Apolipoprotein B messenger RNA editing in rat liver: developmental and hormonal modulation is divergent from apolipoprotein A-IV gene expression despite increased hepatic lipogenesis

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Abstract Rat hepatic apolipoprotein B (apoB) mRNA editing is regulated developmentally as well as by hormonal and nutritional modulation of hepatic lipogenesis, changes previously associated with coordinate modulation of hepatic apoA-IV gene expression. We have examined the effects of dexamethasone administration on apoB mRNA editing and the expression of other apolipoprotein genes in both neonatal and adult rats. Administration of dexamethasone increased hepatic triglyceride content in neonatal rats and increased hepatic but not intestinal apoA-IV mRNA abundance. However, neither the developmental profile nor the extent of hepatic apoB mRNA editing was changed after hormone administration. Dexamethasone produced a dose-dependent increase in adult hepatic triglyceride content and a coordinate fourfold increase in hepatic but not intestinal apoA-IV mRNA abundance, and hepatic and serum apoA-IV protein concentrations. Immunocytochemical localization revealed apoA-IV to be expressed in hepatocytes around the central vein while dexamethasone treatment produced a dose-dependent appearance of fat-filled hepatocytes throughout the lobule that were immunoreactive for apoA-IV. Despite these changes in hepatic triglyceride accumulation there was no change in the extent of hepatic apoB mRNA editing at any dose of dexamethasone. The data suggest that hormonal and metabolic modulation of hepatic apoB mRNA editing may be independent of factors that modulate apoA-IV gene expression despite alterations in hepatic triglyceride content.—**Inui, Y., A. M. L. Hausman, N. Nanthakumar, S. J. Henning, and N. O. Davidson.** Apolipoprotein B messenger RNA editing in rat liver: developmental and hormonal modulation is divergent from apolipoprotein A-IV gene expression despite increased hepatic lipogenesis. *J. Lipid Res.* 1992. **33:** 1843–1856.

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Apolipoprotein B (apoB) is a large, hydrophobic protein synthesized in mammalian liver and small intestine and plays a central role in the assembly, secretion, and metabolism of both triglyceride-rich lipoproteins (chylomicrons and very low density lipoproteins) and low density lipoprotein. The two molecular forms of apoB (apoB-100 and apoB-48) are synthesized in an organ-specific manner as products of a single gene (1). ApoB-100 is synthesized in the liver, is the major structural protein of low density lipoprotein, and contains domains in the carboxyl terminus responsible for ligand binding to the low density lipoprotein receptor. ApoB-48 is synthesized in the small intestinal enterocyte, is colinear with the amino terminal half of apoB-100, and arises as a result of a novel form of posttranscriptional RNA editing (2, 3) in which codon 2153 in apoB-100 mRNA is altered from glutamine (CAA) to an in-frame translational stop codon (UAA) in apoB-48 mRNA. The same process accounts for the production of apoB-48 in the rat liver (4) which differs in this regard from humans and other mammals in that both edited and unedited apoB mRNA species are normally present and actively translated.

Recent studies have demonstrated that hepatic apoB mRNA editing in the rat is regulated developmentally during the peri- and neonatal period and in the adult animal by a variety of modulations including administration of thyroid hormone, fasting, and refeeding a high carbohydrate diet and ethinyl-estradiol treatment (4–7). The functional common denominator in these various

Abbreviations: apo, apolipoprotein; L-FABP, liver fatty acid binding protein; PIPES, 1,4-piperazine-diethanesulfonic acid; SDS, sodium dodecylsulfate; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; MMLV, Moloney murine leukemia virus; PBS, phosphate-buffered saline; EDTA, ethylene diamine tetraacetic acid, sodium salt; TLCK, N-α-tosyl-lysyl-chloromethyl ketone; PVDF, polyvinylidene difluoride; PMSF, phenylmethylsulfonyl fluoride; HRP, horseradish peroxidase; TTBS, Tris-buffered saline containing Tween-20.

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modulations has yet to be identified but the findings suggest that apoB mRNA editing is regulated in settings of altered hepatic complex lipid assembly and/or accumulation. For instance, thyroid hormone administration to hypothyroid animals was found to increase hepatic triglyceride synthesis and secretion rates, a finding consistent with the numerous reports of thyroid hormone-dependent modulation of genes involved in hepatic lipogenesis (reviewed in ref. 8). Additionally, fasting and refeeding a high carbohydrate diet for 24 or 48 h produced a 5- to 30-fold increase in hepatic triglyceride content (5). In addition to the effects on hepatic apoB mRNA editing, resulting in de novo hepatic biosynthesis of virtually only apoB-48, these maneuvers also produced increases in hepatic apoA-IV mRNA abundance and biosynthesis (5, 8). Although the function of apoA-IV is unknown, it is one of the most abundantly expressed mRNA species in the adult mammalian small intestine where it demonstrates a rapid increase in both transcript abundance and biosynthesis in response to dietary triglyceride (9, 10). Furthermore, intestinal apoA-IV mRNA abundance is maximal in the newborn rat where its developmentally modulated expression presumably reflects the onset of suckling and intestinal triglyceride synthesis and secretion (11). This appears to be an important metabolic adaptation as essentially the entire circulating plasma triglyceride pool is derived from the intestine during this period (11). As alluded to above, both intestinal and hepatic apoB mRNA editing undergo temporally distinct developmental regulation during the peri- and neonatal period in the rat (12). Specifically, hepatic apoB mRNA editing increases during the second postnatal week in close temporal association with a transient increase in hepatic triglyceride content (13, 14). Thus, the confluence of these events, in association with the alterations in triglyceride synthesis and accumulation known to occur in the neonatal rat liver, suggested the possibility that aspects of cellular triglyceride metabolism may be of importance in the regulation of both hepatic apoB mRNA editing and apoA-IV gene expression.

We have examined this possibility in studies conducted with both neonatal and adult animals treated with dexamethasone. In the neonatal rat circulating concentrations of corticosterone rise substantially between days 12 and 21 postpartum (15), a time frame similar to that reported when hepatic apoB mRNA editing begins to undergo an increase (12). These programmed alterations in circulating corticosteroid levels have been shown to be an important cue for developmental changes occurring in several organ systems during the third postnatal week (16). For example, sucrase mRNA and enzyme activity demonstrate a coordinated, precocious increase in rat small intestine after corticosteroid administration (17). In regard to the present study, previous work demonstrated that administration of dexamethasone to adult rats increases apoA-IV mRNA abundance (9) but little information is available concerning the effects of this regimen on hepatic lipid metabolism in the neonatal animal.

The results of the present investigation demonstrate that dexamethasone produces an increase in hepatic triglyceride content and apoA-IV gene expression in both neonatal and adult animals but with important differences between these age groups. However, despite the dose-dependent accumulation of hepatic triglyceride and induction of apoA-IV gene expression, hepatic apoB mRNA editing was unchanged in both neonatal and adult animals.

MATERIALS AND METHODS

Animals and treatment protocols

Neonatal rats. Timed pregnant Sprague-Dawley rats were received from Charles River Labs on gestational day 14, housed in individual cages in a temperature- and light-controlled environment (see below) and allowed free access to Purina rat chow. The birth date was designated day 0 and on the following day (day 1) litters were reduced to 9 pups per dam. Neonatal animals were allowed free access to maternal milk and were not removed from the mothers until they were killed, which was scheduled between 1100 h and 1300 h to avoid variation from circadian influences. Litters were weaned by removing the dam on day 21. From neonatal day 7, dexamethasone (Sigma, St. Louis, MO) was injected subcutaneously (0.4 mg/kg, daily for 3 or 6 days, i.e., until day 10 or 13). Dexamethasone was dissolved in absolute ethanol and diluted appropriately with 0.9% NaCl to give a final volume of 50 μl. Control animals were injected with vehicle alone. Liver and small intestine were removed from groups of animals on the day indicated, flash frozen in liquid nitrogen, and aliquots were stored in sterile containers at −80°C prior to analysis.

