Apolipoprotein gene expression in the rabbit: abundance, size, and distribution of apolipoprotein mRNA species in different tissues

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Abstract We have used human apolipoprotein cDNAs as hybridization probes to study the relative abundance and distribution of apolipoprotein mRNAs in rabbit tissues by RNA blotting analysis. The tissues surveyed included liver, intestine, lung, pancreas, spleen, stomach, skeletal muscle, testis, heart, kidney, adrenal, aorta, and brain. We found that liver is the sole or major site of synthesis of apoA-II, apoA-IV, apoB, apoC-I, apoC-II, apoC-III, and apoE, and the intestine is a major site of synthesis of apoA-I, apoA-IV, and apoB. Minor sites of apolipoprotein mRNA synthesis were as follows: apoA-I, liver and skeletal muscle; apoA-IV, spleen and lung; apoB, kidney; apoC-II and apoC-III, intestine. ApoE mRNA was detected in all tissues surveyed with the exception of skeletal muscle. Sites with moderate apoE mRNA (10% of the liver value) were lung, brain, spleen, stomach, and testis. All rabbit mRNAs had forms with sizes comparable to their human counterparts. In addition, hybridization of hepatic and intestinal RNA with human apoA-IV and apoB probes produced a second hybridization band of approximately 2.4 and 8 kb, respectively. Similarly, hybridization of rabbit intestinal RNA with human apoC-II produced a hybridization band of 1.8 kb. The 8 kb apoB mRNA form may correspond to the apoB-48 mRNA, whereas the apoA-IV- and apoC-II-related mRNA species have not been described previously. This study provides a comprehensive survey of the sites of apolipoprotein gene expression and shows numerous differences in both the abundance and the tissue distribution of several apolipoprotein mRNAs between rabbit and human tissues. These findings and the observation of potentially new apolipoprotein mRNA species are important for our understanding of the *cis* and *trans* acting factors that confer tissue specificity as well as factors that regulate the expression of apolipoprotein genes in different mammalian species. —**Lenich, C., P. Brecher, S. Makrides, A. Chobanian, and V. I. Zannis.** Apolipoprotein gene expression in the rabbit: abundance, size, and distribution of apolipoprotein mRNA species in different tissues. *J. Lipid Res.* 1988. **29:** 755–764.

Supplementary key words rabbit apolipoprotein • apolipoprotein gene expression • rabbit lipoproteins

The study of lipoproteins and apolipoproteins in humans and animal models has been sparked by the association of high levels of plasma LDL in humans with risk for development of coronary artery disease (1, 2). The rabbit has been used extensively as an animal model to study diet-induced (3–5) or genetic hypercholesterolemia (6), atherosclerosis (6, 7), and alloxan-induced diabetes (8–11). Recently it was shown that the progression of atherosclerotic lesions in the receptor-deficient WHHL has striking similarities with human atherosclerotic lesions (7). The earlier studies were focused mainly on determinations of lipid, lipoprotein, and apolipoprotein values (3–7). During the last 5 years probes for all the human apolipoproteins and the LDL receptor became available (see review 12 and ref. 13–23). These developments opened new approaches for study of the mechanism and the stimuli that regulate lipoprotein and apolipoprotein synthesis and catabolism. Recently it was shown that treatment of rabbits with 17α-ethinyl estradiol increases both the hepatic LDL receptor binding activity and, concomitantly, the steady state LDL receptor mRNA levels leading to increased catabolism of lipoproteins (24–26). Similar investigations of steady state apolipoprotein mRNA levels can provide information of factors controlling apolipoprotein and lipoprotein synthesis. As a first step in this direction we analyzed the distribution of apolipoprotein mRNA in rabbit tissues. This analysis shows striking differences in the distribution of apolipoprotein between rabbit and human tissues and the presence of two unique species of mRNA homologous to the apoA-IV and apoC-II mRNA forms.

EXPERIMENTAL PROCEDURES

Materials

Radiolabeled nucleotides [α-^{32}P]dATP, [α-^{32}P]dCTP, and [α-^{32}P]dTTP were purchased from New England Nuclear. The Klenow fragment of DNA polymerase I and

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the M13 sequencing primer were purchased from New England Biolabs. DNase I was purchased from Sigma, and agarose and Bio-Gel P-60 were obtained from Bio-Rad. Nitrocellulose filters were obtained from Schleicher & Schuell, Inc.

