrogen treatment by increasing the amount of intrahepatic apoVLDL-II mRNA. The transgene mRNA was transcribed at the authentic transcription initiation sites and had the same size as the avian mRNA (10). Recently, Wijnholds et al. (30) reported that an apoVLDL-II transgene containing 5 kb of 5′ flanking sequence and 400 bp of 3′ flanking region was expressed in a tissue-specific manner and was stimulated by estrogen. Our observations corroborate and extend their findings. Wijnholds et al. (30) did not study the effects of estrogen on apoVLDL-II at the protein level or on the plasma lipids.

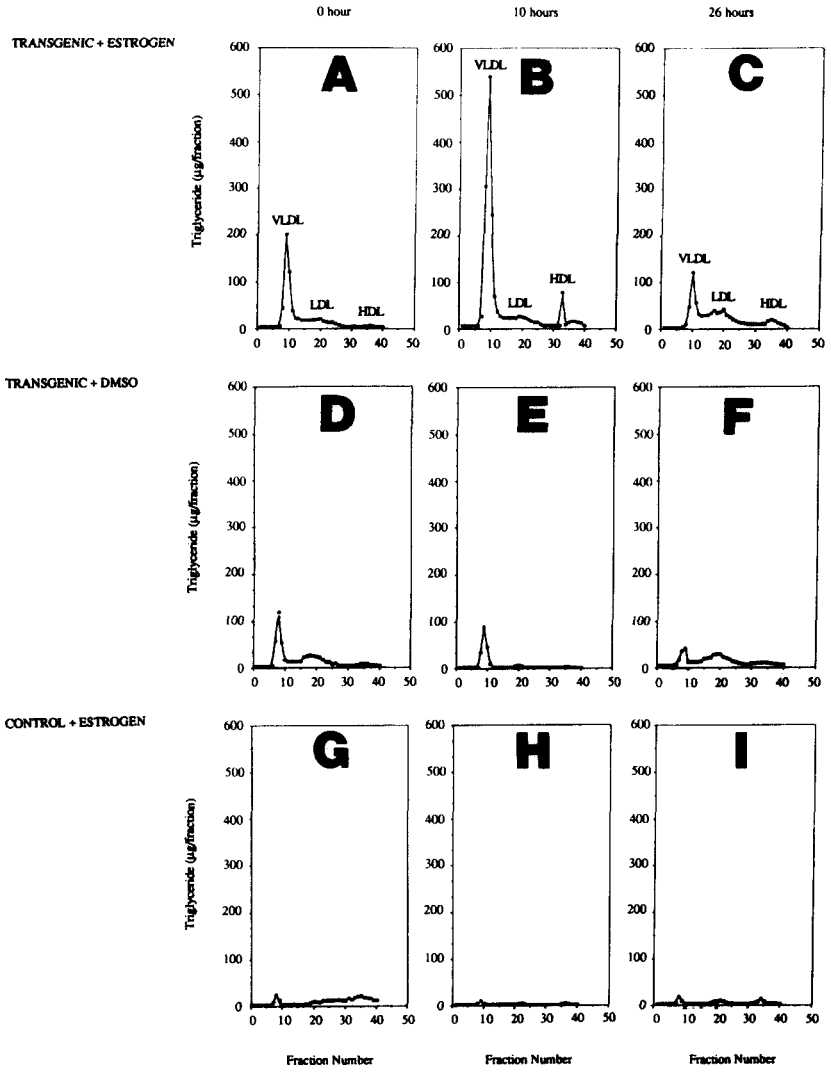

Fig. 7. Fractionation of plasma lipoproteins by FPLC. Plasma from transgenic and control mice at different time points after the injection of estrogen or DMSO was used for the fractionation of VLDL, LDL, and HDL. Mice were anesthetized by intraperitoneal injection of Avertin and 250 µl of plasma obtained from each mouse was subjected to gel filtration chromatography on an FPLC system (Beckman System Gold) with two Superose-6 columns (Pharmacia LKB, Biotechnologies, Inc.) connected in series. Fractions were eluted as reported in Materials and Methods, and 150-µl fractions were assayed for cholesterol and triglycerides by using an enzymatic assay kit from Sigma Diagnostics. Graphs represent one representative fractionation for each time point of estrogen- (panels A, B, C) or DMSO-injected (panels D, E, F) transgenic mice and estrogen-treated control animals (panels G, H, I).

The estrogenic regulation of apoVLDL-II in the cockerel is mediated at the transcriptional level by stimulation of gene transcription (2, 6, 8, 31, 32), and at the posttranscriptional level by stabilization of apoVLDL-II mRNA (2, 5). Part of the elevation in plasma VLDL could also be the result of an anti-lipolytic effect of apoVLDL-II (33). Furthermore, in the cockerel, the increased production of apoVLDL-II in the liver occurs by recruitment of hepatocytes. Using immunoperoxidase staining of histological sections, Lin et al. (29) showed that in untreated cockerels the proportion of hepatocytes that contained immunoreactive apoVLDL-II was extremely low (0.1–0.6%). Interestingly, in the rare cells with detectable immunoreactive apoVLDL-II, the staining was of moderate intensity indicating that these cells have substantial amounts of the protein. After estrogen treatment, the proportion of cells that synthesized apoVLDL-II went up to ~ 10% in 24 h. The production of apoVLDL-II in only a very minor fraction of hepatocytes in the basal state and the gradual recruit-