Adult rats. Male Sprague-Dawley rats weighing 200–250 g were purchased from Charles River Labs. Animals were housed, four per cage, in temperature- (20°C) and light- (on between 6 AM and 6 PM) controlled rooms and fed Purina rat chow ad libitum. All animals were allowed free access to water. Dexamethasone was dissolved in propylene glycol and administered subcutaneously to separate groups at one of the following doses: 0.4 mg/kg, 8 mg/kg, or 50 mg/kg daily as a single injection between 9:00 and 10:00 AM for 4 days. This range was selected to span the various dosage regimens previously used by other investigators (9, 18, 19). Control animals received subcutaneous injections of propylene glycol for 4 days. All experiments were performed 24 h after the last dose. After an overnight fast rats were weighed, anesthetized, and exsanguinated via the abdominal aorta. The liver and small intestine were removed; portions were flash-frozen in

liquid nitrogen and stored as above for subsequent RNA extraction and lipid analysis. Separate aliquots were taken for tissue fixation and immunocytochemistry as described below. Serum was collected and stored at 4°C prior to lipid analysis and small aliquots were frozen at −20°C for apoA-IV assay by ELISA (see below).

Lipid analyses

Serum cholesterol and triglyceride concentrations were determined enzymatically. Hepatic lipids were extracted after homogenization and separated by thin-layer chromatography using Silica G as previously described (5, 8). After triglyceride recovery, fatty acid methyl esters were prepared and analyzed on a Hewlett-Packard 5790A gas-liquid chromatograph interfaced with a Hewlett-Packard 3390A integrator as previously described (5). Hepatic free cholesterol levels were determined by gas-liquid chromatography using coprostanol as an internal standard and injector and detector temperatures as previously detailed (5). After saponification and extraction, total cholesterol was reassayed by gas-liquid chromatography and cholesteryl ester mass was calculated as the difference between total and free cholesterol. Protein concentration was determined colorimetrically (20).

Characterization of apolipoprotein gene expression

Total hepatic and small intestinal RNA was extracted into 5 M guanidine thiocyanate as described (5, 21). RNA (20 μg) was denatured and fractionated through 1% agarose–formaldehyde gels prior to capillary transfer to nylon membranes. Quantitation of apolipoprotein mRNA abundance was additionally determined by slot-blot analysis of serial RNA dilutions (1, 2.5, 5 μg) applied directly to nylon membranes. After fixation at 80°C for 2 h, membranes were prehybridized and subsequently hybridized and washed under conditions of high stringency as previously detailed (5, 8). cDNA probes used in these studies were labeled by random priming and include rat apoA-I (22), apoA-IV (23), L-FABP (24), apoC-III (25), apoE (26), and 18S ribosomal RNA (27). Rat apoB cDNA was a 660-base pair fragment (spanning nucleotides 6280–6940). Blots were exposed to XAR film at −70°C using intensifying screens. mRNA abundance was calculated by scanning laser densitometry (Ultroscan LX, LKB, Gaithersburg, MD) using exposures adjusted for linearity.

ApoB mRNA editing

Direct RNA primer extension was conducted as previously described (28). Samples (10 μg) of total RNA were annealed to a ^{32}P end-labeled 35-mer antisense rat apoB oligonucleotide (5' end at nucleotide 6708) and annealed overnight at 45°C in 50 mM PIPES, pH 6.4, 200 mM NaCl. After ethanol precipitation the samples were suspended in 50 mM Tris-HCl, pH 8.2, 6 mM $MgCl_2$, 10 mM dithiothreitol, 0.5 mM each dATP, dCTP, dTTP, and 0.5 mM dideoxy GTP. Extension was conducted for 90 min at 42°C using 10 units of MMLV reverse transcriptase. After ethanol precipitation, the extension products were resolved by 8% polyacrylamide-urea gel electrophoresis and subjected to autoradiography at −70°C using intensifying screens. Based on the nucleotide sequence of rat apoB cDNA, the extended products corresponded to 43 bases (CAA at nucleotide 6666) or 48 bases (UAA at nucleotide 6666, next C at nucleotide 6661). The ratio of edited to unedited apoB transcript was determined by laser densitometric scanning of the autoradiogram.

In vitro translation of rat apoA-IV mRNA

Total hepatic RNA (final concentration 500 μg/ml) was translated for 30 min at 30°C in a rabbit reticulocyte lysate translation system using [^{35}S]methionine (10). An aliquot (10 μl) of the supernatant was adjusted to 250 μl with NETTAM buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 5 mM Na_2EDTA, 1% Triton X-100, 1 mg/ml BSA, 20 mM methionine, and 0.02% NaN_3) containing 1 mM PMSF and 1 mM benzamidine. Monospecific rabbit anti-rat apoA-IV antiserum (10) was then added and immunoprecipitation was conducted at 4°C overnight. Immune complexes were collected following addition of protein A-agarose (Boehringer-Mannheim, Indianapolis, IN). Immune complexes were washed three times by resuspension in cold NETTAM buffer, 1 mM PMSF, and 1 mM benzamidine. The pellets were subjected to a final wash using 50 mM Tris-HCl, pH 7.4, 1% Triton X-100, 1 mM PMSF, 1 mM benzamidine. Washed immune complexes were dissociated using reducing sample preparation conditions and fractionated on a 10% SDS-polyacrylamide gel. Labeled protein bands were visualized by fluorography after enhancement with 1 M sodium salicylate.

Immunoblot analysis and apoA-IV ELISA

Liver samples were homogenized in PBS (pH 7.5) and protease inhibitors were added at the indicated final concentrations: PMSF (1 mM), benzamidine (1 mM), leupeptin (100 μM), EDTA (5 mM), aprotinin (450 μM), pepstatin (2 μM), TLCK (25 μM). Samples (100 μg total protein) were prepared by incubation in 2% SDS–5% 2-mercaptoethanol and heated to 100°C for 5 min. Samples were fractionated through denaturing SDS-polyacrylamide gels (10% separating, 4% stacking) at 25 mA for 1 h and 35 mA for 4 h at 4°C. The samples were subsequently transferred at 4°C to PVDF membranes (Millipore, Bedford, MA) as previously described (5). The membranes were blocked with Blotto buffer (5% nonfat dry milk, 0.01% antifoam, 0.5% Tween-20) at room temperature for 30 min and incubated with rabbit anti-rat apoA-IV antiserum (1:2500 dilution) or rabbit anti-rat apoA-I antiserum (1:2500 dilution) in Blotto

buffer overnight at 4°C. Blots were washed three times with TTBS (10 mM Tris-HCl, pH 7.5, 150 mM NaCl-0.05% Tween-20) for 5 min and incubated with secondary antibody (1:2500 dilution of donkey anti-rabbit IgG-horseradish peroxidase-linked whole antibody, Amersham, UK) for 2 h at room temperature. Enhanced chemiluminescence detection (ECL, catalog # RPN 2106 Amersham, UK) was performed according to the manufacturer's instructions. The blots were scanned by laser densitometry after exposure to XAR film for 15–20 min.

Serum apoA-IV ELISA

Rat apoA-IV was purified from pooled serum high density lipoprotein using preparative SDS-PAGE and the homogeneous protein was electroeluted, dialyzed, and dissolved in ELISA coating buffer (0.015 M sodium carbonate, 0.03 M sodium bicarbonate). The material was determined to be pure by both single dimension SDS-PAGE and Western blotting (data not shown). Fifty ng per well of the purified rat apoA-IV was applied to 96-well microtiter plates (Micro Test III, Falcon # 3912, Becton-Dickinson, Oxnard, CA). Serum samples were diluted in ELISA buffer (PBS–0.06% Triton X-100–0.05% bovine serum albumin) and 100 μl was applied to uncoated plates followed by 100 μl of anti-apoA-IV antiserum diluted 1:20,000 in ELISA buffer. The samples were incubated at room temperature for 30 min and transferred to a coated plate for an additional 30-min incubation. Plates were washed three times with PBS–0.06% Triton and incubated for 15 min with (1:1000 dilution) biotinylated goat anti-rabbit IgG. After further washes avidin–biotin–HRP complex was added (Vector Laboratories, Burlingame, CA) for a 15-min incubation followed by color development at room temperature. Absorbance was read at 492 nm and the data were analyzed using Microplate Manager interfaced with a Bio-Rad ELISA scanner (Bio-Rad, Richmond, CA). The ELISA assay for rat serum apoA-IV was linear from 20 ng/ml to 2,500 ng/ml (data not shown).