Methods

Isolation of mRNA from rabbit tissues. Male New Zealand white rabbits, 2-2.5 kg, were purchased from Millbrook Farms (Amherst, MA). The animals were housed in the animal facility of Boston University Medical School and were fed Purina Rabbit Chow. Rabbits were killed by injection of an overdose of anesthetic Nembutal. Tissues were obtained within 15 min of killing and were stored at −70°C. For isolation of RNA, the tissues were homogenized to a fine powder under liquid nitrogen. The tissue powder was mixed with 6 ml of a solution of 4 M guanidinium thiocyanate, 0.025 M sodium citrate (pH 7.0), 0.5% sodium N-laurylsarcosinate, and 0.1 M β-mercaptoethanol and forced through a 22-gauge needle. The resultant homogenate was mixed with 6 ml of a solution of 5.7 M CsCl, 0.1 M EDTA, and 0.025 M sodium citrate (pH 7.0) in 12-ml tubes and centrifuged in a fixed-angle Ti50 rotor at 35,000 rpm for 18 hr at 20°C. The pellet was washed with 70% ethanol and dissolved in 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) and 1 mM EDTA (pH 7.5). The RNA was ethanol-precipitated, dissolved in double-distilled water, and stored at −70°C.

Blotting analysis of RNA. For Northern analysis (27), the mRNA was electrophoresed in a 1% agarose gel prepared in a buffer containing 20 mM 3-(N-morpholino) propanesulfonic acid (MOPS), pH 7.0, 5 mM sodium acetate, 1 mM EDTA, 0.23 M formaldehyde, and 0.5 μg/ml ethidium bromide. After electrophoresis, the RNA was blotted onto nitrocellulose filters, baked, and hybridized to the appropriate ^{32}P-labeled apolipoprotein gene probe. The filter was washed for 30 min at 55°C in 0.1% sodium dodecyl sulfate (SDS), 0.15 M sodium chloride, and 0.02 M sodium citrate, pH 7.5, and autoradiographed. Dot blotting analysis of RNA was carried out as described previously (22). The blots were hybridized with ^{32}P-labeled apolipoprotein gene probes. The relative concentration of apolipoprotein mRNAs was estimated by scanning densitometry of the autoradiograms containing the different apolipoprotein mRNA signals. For this purpose the concentration of the RNA analyzed and the exposure time of the film were chosen so that the radioactivity signal obtained was in the linear response range of the film. Alternatively, the nitrocellulose filter bands corresponding to a specific mRNA signal were used for scintillation counting. Prior to counting, the bands were incubated at 100°C for 1 hr in a buffer containing 10 mM Tris-HCl and 1 mM EDTA, pH 7.50. The latter quantification method was used only for the abundant mRNA species.

In preliminary nutritional experiments (not shown) where several animals were fed identical diets, the inter-animal variabilities for apoA-IV and apoE were 10-17%. Preparation of uniformly ^{32}P-labeled apolipoprotein DNA probes was performed as described previously (28). The single-stranded DNA templates used were as follows: *a*) a clone containing the HpaII to HpaII fragment of the human apoA-I gene (13) inserted into the PstI site of M13 mp11 vector; *b*) a clone containing the PstI to PstI insert of apoC-III clone pCIII-607 (18) inserted into the PstI site of the M13 mp11 vector; *c*) a clone containing the first 5' upstream 0.45 kilobase (kb) PstI-PstI fragment of the apoE cDNA clone pE-368 (14) inserted in the PstI site of the M13 mp11 vector; *d*) a clone containing the PstI to PstI insert of the apoC-II cDNA clone pC-II-711 inserted into the PstI site of the M13 vector (15); *e*) a clone containing the insert of the A-IV-2 clone inserted into the EcoRI site of M13 mp11 vector (20,21); and *f*) a clone containing an SstI fragment of apoB B1 clone inserted into the SstI site of M13 mp10 vector (22).

The apoA-II cDNA probe used for hybridization was a generous gift from Dr. Jan L. Breslow, Rockefeller University. The apoC-I clone was a generous gift from Dr. J. Scott of the Clinical Research Center, U.K. The apoA-I probe contained the 3' region and extended to the 5' PstI site of apoA-II cDNA clone (16). The apoC-I clone contained a 420bp PstI to PstI insert of the apoC-I cDNA clone (17). Both inserts were labeled by the Multiprime DNA labeling method (Amersham Corporation) based on the method of Feinberg and Vogelstein (29). Briefly, 2 μg of recombinant plasmid was digested with the appropriate enzymes and the cDNA insert was excised from low gel temperature agarose (Bio-Rad) and placed in a preweighed Eppendorf tube. Water was added at 3 ml/g of agarose and the mixture was boiled for 7 min followed by incubation for 10 min at 37°C. An aliquot of the solution containing 25 ng of DNA was mixed with random sequence hexanucleotides and the Klenow fragment of the DNA polymerase I in the presence of 50 uCi [α-^{32}P]dCTP (3,000 Ci/mmol) in a total volume of 50 μl as specified by the manufacturer. The reaction mixture was incubated at room temperature overnight. Following the addition of 50 μl of 10 mM Tris-HCl, 1 mM EDTA, pH 8.0, the probe was separated from unincorporated nucleotides and agarose fragments by a 20-sec centrifugation through a "spin-column" containing Sephadex G-50 Fine. The specific activity of the labeled probe under these conditions was approximately 10^8-10^9 dpm/μg of DNA. Prior to utilization the probe was boiled for 5 min and added to the hybridization mixture.

