Hepatic expression of genes regulating lipid metabolism in rabbits

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Abstract The liver plays a central role in lipid metabolism and plasma lipoprotein homeostasis. This dynamic process is regulated by a variety of liver-derived proteins. However, the specific liver cells that express these proteins are largely unknown. In the current study we measured mRNA levels for 13 genes encoding proteins involved in lipid metabolism in isolated rabbit hepatic parenchymal and nonparenchymal cells. For these analyses we cloned partial rabbit cDNAs for apolipoprotein A-I (apoA-I), apolipoprotein B (apoB), apolipoprotein E (apoE), cholesteryl ester transfer protein (CETP), hepatic lipase (HL), lipoprotein lipase (LPL), HMG-CoA reductase, LDL-receptor, 7α-hydroxylase, albumin, bile salt-dependent cholesteryl ester hydrolase (CEH), lecithin:cholesterol acyl transferase (LCAT), and plasminogen activator inhibitor protein-1 (PAI-1). The cDNAs provided the basis for developing quantitative RNAse protection assays for each mRNA. These assays were used to determine whether differential patterns of mRNA expression existed between liver and other tissues and between hepatic parenchymal and nonparenchymal cells. The data demonstrate a diverse range in tissue distribution and mRNA abundance. Liver expressed all mRNAs except for LPL and CEH. Messenger RNA levels in isolated liver cell populations normalized to total RNA revealed a cell segregation pattern for hepatic gene expression: parenchymal cells showed higher levels of apoA-I, apoB, apoE, albumin, LCAT, HL, and 7α-hydroxylase mRNAs compared to nonparenchymal cells while nonparenchymal cells showed higher levels of CETP, LDL-receptor, HMG-CoA reductase, and PAI-1 mRNAs compared to parenchymal cells. These data demonstrate the existence of differential mRNA expression patterns in rabbit liver cell populations for genes encoding proteins affecting lipid metabolism.—**Rea, T. J., R. B. DeMattos, and M. E. Pape.** Hepatic expression of genes regulating lipid metabolism in rabbits. *J. Lipid Res.* 1993. **34:** 1901–1910.

Supplementary key words organ-specific gene expression • mRNA quantitation • RNAse protection • tissue distribution

The liver is a key organ in controlling systemic lipid metabolism. Its functions include lipoprotein clearance, intracellular lipid metabolism (i.e., β oxidation, bile acid synthesis), synthesis and secretion of lipoproteins, and remodeling of circulating lipoproteins (1). In many instances these hepatic functions are altered in response to nutritional, hormonal, or pharmacological perturbations (1). Although many studies have focused on characterizing these hepatic functions, relatively few have specifically looked at the role of hepatic nonparenchymal cells (Kupffer, endothelial, and lipocytes) in these processes despite these cells comprising 35% of liver cell number (2). Most studies have focused on either whole liver or only parenchymal cells.

Several studies suggest that hepatic nonparenchymal cells play a large part in the extra- and intracellular metabolism of lipids (3–11). For example, hepatic Kupffer and endothelial cells account for about 30% of the uptake of ^{125}I-labeled tyramine cellobiose LDL in the cholesterol-fed rabbit (3); these same cells can account for 35% of chylomicron remnant clearance (4). Lipocytes (fat-storing cells, Ito cells) serve as the major storage site for retinol in the form of retinyl esters; these cells also contain varying amounts of cholesteryl esters, triglyerides, free cholesterol, and fatty acids (12). In addition to these functions, hepatic nonparenchymal cells have been shown to express scavenger receptors (5), synthesize apolipoproteins (13), and to be the predominant source of cholesteryl ester transfer protein (CETP) mRNA in nonhuman primates (14). Nonetheless, comprehensive data on the expression of lipid-regulating genes in parenchymal and nonparenchymal cells do not exist. Furthermore, there is no study comparing mRNA levels of these genes in hepatic cell types to those levels in other tissues.

Given the importance of hepatic parenchymal and nonparenchymal cells in lipid metabolism and the widespread use of rabbits to study these processes (15), we established two aims for the present study: *1)* clone partial rabbit cDNAs for 13 genes involved in lipid metabolism and develop mRNA quantitation assays for each gene; and *2)* use the assays to survey mRNA abundance in numerous rabbit tissues and hepatic cell populations.

Abbreviations: LDL, low density lipoprotein; CETP, cholesteryl ester transfer protein; PCR, polymerase chain reaction; HL, hepatic lipase; LPL, lipoprotein lipase; CEH, cholesteryl ester hydrolase; LCAT, lecithin:cholesterol acyltransferase; HMG, 3-hydroxy-3-methylglutaryl; PAI-1, plasminogen activator inhibitor protein-1.