Immunocytochemical staining

Liver sections (2–3 mm cubes) were fixed in Bouins solution and embedded in paraffin. Sections (5 μm) were deparaffinized and hydrated by sequential immersion in xylenes and graded alcohols. The sections were incubated in 1% H_2O_2 in methanol for 30 min (to abolish endogenous peroxidase activity), washed in PBS, and incubated with blocking buffer (10% normal goat serum in PBS containing 0.1% bovine serum albumin, 1% Tween-20, 0.5% SDS, and 0.5% sodium deoxycholate) at room temperature for 20 min. These conditions were optimized for the detection of apoA-IV in the livers of animals containing increased cellular triglyceride. After removing excess blocking buffer, incubations were continued with rabbit anti-rat apoA-IV antiserum (1:250 dilution in blocking buffer without goat serum) for 30 min at room temperature. The sections were then washed 5 times with PBS–0.1% bovine serum albumin–0.1% Tween-20, incubated with biotinylated goat anti-rabbit IgG for 30 min at room temperature, followed by incubation with avidin-conjugated horseradish peroxidase complex as suggested by the vendor (Vector Labs, Burlingame, CA). Peroxidase activity was developed with diaminobenzidine tetrahydrochloride and the sections were lightly counterstained with hematoxylin. Pre-immune rabbit serum substituted for the primary antiserum to serve as controls. Sections were photographed at 100 × or 200 × magnification using Ektachrome 400 film with an Olympus BH 2 microscope.

Statistical methods

Both unpaired *t*-tests and one-way analysis of variance (ANOVA) were used to evaluate differences between groups as indicated in the legends to Tables.

TABLE 1. Effects of dexamethasone on body and liver weights and hepatic lipid concentrations in neonatal rats

Group	n	Body Weight	Liver Weight	Liver Wt/ Body Wt	Hepatic Lipid			Serum Lipid[a]		Serum ApoA-IV[a]
					TG	FC	EC	TG	C	
		g	*g*	*%*	*μg/mg protein*			*mg/dl*		*mg/dl*
Day 7										
Control	3	15.2 ± 0.2	0.12 ± 0.01	0.81 ± 0.07	2.7 ± 1.3	15.7 ± 1.7	1.4 ± 1.5	nd	nd	nd
Day 10										
Control	3	20.8 ± 0.7°°°	0.29 ± 0.05°°°	1.41 ± 0.26°°	3.5 ± 1.9	14.3 ± 4.0	2.2 ± 2.1	nd	nd	nd
DX	4	14.5 ± 0.8**	0.24 ± 0.02	1.65 ± 0.22	5.8 ± 1.2*	13.5 ± 1.5	3.9 ± 1.7	nd	nd	nd
Day 13										
Control	4	29.6 ± 1.4°°°	0.29 ± 0.04°°°	0.98 ± 0.10°	5.2 ± 1.4°	17.0 ± 1.0	7.3 ± 5.6	201 ± 57	159 ± 12	25.1 ± 4.2
DX	4	16.5 ± 1.0***	0.29 ± 0.03	1.76 ± 0.24***	15.1 ± 3.1***	14.2 ± 2.2	6.0 ± 1.9	953 ± 100***	355 ± 27***	27.0 ± 4.0

Values are given as means ± SD; n, number; TG, triglyceride; FC, free cholesterol; EC, esterified cholesterol; C, cholesterol; DX, dexamethasone-treated; nd, not determined.

*, **, ***, signifies $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, compared to control using unpaired *t*-tests.

°, °°, °°°, signifies $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, compared to Day 7 control.

[a]Values were determined on five control and four DX-treated animals.

RESULTS

Studies in neonatal rats

Body weights and hepatic lipid content. Dexamethasone treatment was associated with diminished overall weight gain but liver weight was indistinguishable between control and treated animals resulting in an increased liver-weight/body-weight ratio (**Table 1**). Hepatic triglyceride concentration increased over the time period examined in both groups with control animals showing a ~2-fold increase at day 13 compared to day 7. Administration of dexamethasone augmented the developmental increase in hepatic triglyceride concentration which reached ~15 μg/mg protein at day 13. By contrast, hepatic free and esterified cholesterol concentration were uninfluenced by dexamethasone treatment. Additionally, although hepatic cholesteryl ester concentrations showed a trend suggestive of a developmental increase over the period examined; this did not achieve statistical significance (Table 1).

Apolipoprotein and liver fatty acid-binding protein mRNA abundance. Analysis of hepatic RNA from groups of neonatal animals showed a 4-fold and 10-fold increase in apoA-IV mRNA abundance in the dexamethasone-treated group at days 10 and 13, respectively, findings confirmed by both slot-blot and Northern analysis (**Fig. 1A, C**). Additionally, apoC-III mRNA abundance was increased