RESULTS

Distribution of apolipoprotein mRNAs in rabbit tissues

The following rabbit tissues were surveyed for the detection of apolipoprotein mRNA: liver, intestine (duode-

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num and jejunum), lung, pancreas, spleen, stomach, skeletal muscle, testis, heart, kidney, adrenal, aorta, and brain. **Fig. 1** shows autoradiograms obtained from a blotting analysis of RNA isolated from various rabbit tissues. The hybridization probes used in panels A–H were as follows: A) apoA-I, B) apoA-II, C) apoA-IV, DI and DII) apoB, E) apoC-I, F) apoC-II, G) apoC-III, and H) apoE. This analysis shows that apoA-I mRNA of approximately 0.9 kb is made in equal quantities by duodenum and jejunum. Traces of apoA-I mRNA were observed in skeletal muscle and liver (Fig. 1,A). The synthesis of apoA-I mRNA by liver was clearly demonstrated by blotting analysis of polyA + rabbit liver mRNA (panel A, lane t). Fig. 1,B shows that apoA-II mRNA of approximately 0.5 kb is made only by rabbit liver. The abundance of apoA-II mRNA is very low, and its detection was only possible using liver polyA + mRNA (panel B, lane t). Two hybridization bands of 1.4 and 2.4 kb related to apoA-IV mRNA were observed in rabbit liver and jejunum (Fig. 1,C). The 1.4-kb mRNA is comparable in size to its human counterpart. This form is found also in duodenum and spleen (10% of the hepatic value) and in trace amounts in the lung. The 2.4-kb mRNA form has not been described previously. The relative intensity of the two forms of intestinal and hepatic rabbit apoA-IV mRNA forms persisted after oligo dT purification of RNA and following successive washes at 55° and 65°C. Dot blot analysis of apoB (Fig. 1, DI) showed the presence of apoB mRNA in liver, duodenum, jejunum, and kidney. Based on total RNA concentration, the relative abundance of apoB mRNA in duodenum, jejunum, and kidney are 17, 16, and 2%, respectively, of the hepatic value. Northern blotting showed that both the intestinal and hepatic mRNAs contain two apoB forms of approximately 15 and 8 kb in length (Fig. 1, DII). Degradation of the apoB mRNA obtained from kidney did not permit detection of the two mRNA forms in this tissue. ApoC-I mRNA of approximately 0.4 kb was confined to liver. Analysis of 5 μg of intestinal polyA + mRNA failed to detect any hybridization signal in this tissue (Fig. 1,E). Two hybridization bands of apoC-II mRNA of approximately 0.5 and 1.8 kb were observed in the liver and intestine (duodenum and jejunum), respectively (Fig. 1,F). The relative abundance of the intestinal species was 21% of that of the hepatic. The 0.5-kb hepatic mRNA form is comparable in size to its human counterpart. Similarly with the apoA-IV species, the 1.8-kb apoC-II related mRNA form persisted following oligo dT purification (Fig. 1, F: lanes s–v). This suggests that the second mRNA form is not a result of cross hybridization with the 18S ribosomal RNA which electrophoresed in this region. ApoC-III mRNA of 0.5 kb was found in the liver and the intestine (Fig. 1, G). The intestinal apoC-III mRNA levels were 16% of the hepatic value. Finally, Fig. 1, H shows that apoE mRNA of approximately 1.2 kb is present in all tissues surveyed with the possible exception of skeletal muscle. Scanning densitometry showed that sites with moderate apoE mRNA synthesis (10% of the hepatic value) are lung, brain, spleen, testis, and stomach. The apoE mRNA concentration in the other tissues was estimated to be 1–3% of the hepatic value. ApoE mRNA was not detectable in skeletal muscle.

A summary of the tissue distribution and other features of the rabbit apolipoprotein mRNAs is shown in **Table 1.** The major differences in the distribution of apolipoprotein mRNAs between rabbits, humans, and rats are summarized in **Table 2.** The relative abundance of hepatic and intestinal apolipoprotein mRNAs shown in Table 2 is based on analysis of samples obtained from three to six separate animals.

DISCUSSION

The definition of the sites of apolipoprotein synthesis has been the subject of extensive investigation during the last 10 years. On the basis of protein quantification techniques the early studies suggested that the two major sites of apolipoprotein synthesis were the liver and the intestine (30). Further studies of protein synthesis by cells and tissues (31–38) and blotting analysis of RNA (28, 39–41) gave us a more comprehensive picture on the sites of apolipoprotein synthesis in primates and in the rat. The synthesis of rabbit apolipoproteins (42) and apolipoprotein mRNA (43) has received considerably less attention despite the fact that the rabbit is considered a good animal model for the study of genetic or induced hyperlipidemia and atherosclerosis (7).