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MATERIALS AND METHODS

Cloning of cDNAs

We first developed the molecular probes needed to quantitate mRNA levels for genes of interest. The strategy involved *1)* synthesis of first strand cDNA from various RNA templates and *2)* amplification of partial double-stranded cDNAs by the polymerase chain reaction (PCR) (16). RNA templates and the first strand primer varied depending on the target sequence and data from initial PCRs; those parameters are listed in **Table 1.** To amplify double-stranded cDNA for each target, we required PCR primers of sufficient homology to the targeted rabbit sequences. In some cases primers were based on published rabbit sequences; these sequences included CETP, apoA-I, apoE, and HL. For all other sequences we designed "conserved" PCR primers, i.e., primers based on short regions of high homology in other species. To find those regions we aligned all known cDNA sequences of a given gene from various species using the algorithm of Myers and Miller ((17); NALIGN program in PCGene, Intelligenetics, Mountain View, CA) and subsequently designed primers based on high nucleotide homology regions. Synthesized primers contained not only appropriate cDNA sequences but additional sequences to allow directional cloning into EcoRI/EcoRV-digested pBluescript II SK (+); thus, the 5' primer contained sequences for generating an EcoRI site while the 3' primer was designed to give a flush end. Using these primers and first strand cDNA, we tested numerous PCR amplification protocols in order to identify conditions that permitted synthesis of a band of predicted size as assessed by agarose gel electrophoresis; established conditions for the Perkin-Elmer GeneAmp PCR System 9600 are listed in Table 1. After purification of the putative cDNA of interest by gel electrophoresis, it was blunt-ended with T4 DNA polymerase, digested with EcoRI, and directionally ligated into EcoRI/EcoRV-digested pBluescript II SK (+) using standard recombinant DNA techniques. *E. coli* XL-1 Blue (Stratagene, La Jolla, CA) were transformed with the ligation products as suggested by the manufacturer. Initial clone selection was based on insert size and limited sequence data. Those clones of interest were further characterized by sequencing both strands (greater than 80%) of the cDNA insert. These data were then used in homology searches with the Pearson-Lipman algorithm ((18); FastA in the GCG DNA Analysis package, Genetics Computer Group, Inc., Madison, WI). In each instance the highest score was for the gene of interest and there was greater than 80% nucleic acid sequence homology between the rabbit clone and database sequences of other species. Each of the clones derived from the "conserved" PCR primers represents a novel molecular probe for analyzing rabbit gene expression.

Total RNA isolation and mRNA quantitation assays

Total RNA was isolated and mRNA levels were measured as described in detail elsewhere (19, 20). The only variation from the internal standard/RNAse protection assay described (19) was to decrease the amount of probe from 1×10^6 to 0.2×10^6 cpm per hybridization and use the Molecular Dynamics 400E Phosphorimager (Molecular Dynamics, Sunnyvale, CA) for image analysis and band quantitation. In some internal standard/RNAse protection assays, protected probe fragments migrated as multiple bands upon electrophoretic separation on polyacrylamide (e.g., LPL, HL, albumin). We have observed this phenomena previously; it is most likely due to secondary structure of the RNA fragments, which could be related to resistance of the probe to RNAse activity (19).

For the tissue distribution studies, total RNA from two chow-fed New Zealand White rabbits were pooled and served as the source of RNA for mRNA quantitation; pancreas and omental fat were from a single animal. mRNA levels in liver cell populations (see below) as shown in Fig. 2 were from a single animal. Measurements of apoA-I, apoB, and CETP mRNAs in isolated cell populations from three rabbits were nearly identical indicating the reproducibility of the system (data not shown).

Isolation of hepatic cell types from rabbit liver

Male New Zealand white rabbits (2–3 kg; Kuiper) were maintained on a chow diet (ad libitum) with free access to water. Animals were anesthetized with 15 mg/kg of xylazine i.m. (Rompun; Mobay) followed by 50 mg/kg ketamine HCl i.m. (Vetalar; Aveco). To minimize thrombosis, heparin (800 U/kg; Elkins-Sinn) was injected into an ear vein just prior to surgery. A liver sample for RNA isolation was obtained during surgery by ligating the caudal lobe, cutting off a small tissue sample, and immediately freezing the sample in liquid nitrogen. The liver was then surgically removed from the animal and placed in a pre-warmed stainless-steel incubation pan for ex vivo perfusion. The basic two-step method of Seglan was used as follows (21). The liver was cleared by retrograde perfusion through the inferior vena cava with pre-perfusion buffer (149 mM NaCl, 10 mM HEPES, 16.6 mM fructose, 0.5 mM EGTA, 0.0003% phenol red, pH 7.5) for 10 min. After the preperfusion, digestion buffer (6.7 mM KCl, 142 mM NaCl, 10 mM HEPES, 5 mM $CaCl_2$, 16.6 mM fructose, 0.0003% phenol red, 0.2% bovine serum albumin, 160 units/ml trypsin inhibitor, 12.5 U/ml collagenase (Worthington Biochemical Corp., Freehold, NJ), pH 7.5) was perfused through the liver in a non-recirculating fashion for an additional 20 min. At this time the liver was transferred to a fresh dish, the gall bladder was removed, and liver cells were released by cutting the capsule and manually shaking the organ. Cells were passed consecu-