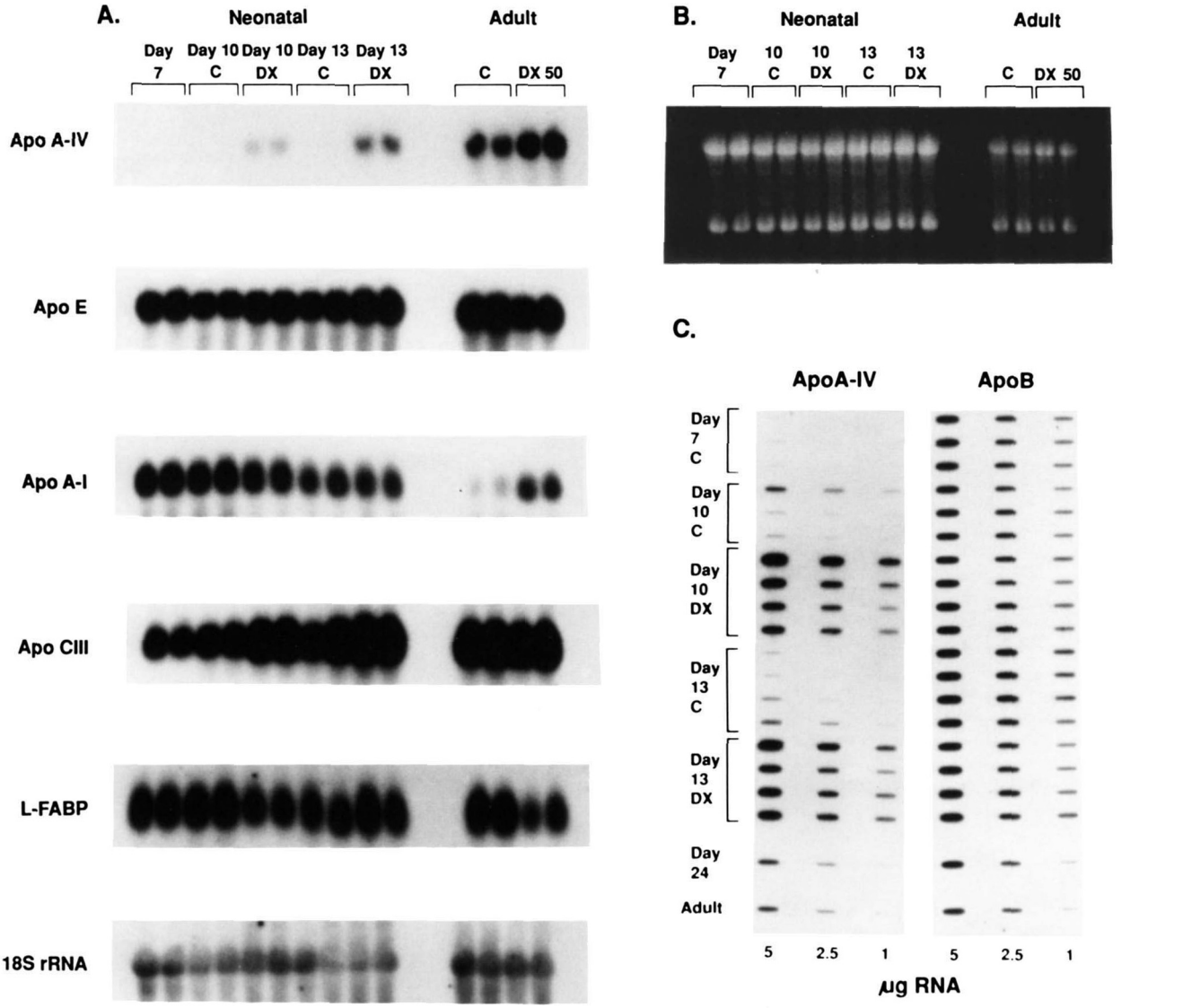


Fig. 1. Hepatic apolipoprotein and L-FABP mRNA abundance in neonatal animals treated with dexamethasone. Groups of 7-day-old neonatal animals were administered dexamethasone (0.4 mg/kg daily, DX) or vehicle (C) for 3 or 6 days and total liver RNA (20 μg) was fractionated through 1% agarose-formaldehyde gels prior to transfer to nylon membranes. A: Northern transfers sequentially probed under high stringency for apolipoprotein and L-FABP transcripts with transfer equivalence validated using 18SrRNA. B: Ethidium bromide-stained gel prior to transfer. After transfer the gel was restained, revealing no residual RNA (not shown). C: Slot-blot analysis of total RNA dilutions probed sequentially with apoA-IV and apoB cDNAs.

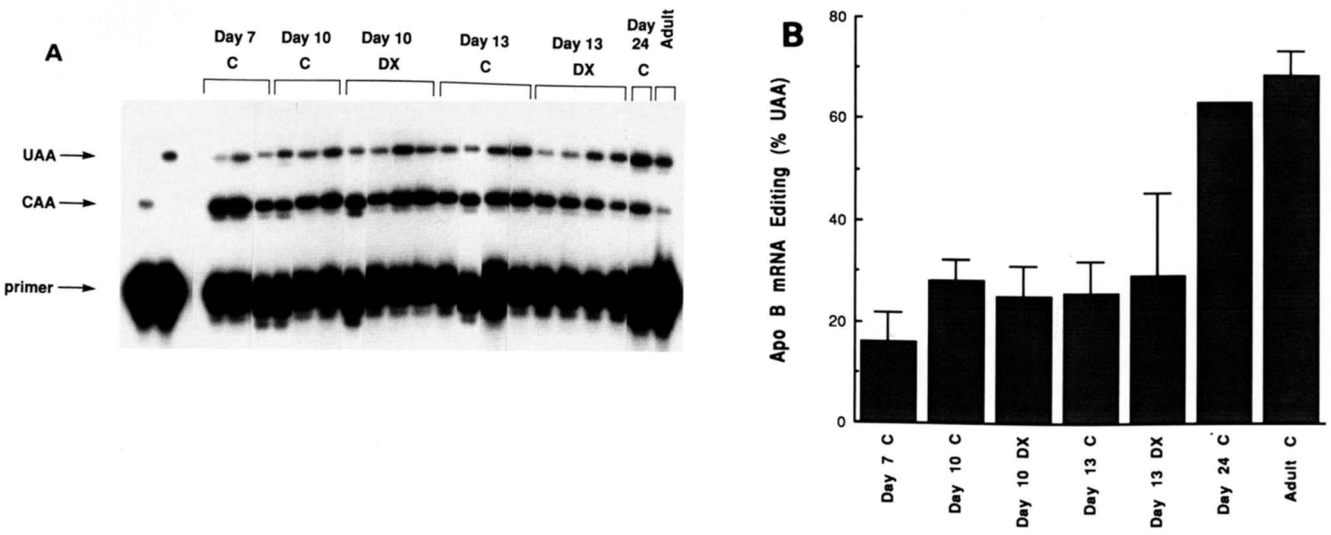


Fig. 2. A: RNA primer extension to determine the proportions of edited and unedited apoB mRNA. Aliquots of total hepatic RNA (10 μg) were annealed to an antisense primer that was reverse-transcribed using ddGTP and dA, C, T-TPs as detailed in Methods. The mobilities of the unreacted primer, unedited (CAA), and edited (UAA) apoB mRNAs are shown. B: Bar graph depicting the ratio of edited to unedited apoB mRNA species determined from the primer extension analysis shown above. The data are plotted as % UAA with the mean and standard deviation for triplicate assays shown. In all cases the standard deviation within each triplicate was less than 5%. The data from day 24 represents the mean of triplicate determinations for a single animal.

2-fold at both day 10 and 13 in the dexamethasone-treated animals (Fig. 1A). By contrast, hepatic apoB, apoE, apoA-I, and liver fatty acid binding protein mRNA abundance were unaltered by dexamethasone administration (Fig. 1A). The developmental increase in hepatic triglyceride content noted in (untreated) suckling rats was associated with an approximately 3-fold increase in hepatic apoA-IV mRNA abundance without accompanying changes in apoB mRNA abundance (Fig. 1C). Parallel analysis of small intestinal RNA demonstrated no differences attributable to dexamethasone treatment in any of the mRNA species examined (data not shown).

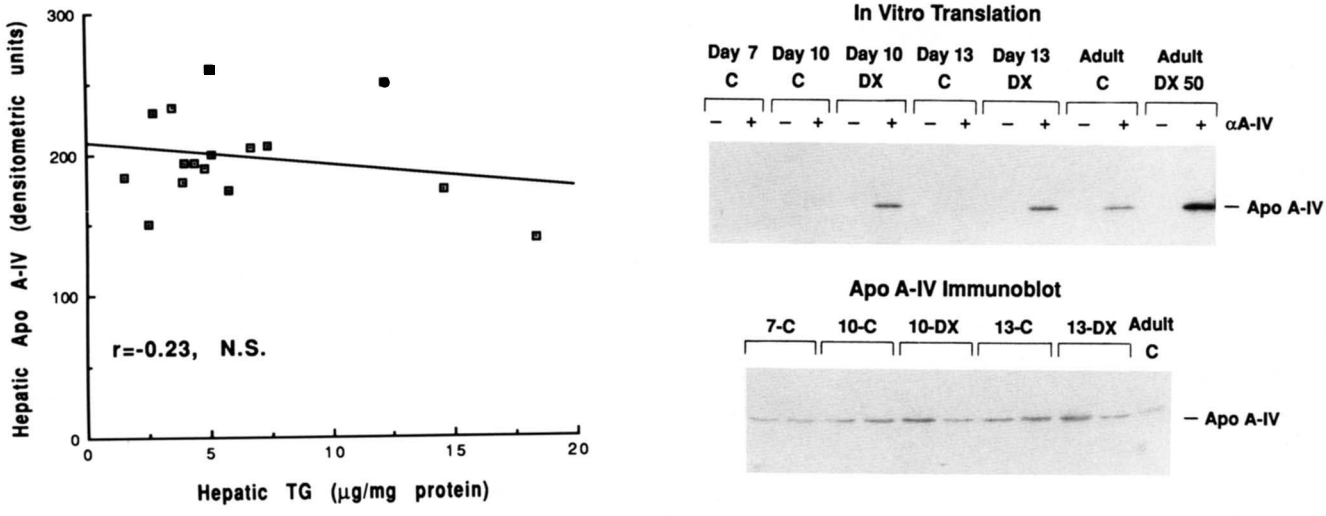


Fig. 3. Hepatic apoA-IV protein abundance is not correlated with hepatic triglyceride accumulation in neonatal animals and is unchanged despite increases in both apoA-IV mRNA abundance and translation. Left panel: Neonatal animals treated with dexamethasone or vehicle, as detailed in the legend to Fig. 1 and Methods, were killed and aliquots of the livers were taken for lipid extraction and triglyceride mass (TG) determination or protein extraction and immunoblot analysis. Lower right panel: Representative immunoblots (100 μg protein) are illustrated. Examination of the group reveals no significant correlation between hepatic TG and apoA-IV. The equation for the regression is $y = -1.627x + 208.2$. Upper right panel: Total hepatic RNAs from neonatal animals treated with dexamethasone (DX) or vehicle (C) together with adult animals that were either untreated (C) or treated with dexamethasone 50 mg/kg (DX 50) were translated in vitro with [^{35}S]methionine in a rabbit reticulocyte lysate. Parallel aliquots of the reaction were immunoprecipitated with anti-apoA-IV antiserum (+) or preimmune rabbit serum (−). The products were analyzed by denaturing SDS-PAGE and the resulting fluorogram is shown.