This study was undertaken in an attempt to elucidate the potential sites of apolipoprotein synthesis in the rabbit as a first step towards understanding the mechanisms and stimuli which control the expression of apolipoprotein genes in vivo. This information in turn may be useful in devising means to control plasma lipoprotein and cholesterol levels in this experimental animal model.

The most striking differences in the abundance and distribution of apolipoproteins between human and rabbit tissues are in apoA-I, apoA-IV, and to a lesser extent in other apolipoproteins. Blotting analysis has shown that the human or monkey liver contains large quantities of apoA-I and apoA-II and minor quantities of apoA-IV mRNA (21, 28 and Zannis, V. I., and M. M. Hussain, unpublished results). This picture is totally reversed in the rabbit where the liver contains only traces of apoA-I and apoA-II mRNA and large quantities of apoA-IV mRNA. The observation of low apoA-I and apoA-II mRNA is compatible with previous reports of low levels of apoA-I synthesis by cell-free translation of rabbit intestinal and hepatic mRNA (42) and the reported absence of apoA-II from rabbit HDL (44). However, it is also possible that the weak radioactivity signal of apoA-II mRNA may be due to limited homology

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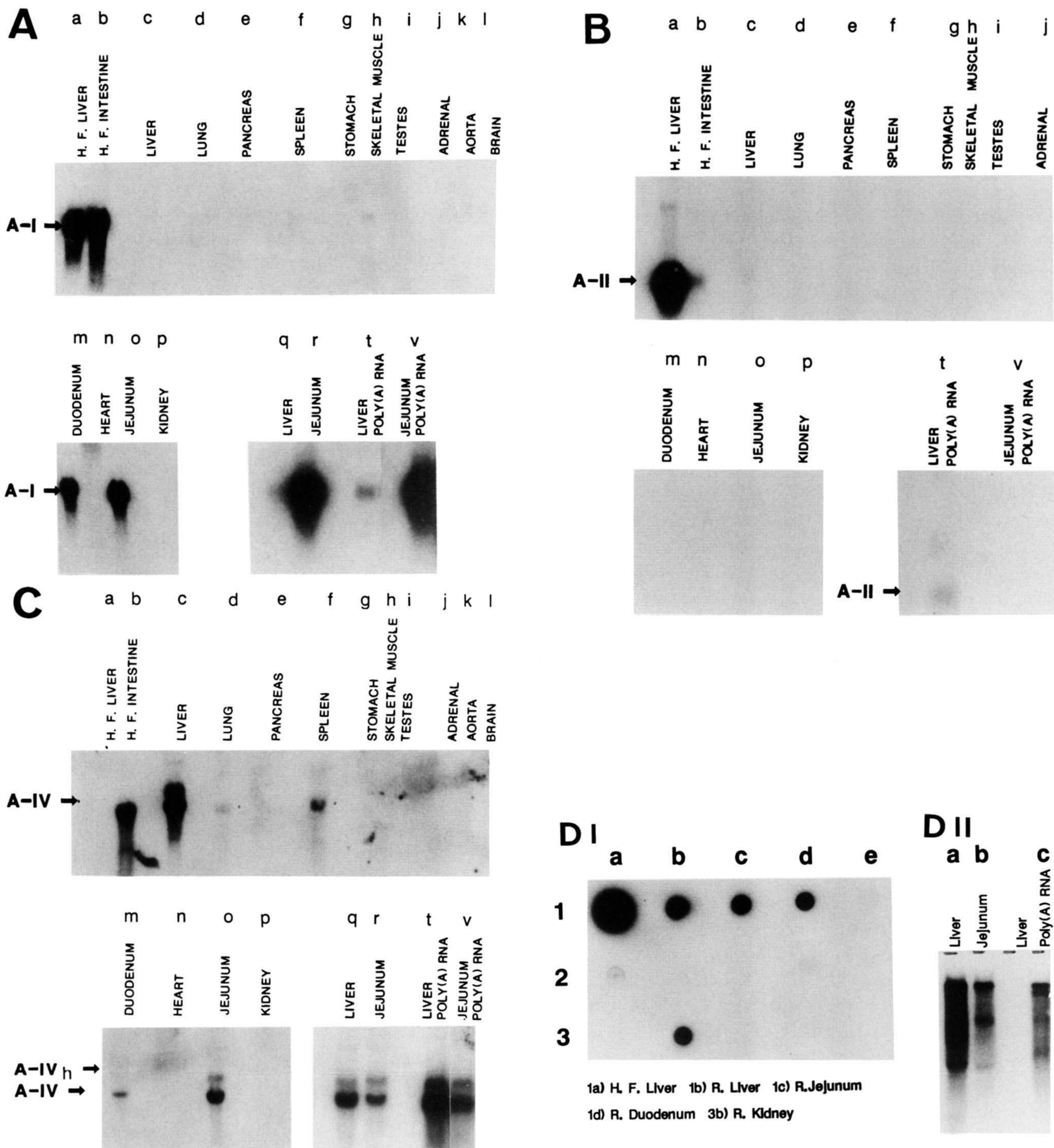