TABLE 1. PCR conditions and characteristics of partial cDNAs

Gene	Total RNA Source	1st Strand cDNA Primer	PCR Primers[a]	PCR Protocol (number of cycles)	Approx. Probe Length	Approx. IS Protected Size	Approx. A Protected Size
CETP	Liver	Downstream primer	5' ATGGAATTCACACCATCTCCAACATCATGGC 3' 5' TTCTGGCAGGAGATCTTGGGC 3'	94°C–15 s/60°C–15 s/ 72°C–15 s (35)	540 b	510 b	445 b
ApoA-I	Liver	Oligo dT	5' ATGGAATTCGGCATTTCTGGCAGCGAGATG 3' 5' CCTGCAGATCCTTGTTCATCTC 3'	94°C–15 s/60°C–15 s/ 72°C–15 s (35)	385 b	350 b	290 b
ApoB	Liver	Downstream primer	5' ATGGAATTCAAATTGGCGTGGAGCTTACTGG 3' 5' TTGCTGTGGGAGTTTTCCCAG 3'	94°C–15 s/60°C–15 s/ 72°C–15 s (35)	300 b[b]	265 b	200 b
ApoE	Liver	Downstream primer	5' ATGGAATTCTTCTGGGATTACCTGCGCTGG 3' 5' TTGCGCAGCTTGCGCAGGTG 3'	94°C–15 s/72°C–30 s (35); 1% DMSO	435 b	400 b	340 b
LCAT	Liver	Downstream primer	5' ATGGAATTCGCTTTGGCAAGACCTACTCTG 3' 5' CCTGGTTGTCACCTGAGGCC 3'	94°C–15 s/60°C–30 s (35)	480 b	450 b	385 b
Albumin	Liver	Downstream primer	5' ATGGAATTCGAGAAGGTCACCAAGTGCTG 3' 5' TCTCAGGGTAGCCTGAGATG 3'	94°C–15 s/37°C–15 s/ 72°C–60 s (35)[c]	475 b	440 b	375 b
HMG-CoA reductase	Liver	Oligo dT	5' ATGGAATTCCATGGCTGGGAGCATAGGAG 3' 5' TCCTTGAACACCTAGCATCTGC 3'	94°C–15 s/60°C–30 s (35)	350 b	320 b	250 b
LDL-receptor	Liver	Downstream primer	5' ATGGAATTCAAGCCCAGGGCCATCGTGGTG 3' 5' CACTGAAGATGGCTTCGTTGATG 3'	94°C–15 s/37°C–15 s 72°C–60 s (35)	420 b	385 b	340 b
7α-hydroxylase	Liver	Downstream primer	5' ATGGAATTCCATCTCAAGCAAACACCATTCC 3' 5' TGCATTAACTGTGGATAAAGAGC 3'	94°C–60°C–15 s 72°C–15 s (35)	425 b	395 b	330 b
Hepatic lipase	Liver	Downstream primer	5' ATGGAATTCTCCAGCCTGGCTGCCACTTC 3' 5' TTGAAGGGGGACTGGGCTCG 3'	94°C–15 s/60°C–30 s (35)	400 b	365 b	300 b
Lipoprotein lipase	Fat	Downstream primer	5' ATGGAATTCTGGACCAACTAGTGAAGTGCTC 3' 5' GGAACACTTTGTAGGGCATCTG 3'	94°C–15 s/60°C–30 s (35)	340 b	310 b	245 b
CEH	Pancreas	Downstream primer	5' ATGGAATTCATGGAGGTGCCTTCCTCATGG 3' 5' GCCGGATGAGGCCCTTGTTG 3'	94°C–15 s/60°C–30 s (35)	430 b	395 b	330 b
PAI-1	Liver	Downstream primer	5' ATGGAATTCCCGTGGAACAAGAATGAGATCAG 3' 5' TGAGCCATCATGGGCACAGAG 3'	94°C–15 s/50°C–30 s (35)[d]	455 b	425 b	360 b

[a]Upper sequence is the 5' primer; lower sequence is the 3' primer.
[b]The original amplification product was approximately 355 bp but digestion with Eco RI (used to prepare product for directional cloning) produced two smaller fragments one of which was subsequently cloned and used to generate the apoB probe.
[c]Amplification product eluted from agarose gel and reamplified under the same PCR conditions.
[d]Amplification product eluted from agarose gel and reamplified under the following conditions: 94°C–15 s/72°C–15 s (35).