Developmental and hormonal regulation of hepatic apoB mRNA editing. The proportion of hepatic edited apoB mRNA (% UAA) was 16 ± 5% on neonatal day 7, increasing to 28 ± 4% on day 10, 27 ± 6% on day 13, and close to the adult level of 63% on day 24 (**Fig. 2**). These values are similar to those reported recently by other workers (12) and were no different in dexamethasone-treated neonatal rats (25 ± 6% day 10 and 29 ± 16% on day 13, Fig. 2).

Apolipoprotein A-IV gene expression and hepatic triglyceride content. In view of the marked hepatic triglyceride accumulation and increased apoA-IV mRNA abundance, the abundance of apoA-IV protein was examined semiquantitatively in the livers of these animals and the data were analyzed in comparison to hepatic triglyceride content. The results show that hepatic apoA-IV protein (and apoA-I, data not shown) is present at comparable levels in all neonatal animals and unrelated to hepatic triglyceride content (**Fig. 3**). In vitro translation of total liver RNA followed by immunoprecipitation of apoA-IV confirmed the data obtained by hybridization analysis, i.e., a 5- to 6-fold translational increase in apoA-IV in dexamethasone-treated animals at both day 10 and 13 (Fig. 3). These findings suggest that while hepatic apoA-IV mRNA abundance and in vitro translation are increased by dexamethasone treatment, hepatic apoA-IV protein abundance in the neonatal animal is regulated by mechanisms independent of these changes. Additionally, serum apoA-IV concentrations were indistinguishable in 13-day-old suckling rats treated from day 7 with either vehicle or dexamethasone injection (Table 1). Taken together, the data are consistent with findings alluded to above that the circulating lipoprotein pool in suckling rats is derived largely from the small intestine where dexamethasone treatment does not modulate apoA-IV mRNA abundance.

Studies in adult rats

Body weights, hepatic and serum lipid, and apoA-IV protein concentration. As shown in **Table 2,** dexamethasone treatment produced a dose-dependent increase in liver weight and an increase in the liver-weight/body-weight ratio. Additionally, dexamethasone produced a dose-dependent increase in both serum and hepatic triglyceride concentration, without accompanying alterations in cholesterol concentration. Serum apoA-IV concentration increased after dexamethasone treatment and, as illustrated in **Fig. 4A,** demonstrated significant correlation with hepatic triglyceride content ($r = 0.91$, $P < 0.001$). Western blotting analysis of hepatic apoA-IV protein is illustrated in Fig. 4B and shows an increase in hepatic apoA-IV protein concentration in animals treated with dexamethasone that is highly correlated with hepatic triglyceride concentration ($r = 0.93$, $P < 0.001$). Additionally, as shown in Fig. 4C, serum apoA-IV concentration was highly correlated with hepatic apoA-IV protein abundance ($r = 0.94$, $P < 0.001$). These findings stand in marked contrast with the results presented above in neonatal animals.

Apolipoprotein and liver fatty acid binding protein mRNA abundance. Hepatic apoA-IV mRNA abundance increased ~4-fold and apoA-I mRNA abundance increased ~2-fold in animals treated with dexamethasone at doses of 8 and 50 mg/kg, respectively, (**Fig. 5**). ApoC-III mRNA abundance increased less than 2-fold while apoE mRNA abundance decreased by 30% and hepatic L-FABP mRNA abundance decreased by 70% in animals treated with 8 and 50 mg/kg dexamethasone (Fig. 5). By contrast, hepatic apoB mRNA abundance was unaltered at any dose. These changes are divergent from those described above for neonatal animals, particularly in regard to L-FABP mRNA abundance which was unchanged in this setting. It should be emphasized, however, that the doses of dexamethasone used in the adult animals were substantially higher than those used in neonatal rats. In keeping with the findings in neonatal animals, however, there was no change in mRNA abundance for any of these species in small intestinal RNA (data not shown).

Hormonal regulation of hepatic apoB mRNA editing. Edited apoB mRNA (% UAA) accounted for 68 ± 5% total

TABLE 2. Effects of dexamethasone on body and liver weight, and hepatic and serum lipid concentrations of adult rats

Group	n	Body Weight	Liver Weight	Liver Wt/ Body Wt	Hepatic Lipid			Serum Lipid		Serum ApoA-IV
					TG	FC	EC	TG	C	
		g	*g*	%	*μg/mg protein*			*mg/dl*		*mg/dl*
Control	4	238 ± 7	8.8 ± 0.6	3.7 ± 0.1	1.6 ± 0.2	8.1 ± 0.9	0.3 ± 0.5	46 ± 7	111 ± 3	15.9 ± 2.3
DX, 0.4 mg/kg	4	224 ± 16	9.8 ± 1.2	4.3 ± 0.2**	2.1 ± 0.6	8.1 ± 0.9	0.4 ± 0.5	112 ± 33**	81 ± 18	18.6 ± 1.4
DX, 8 mg/kg	4	202 ± 16**	13.5 ± 1.1***	6.7 ± 0.1***	5.2 ± 1.1***	7.9 ± 1.1	0.5 ± 0.3	228 ± 16***	108 ± 11	28.5 ± 3.1***
DX, 50 mg/kg	4	197 ± 8***	14.6 ± 1.2***	7.5 ± 0.8***	7.1 ± 2.0**	9.6 ± 0.7*	1.0 ± 0.7	250 ± 63***	127 ± 12*	45.1 ± 6.1***
ANOVA		$P < 0.005$	$P < 0.005$	$P < 0.005$	$P < 0.005$	NS	NS	$P < 0.005$	NS	$P < 0.005$

Values are given as mean ± SD; n, number; TG, triglyceride; FC, free cholesterol; EC, esterified cholesterol; C, cholesterol; DX, dexamethasone; NS, not significant; ANOVA, one-way analysis of variance.
*, **, ***, signifies $P < 0.05$, $P < 0.01$, $P < 0.001$, respectively, compared to control using unpaired *t*-tests.