Fig. 1 Blotting analysis of RNA isolated from various rabbit and control human tissues. With the exception of panel DI, the RNA was electrophoresed on 1% agarose gels, transferred to nitrocellulose filters, and hybridized with the appropriate ^{32}P-labeled cDNA probes. The blots in panels A–H were hybridized with the following probes as described in Methods: A) apoA-I; B) apoA-II; C) apoA-IV; DI and DII) apoB; E) apoC-I; F) apoC-II; G) apoC-III; H) apoE. In panels A–C and E–H, lanes a and b contain 10 μg of RNA isolated from fetal human liver and intestine. Lanes c–p contain 30 μg of total rabbit RNA as follows: c) liver, d) lung, e) pancreas, f) spleen, g) stomach, h) skeletal muscle, i) testis, j) adrenal, k) aorta, l) brain, m) duodenum, n) heart, o) jejunum, p) kidney; and lanes q and r contain 20 μg of total rabbit RNA from liver and jejunum, respectively. Lanes s and u contain 1 μg of polyA$^+$ mRNA and t and v contain 5 μg of polyA+ mRNA isolated from rabbit liver and jejunum. The sizes of rabbit apolipoprotein mRNAs were estimated from the sizes of the corresponding human mRNAs and the 18S and 28S ribosomal RNAs. A-IVh and CIIh designate 2.4 and 1.8 mRNA species, respectively, which cross-react with the human apoA-IV and apoC-II cDNA probes, respectively. Panel DI represents a dot blotting and panel DII a Northern blotting analysis, respectively, of RNA isolated from the following tissues: row 1: a) 10 μg of human liver; all other wells contain 20 μg of the rabbit RNAs as follows: row 1: b) liver, c) jejunum, d) duodenum, e) lung; row 2: a) testis, b) pancreas, c) brain, d) spleen, e) adrenal; row 3: a) stomach, b) kidney, c) skeletal muscle, d) heart. Panel DII, lanes a–c contain the following samples: a and b: 20 μg of total RNA isolated from rabbit liver and jejunum, respectively, and lane c: 5 μg of polyA+ mRNA isolated from rabbit liver. The size of apoB mRNA was estimated using as standards the 28S and 18S ribosomal RNA and the human apoA-I, apoA-IV, and apoE mRNAs.

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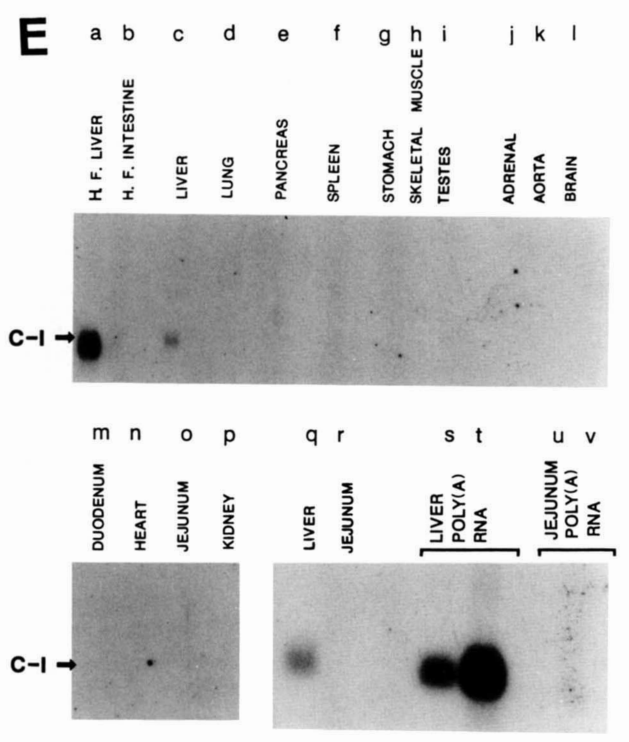