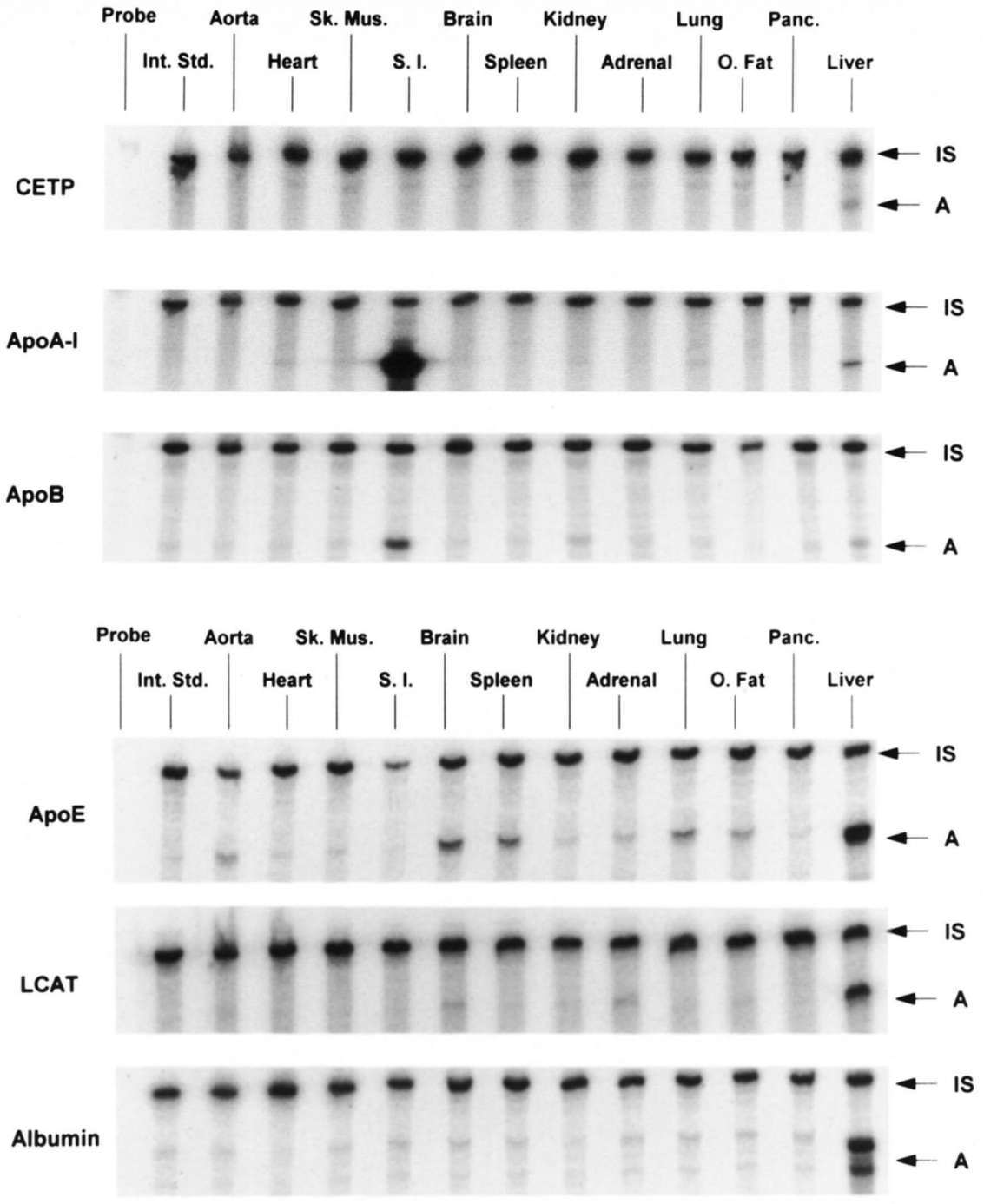
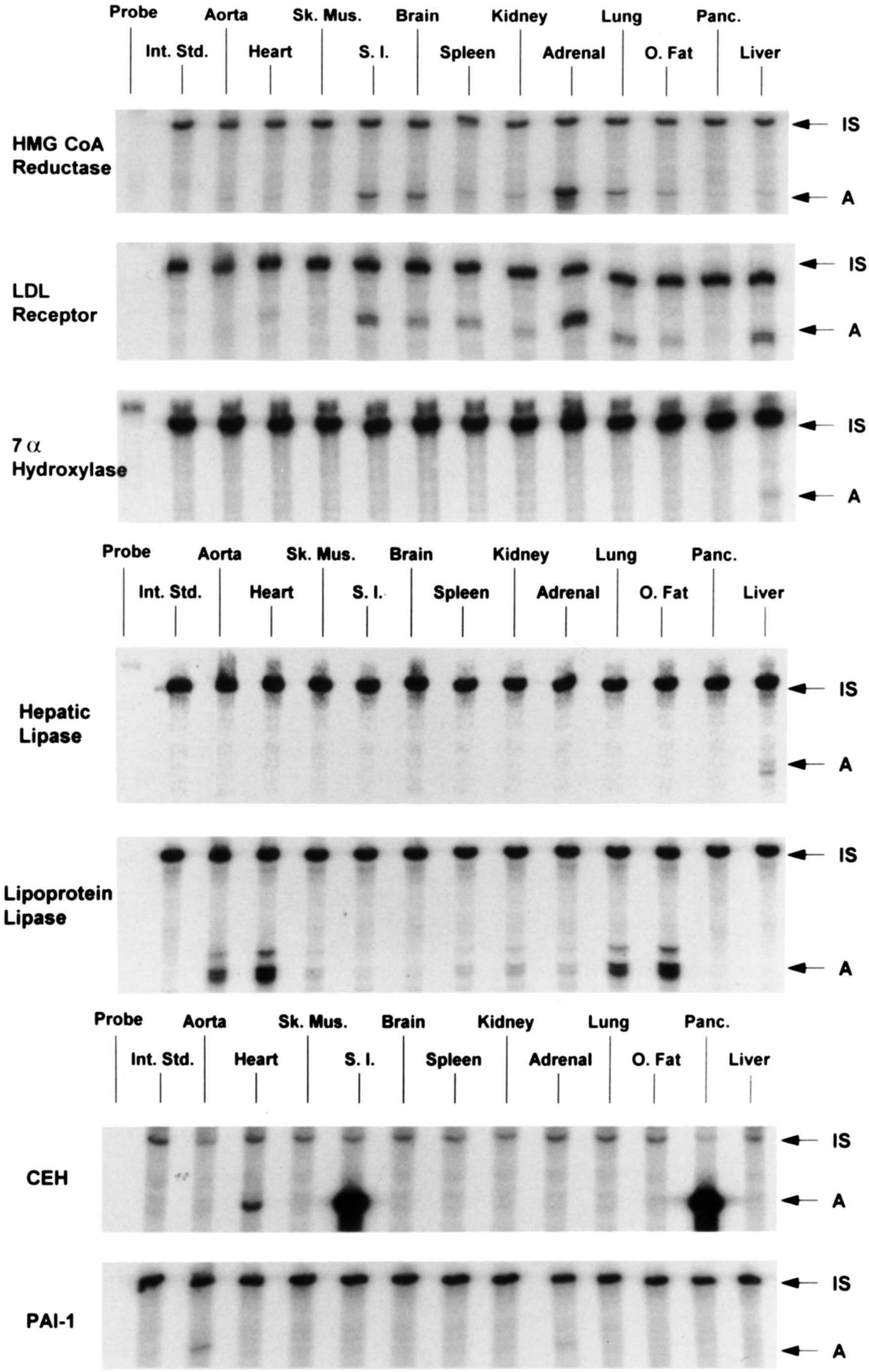