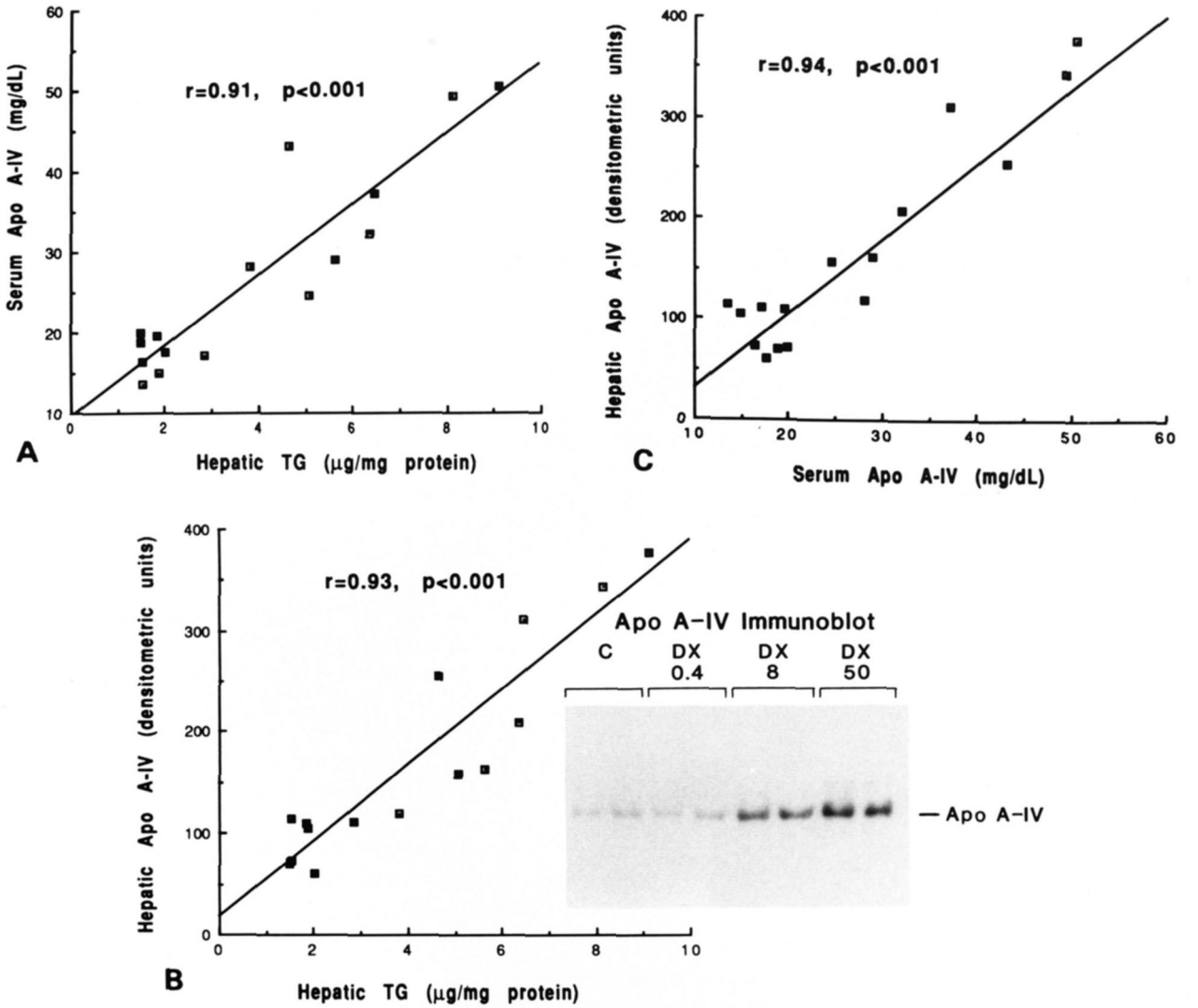


Fig. 4. Adult animals treated with dexamethasone. Hepatic triglyceride content is correlated with both serum apoA-IV concentration and hepatic apoA-IV content, and serum apoA-IV concentration is correlated with hepatic apoA-IV content. A: Serum apoA-IV was determined by competitive ELISA and compared to hepatic triglyceride content that was determined by gas–liquid chromatography after total hepatic lipid extraction. The equation for the regression is y = 4.36x + 9.59. B: Hepatic apoA-IV content was determined semiquantitatively by immunoblot analysis of hepatic proteins (100 µg protein) after denaturing SDS-PAGE and transfer to PVDF membranes. The inset shows a representative analysis. C, control animals, DX, dexamethasone-treated animals with the numerical suffix indicating the dosage used. The equation for the regression is y = 37.14x + 16.85. C: Hepatic apoA-IV content was determined semiquantitatively by immunoblot analysis of hepatic proteins (100 µg protein) as described above and compared to serum apoA-IV concentration which was determined by competitive ELISA. The equation for the regression is y = 7.89x − 47.92.

hepatic apoB mRNA in adult controls (**Fig. 6**), proportions that were unchanged after dexamethasone treatment (62 ± 3%, 69 ± 5%, and 72 ± 4% at doses of 0.4, 8, and 50 mg/kg, respectively).

Immunolocalization of apoA-IV in adult rat liver and modulation by dexamethasone. Light microscopic examination showed a progressively more severe steatosis in the livers of dexamethasone-treated rats at doses of 8 and 50 mg/kg (**Fig. 7A**). Immunocytochemical staining revealed apoA-IV protein to be localized principally within hepatocytes around the central vein (Fig. 7B, upper left panel). The distribution of immunoreactive apoA-IV in animals treated with 0.4 mg/kg dexamethasone was similar to controls but with a well-demarcated area of immunoreactive cells surrounding the central vein. Immunoreactive cells extended throughout the lobule at higher doses, with isolated mid-zonal hepatocytes displaying intense staining (arrowheads, Fig. 7B, lower left panel). Uniformly intense immunoreactivity was found, with fat-filled hepatocytes throughout the lobule, in animals treated with 50 mg/kg

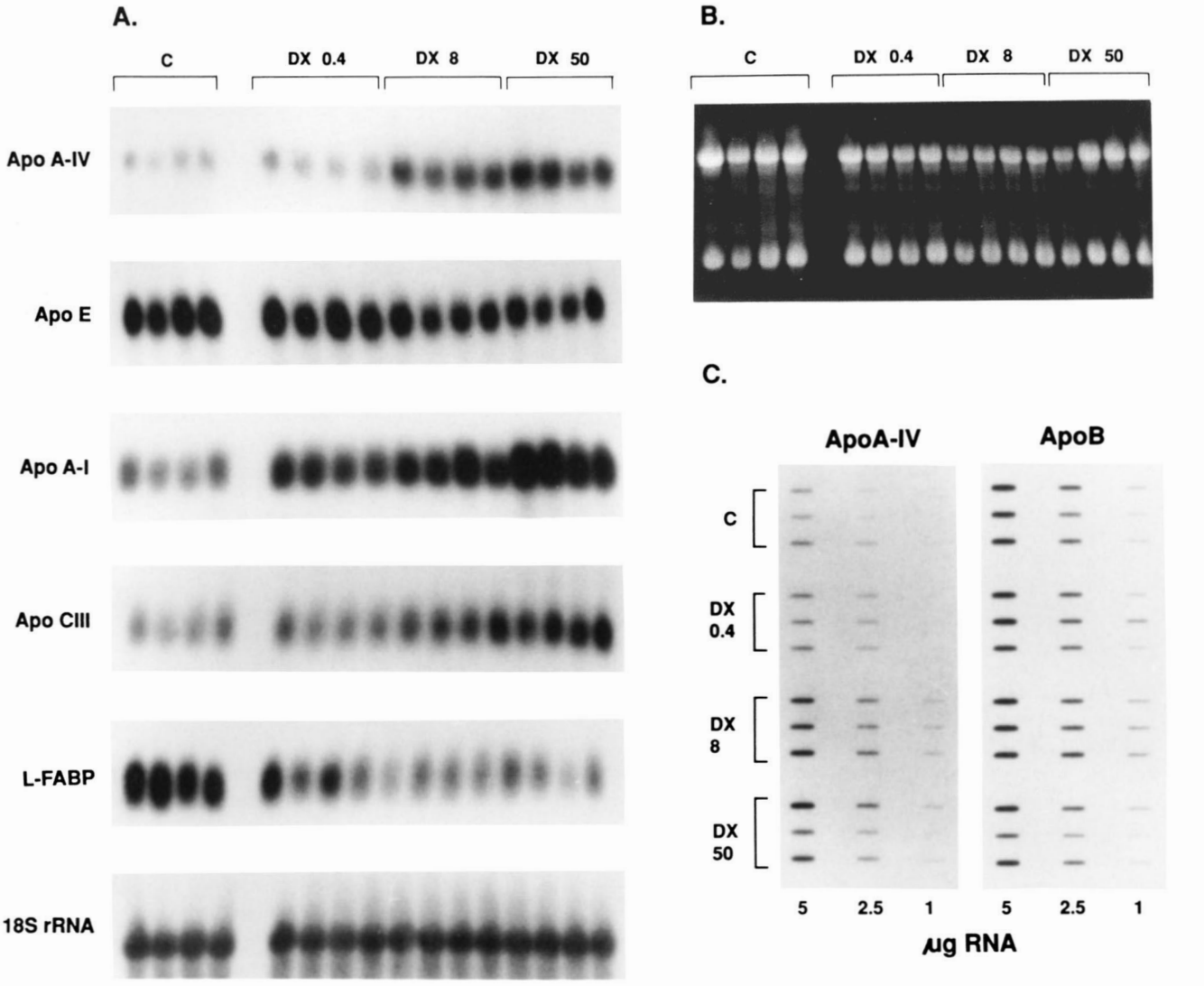


Fig. 5. Hepatic apolipoprotein and L-FABP mRNA abundance in adult animals treated with dexamethasone. Groups of animals were administered dexamethasone at increasing doses (DX, 0.4 mg/kg, 8 mg/kg or 50 mg/kg) or vehicle (C) for 4 days and total liver RNA (20 μg) was fractionated through 1% agarose-formaldehyde gels prior to transfer to nylon membranes. A: Northern transfers sequentially probed under high stringency for apolipoprotein and L-FABP transcripts with transfer equivalence validated using 18SrRNA. B: Ethidium bromide-stained gel prior to transfer. After transfer the gel was restained, revealing no residual RNA (not shown). C: Slot-blot analysis of total RNA dilutions probed sequentially with apoA-IV and apoB cDNAs.

dexamethasone. Control sections treated with non-immune rabbit serum showed no staining (data not shown).