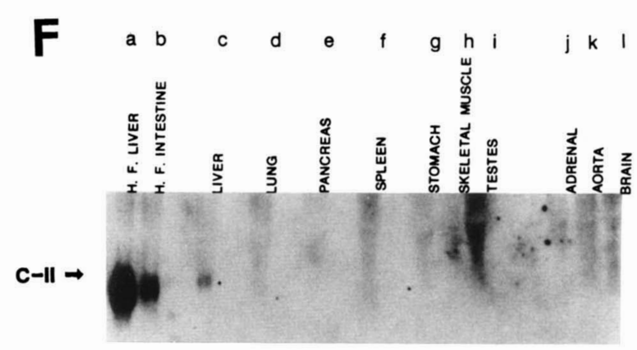

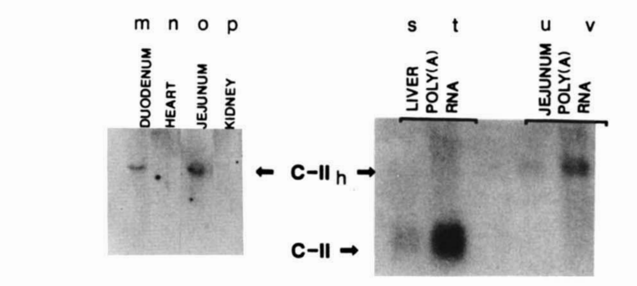

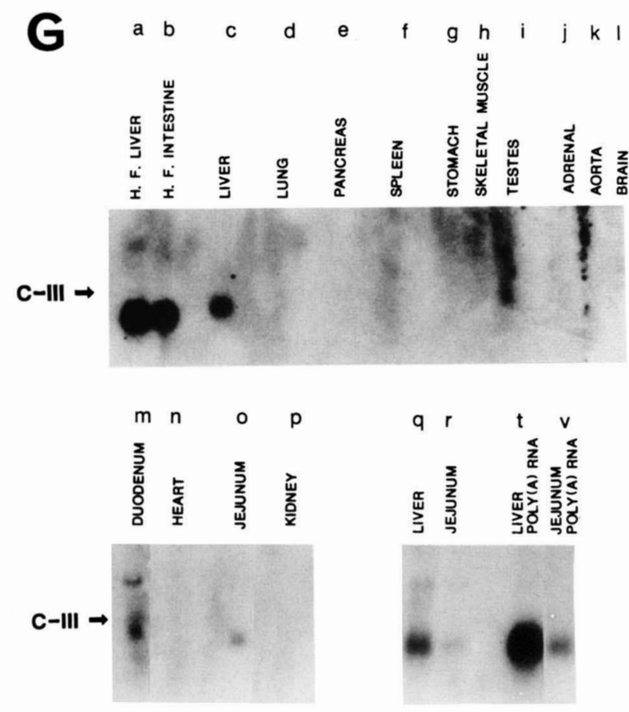

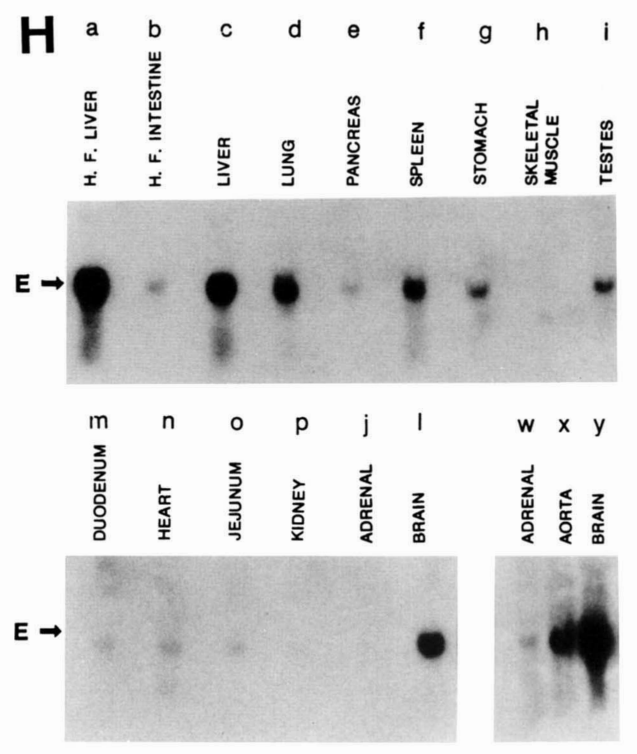

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TABLE 1. Distribution of Apolipoprotein mRNAs in rabbit tissues

Apolipoprotein	Major Site(s)	Minor Site(s)	Hybridization Bands	Size
				kb
ApoA-I	Intestine	Liver, skeletal muscle	1	0.9
ApoA-II	Liver		1	0.5
ApoA-IV	Liver, intestine	Spleen, lung	2	1.4 and 2.4
ApoB	Liver	Kidney	2	8 and 15
ApoC-I	Liver		1	0.4
ApoC-II	Liver	Intestine	2	0.5 and 1.8
ApoC-III	Liver	Intestine	1	0.5
ApoE	Liver	Several tissues	1	1.2

between the human and rabbit apoA-II mRNA. This question will be addressed in the future utilizing homologous rabbit cDNA probes.