Fig. 1. Total RNA was isolated from each tissue (Sk. mus., skeletal muscle; S.I., small intestine; O. fat, omental fat; panc., pancreas) and the abundance of the indicated mRNA was measured by the internal standard/RNAse protection assay. The probe and internal standard lanes were from samples that contained either probe alone or probe plus an amount of internal standard equal to that in the tissue sample hybridization; they were treated the same as the tissue samples after hybridization. The amount of internal standard and total RNA for each analysis was as follows (except for aorta and adrenal gland analyses which contained 20 μg of total RNA instead of 30 μg): CETP, 30 pg internal standard/30 μg total RNA; apoA-I, 20 pg/30 μg; apoB, 50 pg/30 μg; apoE, 40 pg/5 μg; HL, 30 pg/30 μg; LDL-receptor, 20 pg/30 μg; HMG-CoA reductase, 20 pg/30 μg; 7α-hydroxylase, 50 pg/30 μg; LCAT, 40 pg/30 μg; albumin, 50 pg/2.5 μg; LPL, 40 pg/30 μg; CEH, 50 pg/30 μg; PAI-1, 30 pg/30 μg. IS, probe protected by internal standard; A, probe protected by authentic mRNA.



tively through stainless-steel meshes of 250, 106, and 75 μm opening size and then pelleted by centrifugation at 50 *g* for 3 min. The supernatant was saved (containing nonparenchymal cells) and the pellet was washed three more times with suspension buffer (DMEM, 0.2% bovine serum albumin, 0.4 mg/ml fructose, 11 μM insulin, 5% fetal bovine serum, pH 7.2). A sample of the final pellet containing parenchymal cells was frozen. The pooled supernatants were spun at 50 *g* for 3 min to remove contaminating parenchymal cells and the resulting supernatant was spun at 600 *g* for 7 min to pellet the nonparenchymal cells. The nonparenchymal cells were resuspended in Gey's balanced salt solution (Gibco, Gaithersburg, MD) and treated with 0.05% pronase E for 1.5 h at 39°C to destroy contaminating parenchymal cells. After protease treatment, cells were again spun at high speed and the final pellet was frozen for subsequent RNA isolation. Less than 1% of the nonparenchymal cell population were parenchymal cells as assessed by light microscopy.