DISCUSSION

These studies demonstrate that hepatic apoB mRNA editing responds to developmental cues in the neonatal rat but appears to be uninfluenced by dexamethasone administration both with respect to the extent of editing (i.e., % UAA) and the timing of the developmental increase. These findings were recapitulated in the adult animal where dexamethasone administration produced a dose-dependent accumulation of hepatic triglyceride, to levels more than 2-fold higher than those seen in adult animals after fasting and 24 h refeeding a high carbohydrate diet, in which context hepatic apoB mRNA editing increased from 37% to 79% (5). Taken together the data suggest that the regulation of apoB mRNA editing can be uncoupled from alterations in hepatic triglyceride metabolism.

Aspects of the studies in neonatal animals merit further discussion. A variety of changes have been demonstrated in both hepatic and intestinal apolipoprotein gene expression and hepatic triglyceride synthesis and secretion during the neonatal period (9, 11–14, 29–31). During the immediate peri- and postnatal periods there is a dramatic

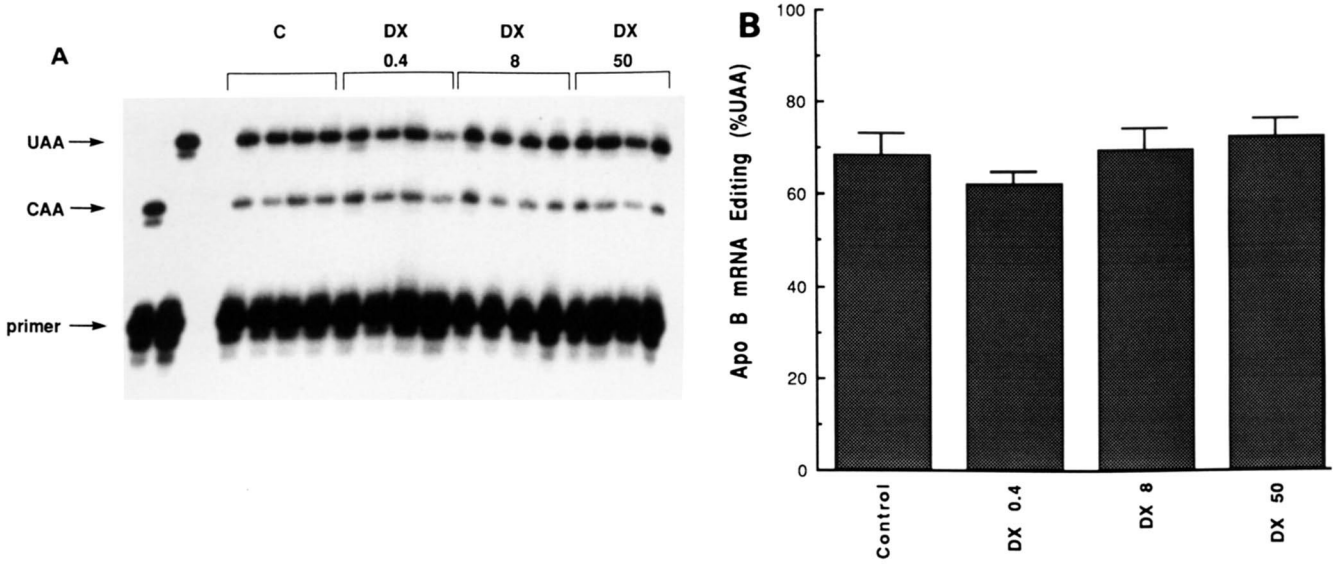
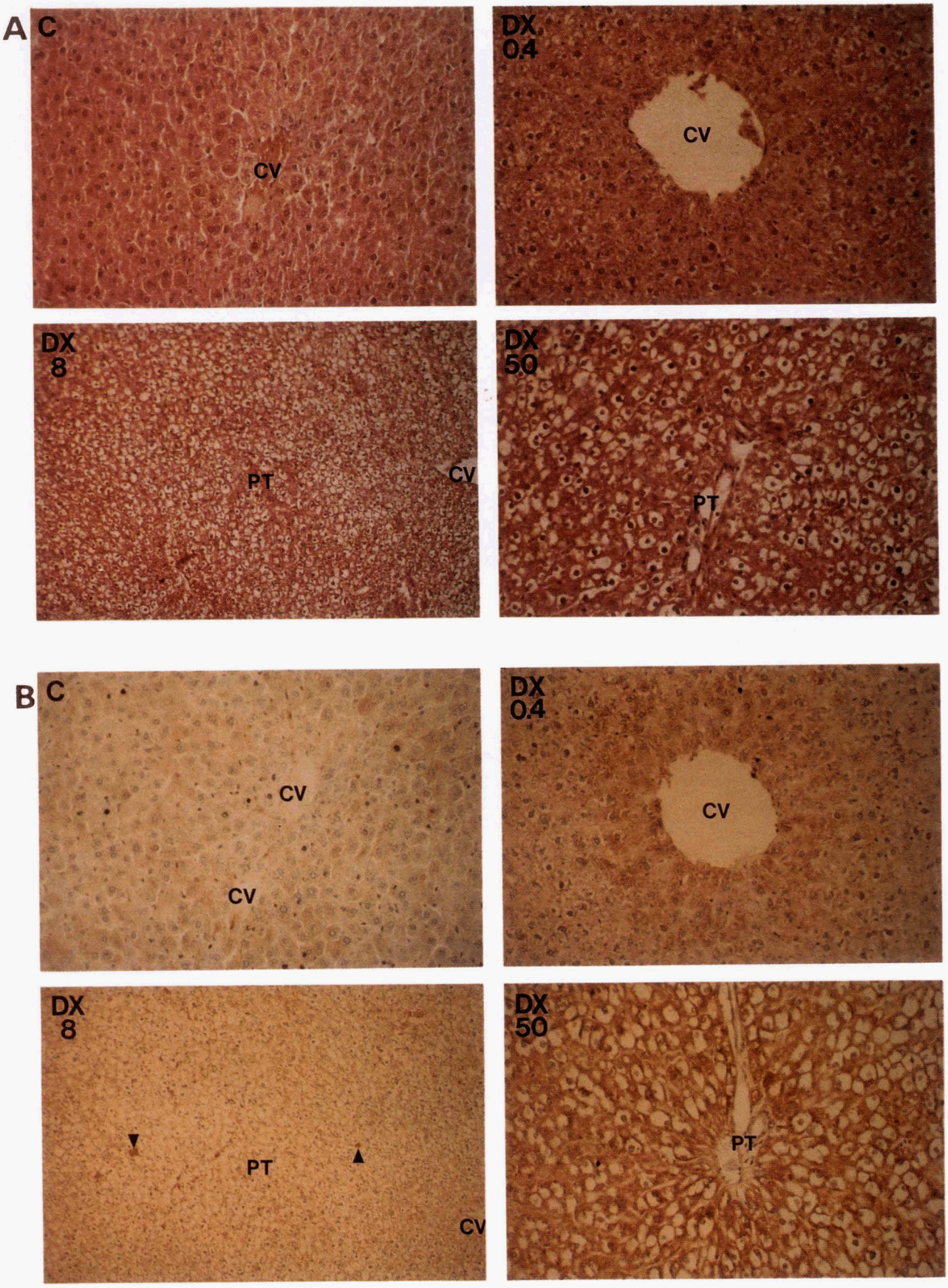