ApoA-I and apoA-IV are activators of LCAT (45, 46). It was reported that apoA-I as a component of HDL promotes cholesterol efflux from the cells (47, 48). In this capacity, apoA-I may play an important role in the reverse transport of cholesterol, and the regulation of its synthesis may be crucial for maintenance of cellular cholesterol homeostasis (49, 50). In this context, recent studies have shown that tissue apoA-I and apoA-IV mRNA levels are controlled by hormonal factors (51) and are developmentally regulated (41, 51). The observation of only traces of apoA-I mRNA in the rabbit tissues suggests that the rabbit may offer a good animal model for the study of regulation of hepatic apoA-I gene expression in vivo since relatively small increases in mRNA levels are readily distinguishable from the control values.

The physiological significance of apoA-II remains unclear. There are conflicting reports on its role in the activation of hepatic triglyceride lipase (52, 53) and the inhibition of lecithin:cholesterol acyltransferase (45, 54). The observed very low levels of hepatic apoA-II mRNA and the reported absence of apoA-II from rabbit HDL (44) suggest that this protein either may not have an important physiological function in the rabbit or that its function(s) can be assumed by other apolipoproteins.

The importance of large levels of hepatic apoA-IV mRNA in the rabbit as compared to humans is not clear. We have observed that both hepatic and intestinal apoA-IV mRNA increase in the rabbit in response to 6% corn oil feeding (55), suggesting that apoA-IV may have a role in triglyceride transport (11).

Previous studies indicate that the liver synthesizes apoB-100 and apoB-48 whereas the intestine synthesizes only the apoB-48 form (33,37 and review 56). The present study shows that both liver and intestine contain apoB-100 mRNA and an 8-kb mRNA form that may correspond to apoB-48 mRNA. This would imply that apoB-100 and apoB-48 protein forms may be synthesized by both liver and intestine. The notion that the intestine is capable of synthesizing apoB-48 is supported by recent findings showing apoB-48 synthesis by colon carcinoma cells (Caco-2) which upon differentiation resemble intestinal epithelial cells (57). It is also possible that there may be species differences in the synthesis of apoB-48 mRNA or that synthesis of apoB-48 in the intestine is regulated at the post-transcriptional or translational level. As shown in Table 1, minor sites of apoB and apoA-I synthesis are the striated muscle and the kidney, respectively. It is interesting that the same tissues express apoA-I and apoB in avian species (32, 35) but not in primates and rats (19, 28 and Zannis, V. I., and M. M. Hussain, unpublished results). It is also interesting that apoA-IV mRNA which was found exclusively in liver and intestine in primates and rats (21, 28, and Zannis, V. I., unpublished results) is also found in the spleen and the lung of the rabbit.

The tissue specificity in the expression of apoC-I, apoC-II, and apoC-III and the relative abundance of these apolipoprotein mRNA species are similar to those reported for humans and the rat (Table 2). The only difference is the relative smaller quantities of apoC-III mRNA in the rabbit as compared to that in the human intestine (28).

A unique finding of the present study is that the rabbit intestine lacks the 0.5-kb apoC-II mRNA form seen in liver. Instead it has a second mRNA form 1.8 kb in length which hybridizes with the human apoC-II probe. Two species of mRNA (1.4 and 2.4 kb) were also observed in the rabbit liver and intestine following hybridization of the corresponding RNAs with the human apoA-IV probe. The present data do not allow us to distinguish whether the higher molecular weight forms obtained by hybridization with the human apoC-II and apoA-IV probes represent additional apoC-II and apoA-IV mRNA species coding for apoC-II and apoA-IV, respectively, or whether they represent mRNA species coding for homologous proteins. The origin of these novel mRNA species, their translatability to the corresponding proteins, and the regulation of their synthesis comprise a large undertaking and will be the subject of future investigation.

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TABLE 2. Tissue distribution and relative abundance of apolipoprotein mRNA in rabbits, rats, and humans