RESULTS

Fig. 1 and **Table 2** show the abundance of mRNAs in several tissues measured with the internal standard/RNAse protection assay and the corresponding rabbit specific probe. Only LPL and CEH were not detectable in the liver under these assay conditions while all other genes showed some level of expression in that organ. Of this group CETP, albumin, 7α-hydroxylase, and HL mRNAs were detectable only in the liver while apoA-I mRNA was present in liver, small intestine, heart, and lung with the small intestine having the highest levels. The observed apoA-I expression levels and tissue distribution are commensurate with those previously published (22, 23). ApoB mRNA was present in both liver and small intestine with the small intestine having a slightly higher concentration. ApoE was expressed in many tissues with the highest level in liver. LCAT also showed fairly widespread distribution with noticeable levels in liver, adrenal, brain, and aorta. Both HMG-CoA reductase and LDL-receptor mRNAs were present in several peripheral tissues with the highest level of each in the adrenal gland. Lipoprotein lipase mRNA was present in omental fat, lung, heart, and aorta, and to lesser extents in other tissues. CEH showed expression in small intestine, pancreas, and heart while PAI-1 was detectable in aorta and at much lower levels in other tissues.

To further assess the expression of these genes in liver, we measured mRNA levels in isolated hepatic parenchymal and nonparenchymal cells (**Fig. 2** and **Table 3**). ApoA-I, apoB, LCAT, albumin, 7α-hydroxylase, and HL mRNAs were present in the parenchymal cell population but undetectable in nonparenchymal cells. PAI-1 mRNA was detectable only in nonparenchymal cells. CETP, apoE, HMG-CoA reductase, and LDL-receptor appeared to be present in both cell types although at markedly different levels. For instance, CETP, apoE, HMG-CoA reductase, and LDL-receptor mRNA levels were 35-, 18-, 2.5-, and 2-fold higher, respectively, in nonparenchymal cells than in parenchymal cells when expressed as picograms per microgram of total RNA (Table 3). However, when the data were expressed as molecules per cell, only CETP mRNA was more prevalent in nonparenchymal cells; this is because parenchymal cells contain 23-fold more total RNA per cell than nonparen-

TABLE 2. Estimates of mRNA concentration in various rabbit tissues

	mRNA Levels											
Tissue	CETP	ApoA-I	ApoB	ApoE	LCAT	Albumin	HMG-CoA Reductase	LDL Receptor	7-α Hydroxylase	Hepatic Lipase	Lipoprotein Lipase	CEH	PAI-1
							picogram/μg total RNA						
Aorta				6.40			0.63	0.94			7.60		1.61
Heart		0.22		1.23			0.31				8.90	11.3	
Skeletal muscle				1.20							0.68		
Small intestine		54.2	23.8				1.61	1.89				459	
Brain				9.00	0.39		1.71	1.09					
Spleen				5.38			0.90	1.28			0.67		
Kidney		0.12	4.23	1.52			0.89	0.57			1.16		
Adrenal				1.83	0.76		9.35	9.50			1.04		0.84
Lung		0.15		5.30			1.56	1.67			4.70		
Omental fat				3.02			0.91	0.76			8.43		
Pancreas				1.23			0.29					2930	
Liver[a]	1.47	0.33	13.9	32.3	2.19	163	1.44	1.54	0.61	1.15			

Data are from the gels shown in Fig. 1, except for the liver data. mRNA mass values were calculated as described (19) with authentic mRNA molecular weights from published values (22, 28–30, 34–38, 41–48). Where no number appears, the authentic mRNA signal was either absent or too low to permit reliable quantitation under these assay conditions.

[a]Liver data are mean values from several liver analyses. The corresponding sample sizes for these data are as follows: CETP, n = 10; apoB, HMG-CoA reductase, LDL-receptor, n = 8; apoA-I, apoE, n = 7; LCAT, 7α-hydroxylase hepatic lipase, n = 5; albumin, n = 4.