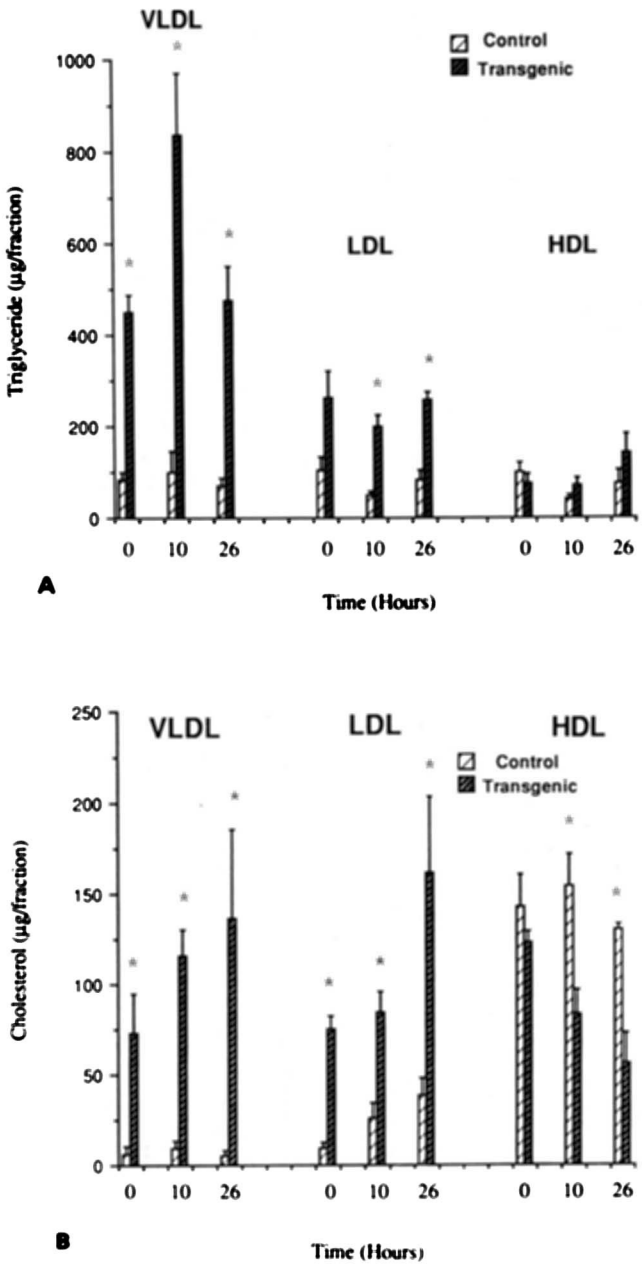

Fig. 8. Lipoprotein triglyceride and cholesterol levels in transgenic and control mice. VLDL, LDL, and HDL were separated from the plasma of transgenic and control mice as described in the legend to Fig. 7. Triglyceride (panel A) and cholesterol (panel B) concentrations are plotted versus time after estrogen injection of transgenic and control mice. Bars represent mean ± SEM, n = 4; • = $P < 0.01$.

ment of other hepatocytes to produce the protein after estrogen treatment indicates that avian hepatocytes are biochemically heterogeneous with respect to apoVLDL-II synthesis and have differential responsiveness to estrogen. In this study, we found that the interesting phenomenon of cellular recruitment appears to occur also in transgenic mice. Again, in these animals, in the rare cells that contain immunoreactive apoVLDL-II in the basal state, the degree of staining was moderately intense, indicating that these cells contain substantial amounts of apoVLDL-II while neighboring cells were entirely negative. Recruitment of hepatocytes into synthesizing this protein occurred after a single dose of estrogen. Within 24 h of hormone treatment, some 40–60% of liver cells were producing the protein. Therefore, the transgene contained sufficient information to allow apoVLDL-II to be expressed in the basal state and after estrogen treatment in a pattern very similar to that in the cockerel. These immunocytochemical studies in transgenic mice indicate that, like avian liver cells, mammalian hepatocytes are biochemically heterogeneous.

The plasma lipoprotein response of transgenic mice to estrogen treatment is very reminiscent of that in the cockerel (2, 3). In the avian species, a response was demonstrated within 10 h of estrogen treatment and the VLDL produced was greatly enriched in triglycerides. These characteristics were reproduced in the transgenic mice. The 5′ flanking region included in the transgene has been characterized by a number of laboratories (7, 8, 34). It contains putative regulatory sequences that confer tissue specificity and estrogen responsiveness to the apoVLDL-II gene but no additional coding sequences. In the transgenic mice created in this study, the only difference between them and controls is the presence of the integrated transgene for an apolipoprotein, apoVLDL-II. The fact that the plasma lipoproteins in control animals did not change after estrogen treatment indicates that in the transgenic mice, the increased synthesis of a single apolipoprotein, apoVLDL-II, by itself, is sufficient to result in an increased production of the lipoprotein particles (VLDL in this case) that normally associate with the protein. ApoVLDL-II was the first vertebrate apolipoprotein gene cloned. The generation of transgenic mice expressing this estrogen-responsive apolipoprotein gene provides an in vivo model for the steroid hormone regulation of lipoprotein metabolism. ■

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