Fig. 6. A:RNA primer extension to determine the proportions of edited and unedited apoB mRNA. Aliquots of total hepatic RNA (10 μg) were annealed to an antisense primer that was reverse-transcribed using ddGTP and dA, C, T-TPs as detailed in Methods. The mobility of the unreacted primer, unedited (CAA), and edited (UAA) apoB mRNAs are shown. B: Bar graph depicting the ratio of edited to unedited apoB mRNA species determined from the primer extension analysis shown above. The data are plotted as % UAA with the mean and standard deviation for triplicate assays are shown. In all cases the standard deviation within each triplicate was less than 5%.

increase in hepatic lipogenesis with a massive, transient accumulation of triglyceride extending into the suckling period that has been shown to occur in association with a virtually complete block in the ability of the liver to secrete triglyceride-rich lipoproteins (14, 30). The molecular basis for this transient block in hepatic triglyceride secretion is unknown but is temporally coincident with a gradual increase in apoB-48 synthesis that has recently been shown to be produced by corresponding alterations in the proportion of edited apoB mRNA (12). The present studies examined the extent to which these developmentally programmed events, i.e., hepatic triglyceride accumulation and apoB mRNA editing, could be coordinately modulated by glucocorticoid administration. That dexamethasone administration fails to modulate hepatic apoB mRNA editing in the suckling animal despite further enhancement of hepatic triglyceride accumulation suggests that the development of hepatic apoB mRNA editing is genetically imprinted rather than reflecting a temporarily higher threshold for apoB-48 production driven, for example, by rising cellular triglyceride levels. These suggestions require cautious interpretation, however, as the underlying mechanism(s) leading either to triglyceride accumulation in neonatal rat hepatocytes or the augmentation of this response after dexamethasone administration have yet to be established. Other studies, however, have shown that dexamethasone treatment increases lipogenesis and augments triglyceride-rich lipoprotein secretion from adult hepatocytes (32–35).

In many regards the findings summarized above are analogous to the massive hypertriglyceridemia and hepatic triglyceride accumulation occurring transiently during the suckling period in the fld/fld mouse in which affected animals show a ~100-fold elevation of hepatic apoA-IV mRNA that temporally mirrors the pattern of hepatic triglyceride accumulation (36). Interestingly, despite this increase in hepatic apoA-IV mRNA abundance, hepatic apoA-IV protein levels varied by less than 2-fold (36). The present studies demonstrate a similar divergence of hepatic apoA-IV mRNA and protein abundance in the neonatal but not adult animal treated with dexamethasone. One possible explanation is that during the neonatal period the bulk of hepatic apoA-IV protein is of intestinal origin. The demonstration of similar values

Fig. 7. A: Hematoxylin-stained liver sections from control adult animals (C) or from animals treated with increasing doses of dexamethasone (DX), where the suffix in each case refers to the dose. CV, central vein; PT, portal triad. Note the progressive steatosis ultimately involving cells throughout the lobule. B: Immunocytochemical localization of apoA-IV. Sections from the respective animals listed above were examined using avidin-biotin immunoperoxidase staining with an anti-apoA-IV antiserum. Control animals (C) demonstrate staining principally around the central vein (CV) which is more clearly revealed in animals treated with the lowest dose of dexamethasone (DX 0.4 mg/kg). At higher doses of dexamethasone, there is progressively more widespread immunostaining which extends to the portal triad (PT). Note the occasional intensely stained hepatocyte at the 8 mg/kg dosage regimen (arrowheads). At the highest dose of dexamethasone, there is uniformly intense staining of fat-filled hepatocytes throughout the lobule.



for serum apoA-IV in both control and dexamethasone-treated 13-day-old rats would additionally support this contention. On the other hand, the coordinated regulation of hepatic apoA-IV mRNA and protein abundance in adult animals, the associated increase in serum apoA-IV concentration, and the significant correlation of both parameters with hepatic triglyceride content after dexamethasone treatment suggest that hepatic apoA-IV gene expression in the adult animal is regulated by alterations in cellular triglyceride metabolism. The concordance of findings with respect to hepatic apoA-IV mRNA abundance and serum apoA-IV levels in the adult rat lends itself to the speculation that synthetic events in the liver may be of importance in the regulation of circulating apoA-IV levels in this species. A report that circulating serum apoA-IV was correlated in fasting human subjects with serum triglyceride concentrations, in the absence of chylomicronemia, was argued by the authors to raise the possibility that hepatic synthesis of apoA-IV may be of importance in some individuals (37). Clearly, this speculation needs to be tested directly.

The function of apoA-IV in the context of cellular lipoprotein assembly and secretion is unknown. Several lines of evidence however implicate a strong association between apoA-IV gene expression and triglyceride synthesis and secretion. Small intestinal apoA-IV mRNA abundance in the neonatal rat and pig is dramatically induced coincident with the onset of suckling (9, 38). Additionally, apoA-IV mRNA abundance and synthesis are increased in the adult rat and pig small intestine after a triglyceride bolus (10, 38). Furthermore, in the adult small intestine, a tissue in which triglyceride assembly and secretion are functionally coupled to the expression of predominantly edited apoB mRNA, apoA-IV can be demonstrated to be associated with intracellular triglyceride-rich lipoprotein particles (39). The present findings that dexamethasone administration to adult animals produces hepatic triglyceride accumulation, taken together with numerous previous reports of augmented hepatic triglyceride secretion, in both the in vivo and in vitro setting (32–35), raise the possibility that apoA-IV may assume a functional role in facilitating triglyceride export particularly as apoB mRNA editing appears to be unregulated in this context. This possibility will need to be formally addressed in future studies. Whether hepatic apoA-IV gene expression and apoB mRNA editing undergo coordinate modulation in other models of altered hepatic triglyceride metabolism is currently under investigation. An additional point that bears emphasis is the uncertainty concerning the mechanism(s) underlying induction of hepatic apoA-IV mRNA abundance by either alterations in cellular triglyceride content or dexamethasone administration. For instance, the present studies do not exclude the possibility that dexamethasone produces an effect on apoA-IV gene expression independent of its effects on triglyceride accumulation.

The present studies suggest that hepatic apoA-IV is expressed predominantly in pericentral hepatocytes in the normal adult rat and that with the onset of progressive steatosis, apoA-IV becomes detectable throughout the lobule, eventually being expressed in virtually all hepatocytes. Further studies using in situ hybridization will be necessary to confirm the colocalization of apoA-IV mRNA and protein and to establish the presence of a gradient of gene expression within the liver lobule. The findings of increased hepatic apoA-IV gene expression in dexamethasone-treated adult animals contrasts with the ~3-fold reduction in mRNA abundance for liver fatty acid binding protein (L-FABP) in the same animals. L-FABP is a member of a family of related genes with a presumed function in the compartmentalization and intracellular transport of long-chain fatty acids (reviewed in ref. 40). Previous work demonstrated that L-FABP is expressed during late gestation in the rat liver and small intestine with a developmental increase at birth, greater in the gut than the liver (31). During the suckling period, however, there is little change in the abundance of hepatic L-FABP mRNA suggesting that cellular triglyceride accumulation per se is not a potent stimulus to the expression of this abundant gene. This impression would be supported by the present findings that hepatic L-FABP mRNA abundance decreased with increasing triglyceride accumulation. Studies have shown that L-FABP mRNA abundance and protein turnover may be modulated in the adult animal by clofibrate and by gender status, suggesting that this gene is responsive to changes in hepatic lipid metabolism, but the cellular and molecular basis for such interaction is still unclear (41).

These studies clearly illustrate the difficulties of attempting to resolve such complex metabolic interrelationships using a whole animal model, and work is focussed on cell lines in which apoB mRNA is edited either in response to hormonal or metabolic stimuli. Such studies will form the basis for future reports.

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