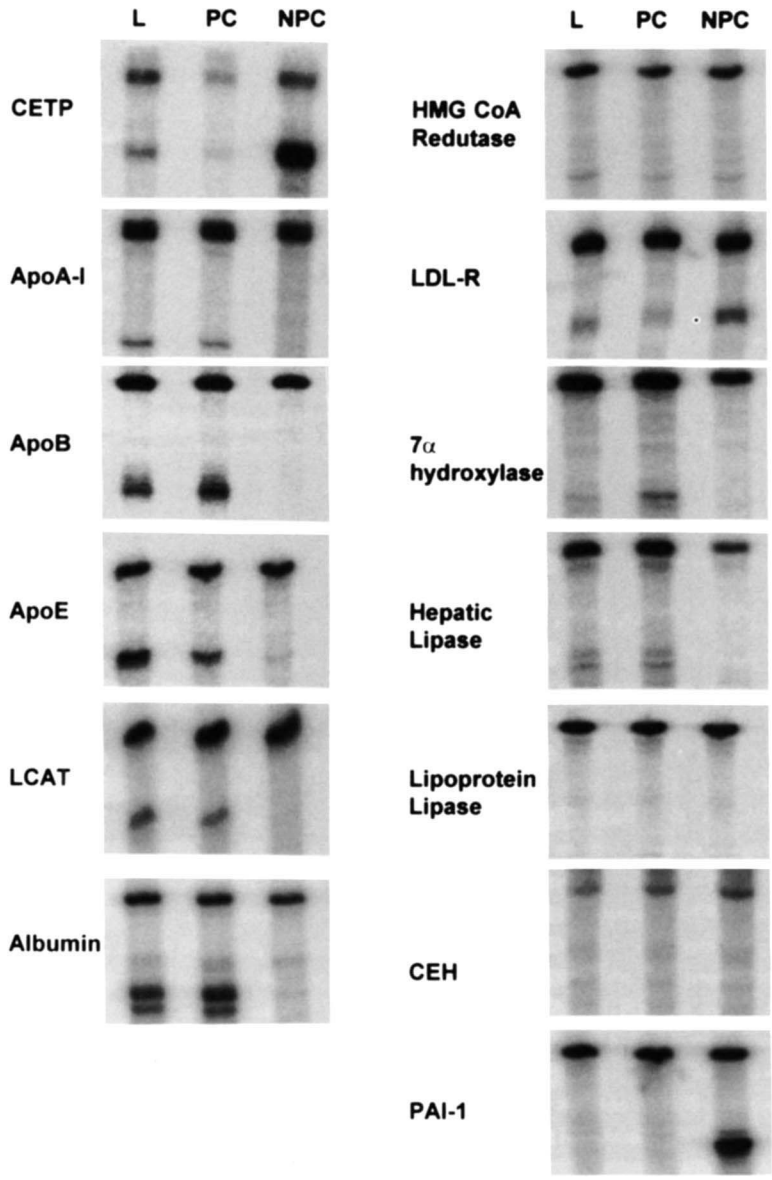


Fig. 2. Total RNA from liver (L), parenchymal cells (PC), and nonparenchymal cells (NPC) was isolated from tissue or the isolated cell population and the abundance of the indicated mRNA was measured by the internal standard/RNAse protection assay. The amount of internal standard and total RNA used for each analysis is the same as described in the legend to Fig. 1. The top band in each analysis is probe protected by internal standard and the bottom band is probe protected by authentic mRNA. CEH and LPL do not have detectable levels of hepatic mRNA under these assay conditions (see Fig. 1).

chymal cells (Table 3). Furthermore, normalizing the mRNA values for the contribution of each cell population to liver cell number revealed that nonparenchymal cells contributed heavily only to total hepatic CETP mRNA mass but not the total liver mass of apoE, LDL-receptor, or HMG-CoA reductase mRNAs.

DISCUSSION

The rabbit is widely used as a model for studying normal and pathological lipoprotein metabolism. However, a comprehensive study of tissue-specific expression of genes affecting these processes has not been undertaken. A primary reason has been the lack of rabbit-specific probes which, in turn, has lead to relatively insensitive mRNA quantitation methods. This problem is exacerbated with low abundance mRNAs and when little RNA is available for analysis (e.g., hepatic nonparenchymal cells). With these limitations in mind we had two principle objectives in the present study: *1)* clone partial rabbit cDNAs for 13 genes involved in lipid metabolism and develop mRNA quantitation assays for each gene and *2)* use the assays to

TABLE 3. Concentrations of CETP, apoE, HMG-CoA reductase, and LDL-receptor mRNAs in liver cell populations

	mRNA Levels						
	pg/μg Total RNA (mean ± SEM)[a]			Molecules/Cell (mean)[b]		Molecules/μg Liver (mean)[c]	
	Liver	PC	NPC	PC	NPC	PC	NPC
CETP (n = 7)	1.54 ± 0.27	0.59 ± 0.10	20.7 ± 4.8	30	46	3800	3100
ApoE (n = 4)	34.1 ± 5.5	23.5 ± 4.9	1.28 ± 0.44	2320	5	290,000	360
HMG-CoA reductase (n = 4)	2.23 ± 0.74	0.63 ± 0.09	1.50 ± 0.35	26	3	3200	175
LDL-receptor (n = 4)	1.64 ± 0.28	0.86 ± 0.06	1.73 ± 0.26	18	2	2300	110

[a]Differences between all PC (parenchymal cells) and NPC (nonparenchymal cells) values are statistically significant ($P < 0.05$).
[b]To determine total RNA/cell, parenchymal and nonparenchymal cells were isolated and counted. Approximately equal numbers of cells were then used to isolate total RNA in triplicate as described in Materials and Methods. To correct for the efficiency of isolation, a ^{3}H-radiolabeled RNA was added to each sample after the initial cell disruption in chaotropic agent. PC and NPC contained 64.8 and 2.8 μg of total RNA per 1×10^6 cells, respectively.
[c]The total number of cells per gram of liver was assumed to be 192×10^6 (50) and calculations were based on PC and NPC contributing 65% and 35% to liver cell number, respectively.

survey mRNA abundance in numerous rabbit tissues and hepatic cell populations.