Tissue	Species	ApoA-I	ApoA-II	ApoA-IV	ApoB	ApoC-I	ApoC-II	ApoC-III	ApoD	ApoE
Liver	Rabbit	+	+	++++	++++	++	++	++	*	++++
	Rat[a,b,c]	++	*	+	++++	+	*	++++	*	++++
	Human[d–i]	++++	++++	-(+)[j]	++++	++++	++++	++++	++	++++
Intestine	Rabbit	++++	-	+++	+++	-	++	++	*	++
	Rat	++++	*	++++	+	*	*	++	*	-
	Human	++++	+	++++	+++	+	+++	++++	+++	++
Brain	Rabbit	-	-	-	-	-	-	-	*	+++
	Rat	±[k]	*	±	-	*	*	-	*	*
	Human	-	-	-	-	*	-	-	*	++
Spleen	Rabbit	-	-	++	-	-	-	-	*	+++
	Rat	±	*	±	-	*	*	-	*	++++
	Human	-	-	-	-	*	-	-	+++	++
Testes	Rabbit	-	-	-	-	-	-	-	*	+++
	Rat	±	*	±	*	*	*	*	*	+++
	Human	+++	*	*	-	*	-	-	*	+++
Lung	Rabbit	-	-	+	-	-	-	-	*	+++
	Rat	±	*	±	*	*	*	-	*	++++
	Human	-	-	-	-	*	-	*	*	+
Aorta	Rabbit	-	-	-	-	-	-	-	*	+++
	Rat	*	*	*	*	*	*	*	*	*
	Human	*	*	*	*	*	*	*	*	*
Kidney	Rabbit	-	-	-	++	-	-	-	*	+
	Rat	±	-	±	-	*	*	-	*	++++
	Human	++	-	-	-	*	-	-	++++	+++
Heart	Rabbit	-	-	-	-	-	-	-	*	++
	Rat	±	*	±	-	*	*	-	*	++
	Rabbit	+	-	-	-	-	-	-	*	++
Stomach	Rabbit	-	-	-	-	-	-	-	*	+++
	Rat	±	*	±	++++	*	*	*	*	*
	Human	+	-	-	-	-	-	-	*	++
Pancreas	Rabbit	-	-	-	-	-	-	-	*	++
	Rat	±	*	±	*	*	*	*	*	*
	Human	-	-	-	-	-	-	-	++++	++
Adrenal	Rabbit	-	-	-	-	-	-	-	*	+
	Rat	±	*	±	*	*	*	*	*	+++
	Human	++	-	-	-	-	-	-	++++	++++
Striated muscle	Rabbit	+	-	-	-	-	-	-	*	-
	Rat	-	*	-	*	*	*	-	*	*
	Human	-	-	-	-	-	-	*	*	+

Abbreviations indicate abundance of mRNA as follows: -, absence; +, traces (barely detectable on blotting analysis of total RNA); ++, small quantities; +++, moderate quantities; ++++, large quantities; *, data not available.

[a] Data on rat apoA-I and apoA-IV mRNA obtained from refs. 41 and 51 and on rat apoC-III from ref. 41.

[b] Indirect data on rat apoE mRNA obtained from ref. 39.

[c] Data on rat apoB mRNA obtained from ref. 19.

[d] Data on fetal human apoA-I, apoC-II, apoC-III and apoE mRNA obtained from ref. 28.

[e] The findings on human apoA-I, apoC-II and apoC-III mRNA in fetal human pancreas reported in this table differ from those of ref. 28. The new data are based on three separate analyses of different mRNA preparations and suggest that the pancreatic tissue used initially in ref. 28 might have been contaminated by intestinal tissue.

[f] Data on fetal human apoA-IV mRNA obtained from ref. 21.

[g] Data on fetal human apoB mRNA obtained from ref. 22.

[h] Data on fetal human apoA-II and apoC-I mRNA (Zannis, V. I., and M. M. Hussain, unpublished results).

[i] Data on human apoD mRNA obtained from ref. 23.

[j] The designation on -(+) indicates absence in fetal and presence in adult human liver.

[k] The designation ± indicates disagreement between references 41 and 51 on the minor sites of synthesis of rat apoA-I and apoA-IV mRNA.

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Protein and mRNA analyses have suggested that, although the liver is the major site of apoE synthesis, all other tissues tested are capable of synthesizing apoE or apoE mRNA (28, 40). Quantitative studies in nonhuman primates suggest that peripheral tissues may contribute 20-40% of the total plasma apoE pool (40).

The distribution of apoE mRNA in rabbit and human tissues has only quantitative differences. Thus, lung, which is a major site of apoE mRNA synthesis in the rabbit, is a minor site in humans. The opposite pertains to the kidney which is a major site of apoE mRNA synthesis in humans but only a minor site of synthesis in rabbits. ApoE is an ubiquitous protein which is synthesized by a variety of tissues (39, 40) including the cells of the central and peripheral nervous system (38). It has been observed that patients with familial apoE deficiency who cannot synthesize apoE do not have adverse clinical symptoms other than the lipid and lipoprotein abnormalities and premature cardiovascular disease (58, 59). This suggests that the major function of apoE is the clearance of triglyceride-rich lipoproteins (58). ApoE may also play an important role in the redistribution of the cholesterol among tissues by its participation in the reverse transport of cholesterol (60). The latter function may account for the ubiquitous nature of this apolipoprotein.

The isolation of cDNA probes for rabbit apolipoproteins in the future will allow detailed studies of the mechanisms and stimuli which control apolipoprotein gene expression in vivo.

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