We successfully cloned the cDNAs of interest by the polymerase chain reaction (PCR; (16)). This was accomplished despite the fact that no sequence data existed for several of the genes in rabbits. To overcome lack of these data we designed primers by aligning all available species cDNA sequences for the genes of interest and identifying regions of significant nucleic acid homology. The primers designed for our cloning strategies will be useful for cloning homologous genes from other species where such clones are not available. After amplification with these primers and product cloning, the identity of individual cDNA clones was confirmed by sequence homology to published data. Using these cDNAs we developed an internal standard/RNAse protection assay for each gene of interest. This highly sensitive assay can, in general, detect $1–5 \times 10^7$ molecules in an RNA sample (depending on probe background, size of probe, amount of internal standard, etc., (19)) using a species-specific probe. These cDNAs constitute one of the largest collections of rabbit-specific probes for analyzing mRNA expression for genes affecting lipid metabolism. With these probes, we performed a comprehensive study of mRNA abundance in numerous rabbit tissues and hepatic cell populations.

A major finding from our survey was the hepatic cellular distribution of CETP and PAI-1 mRNAs. Rabbit nonparenchymal cells contain about 35-fold more CETP mRNA than parenchymal cells. This cellular distribution in rabbit liver cells is qualitatively similar to our observations in nonhuman primates (*Cynomolgus macaques;* (14)). However, in the cynomolgus monkey, parenchymal cell CETP mRNA was not detected despite using protocols and RNAse protection assays essentially identical to the ones reported here. Primate parenchymal cells may have the capacity to synthesize CETP as some initial CETP characterization was performed in HepG2 cells, which are presumably of parenchymal origin (24). Indeed, rabbit parenchymal cells contain low levels of CETP mRNA when normalized to total RNA. However, the mRNA level can be normalized in several ways, providing alternative ways to view the data. For instance, using purified cell populations and correcting for yield efficiencies by radiolabeled RNA, we found that, in rabbits, hepatic parenchymal cells contain 23-fold more total RNA per cell than nonparenchymal cells. Furthermore, parenchymal and nonparenchymal cells contribute about 65% and 35%, respectively, to liver cell number (2). When these factors are taken into account, hepatic nonparenchymal cells contain more CETP than parenchymal cells on a per cell basis but each cell type contributes roughly equal amounts to total liver CETP mRNA mass.

The same normalizing factors must be considered when interpreting LDL-receptor, HMG-CoA reductase, and apoE mRNA levels in parenchymal versus nonparenchymal cells. For example, although there was 2-fold more LDL-receptor mRNA per microgram of total RNA in nonparenchymal cells, when the differences in total RNA per cell and the contribution of each cell population to liver cell number are taken into account, parenchymal cells contribute the vast majority of total liver LDL-receptor mRNA mass. This is consistent with parenchymal cells being responsible for ~90% of total hepatic LDL catabolism via the LDL receptor pathway (25).

Another striking finding in this study was the large amount of PAI-1 mRNA in hepatic nonparenchymal cells. The significance of this finding is apparent in light of the recent study by Simpson et al. (26). Using immunohistochemical methods, Simpson et al. (26) identified PAI-1 antigens associated with human hepatic parenchyma and Kupffer cells. Nonetheless, the authors were unable to determine the origin of PAI-1 antigen. Our data in rabbits suggests that hepatic nonparenchymal cells have the capacity to synthesize PAI-1 and may be, at least in rabbits, the major source of circulating PAI-1.

In summary, we have shown in rabbit that *1)* the tissue distribution of mRNAs for genes involved in lipid metabolism generally follows that reported for other species (22, 23, 27–49), and *2)* there exist differential patterns of expression in hepatic parenchymal and nonparenchymal cells for several genes affecting lipid metabolism. The expression patterns are as follows when mRNA levels are normalized to total RNA: parenchymal cells have higher levels of apoA-I, apoB, apoE, albumin, LCAT, HL, and 7α-hydroxylase mRNAs compared to nonparenchymal cells while nonparenchymal cells have higher levels of CETP, HMG-CoA reductase, LDL-receptor, and PAI-1 mRNAs compared to parenchymal cells. Alternatively, when data are normalized to account for the abundance of hepatic parenchymal and nonparenchymal cells and the relative amounts of total RNA in each cell type, only CETP and PAI-1 mRNAs from nonparenchymal cells are major contributors to the total liver mass of their respective mRNAs. This partitioning of gene expression suggests that hepatic parenchymal and nonparenchymal cells may play specialized interactive and perhaps unique roles in the intra- and extracellular transport of lipids. Further studies at the genetic level, particularly with nonparenchymal cells, will further define their role in the biochemical and metabolic processing of lipids in vivo.

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