pleted red cells (see Table 1) were added at a final hematocrit of 0.2 percent.
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20. Supported in part by NIH grants HL19725, HL28935, and HL07062. Part of this work was presented at the national meeting of the Association of American Physicians, Washington, D.C., May 1984. B.S.v.A. is a postdoctoral associate in medicine of the University of Minnesota, also supported by "De Drie Lichten." We gratefully acknowledge technical assistance by R. L. Boyd and L. P. Barker, and help in pathological anatomical studies by G. Frizzera.
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24 September 1984; accepted 8 November 1984

Distinct Hepatic Receptors for Low Density Lipoprotein and Apolipoprotein E in Humans

Abstract. *Since the liver is a central organ for lipid and lipoprotein synthesis and catabolism, hepatic receptors for specific apolipoproteins on plasma lipoproteins would be expected to modulate lipid and lipoprotein metabolism. The role of hepatic receptors for low density lipoproteins and apolipoprotein E–containing lipoproteins was evaluated in patients with complementary disorders in lipoprotein metabolism: abetalipoproteinemia and homozygous familial hypercholesterolemia. In addition, hepatic membranes from a patient with familial hypercholesterolemia were studied and compared before and after portacaval shunt surgery. The results establish that the human liver has receptors for apolipoproteins B and E. Furthermore, in the human, hepatic receptors for low density lipoproteins and apolipoprotein E are genetically distinct and can undergo independent control.*

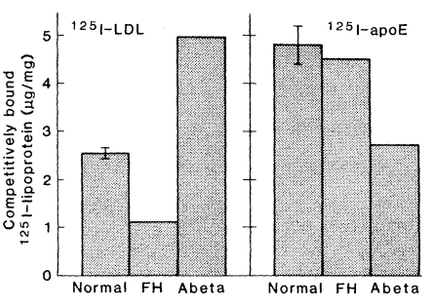
In addition to solubilizing lipid for transport in the blood, the plasma apolipoproteins affect various specific physiologic functions (*1*). Apolipoprotein B (apoB), the primary protein constituent of the cholesterol-carrying low density lipoproteins (LDL), mediates cholesterol delivery to cells by interacting with a specific protein receptor (*2*). Although apolipoprotein E (apoE) is important in the metabolism of triglyceride-rich lipoproteins (*3, 4*), the exact mechanism by which it modulates triglyceride-rich lipoprotein catabolism has not been clearly defined. Lipoproteins containing apoE bind to cultured fibroblasts (*5*) as well as to canine liver (*6*). The binding of apoE-containing lipoproteins can be competed for by apoB-containing lipoproteins; this receptor has therefore been designated the hepatic B, E, or LDL receptor. The LDL receptor is affected by drug treatment, biliary diversion, and diet (*6, 7*). A separate hepatic apoE receptor has been reported, but the physiologic importance of the hepatic apoE receptor in mammals has not been definitely established.

Lipoprotein transport in normal subjects and patients with dyslipoproteinemia can be elucidated by evaluating individuals with specific inborn errors of lipoprotein metabolism. Patients with abetalipoproteinemia do not secrete lipoproteins containing apoB (*8*). The normal ligand in the LDL-receptor cholesterol transport system is missing in these patients, and therefore compensatory changes in the lipid and lipoprotein transport system are required for lipid homeostasis (*8*). Individuals with homozygous familial hypercholesterolemia (FH) lack the LDL receptor (*9*). Therefore, from the standpoint of the interaction of LDL with its receptor, abetalipoproteinemia and FH are complementary genetic defects. We evaluated the hepatic LDL and apoE receptors in humans by analyzing the binding of apoB- and apoE-containing lipoproteins to hepatic membranes from normal subjects and patients with abetalipoproteinemia and homozygous FH.

Patients with abetalipoproteinemia and homozygous FH showed the clinical, biochemical, and genetic criteria for their respective diagnoses (*8, 9*). A therapeutic portacaval anastomosis was performed on the subject with FH in order to decrease the concentration of plasma LDL cholesterol. Hepatic tissues were obtained from normal subjects, from the patient with abetalipoproteinemia, and from the patient with FH after informed consent had been given. Biopsy specimens were immediately placed in a chilled beaker, and hepatic membranes were prepared at 4°C as described (*10*). The membrane pellets were frozen in dry ice and stored in liquid nitrogen until they were used for binding assays.

For lipoprotein quantitation, blood obtained from the subjects after a 12- to 14-hour overnight fast was collected in 0.1 percent EDTA, and the plasma was separated at 4°C in a refrigerated centrifuge. Plasma cholesterol, triglycerides, and lipoproteins were quantitated on an analytic spectrophotometer (Gilford 3500) as described (*10, 11*). Apolipoproteins B and E were determined by radial immunodiffusion and radioimmunoassay, respectively (*11, 12*).

Low density lipoproteins (density, 1.030 to 1.050 g/ml) were used for the binding studies of apoB-containing lipoproteins. These lipoproteins were isolated (*13*), dialyzed against phosphate-buffered saline (*p*H 7.0), and sterilized by filtration. Radioimmunoassay showed that LDL was devoid of apoE. The LDL



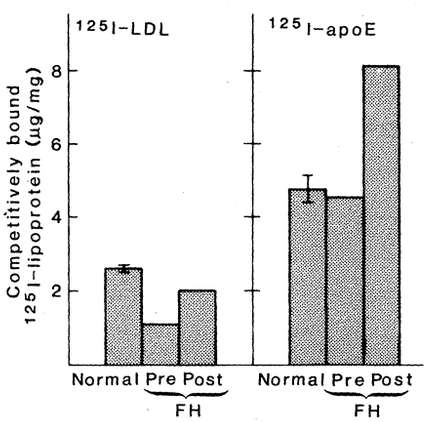


Fig. 1 (left). The binding of apoB (LDL) and apoE particles to hepatic membranes from three normal, one FH, and one abetalipoproteinemia (Abeta) subject. Hepatic membrane protein (0.1 to 0.2 mg) from normal and dyslipidemic subjects was incubated for 30 minutes at 37°C with 10 μg of ^{125}I-labeled lipoprotein. Bound and free ^{125}I-lipoproteins were then separated by a 3-minute, 100,000*g* ultracentrifugation. Competitively bound lipoprotein is the difference in binding observed in the absence and presence of tenfold excess unlabeled homologous lipoprotein. Values for normal subjects represent the mean ± standard error of the mean of the three samples. Fig. 2 (right). The binding of apoB (LDL) and apoE lipoproteins to three normal and one homozygous FH hepatic membrane before (pre) and after (post) portacaval shunt surgery. The competitive binding of ^{125}I-lipoproteins was determined as the difference in binding in the absence and presence of tenfold excess unlabeled lipoprotein to 100 to 200 μg of membrane proteins. Normal values represent the mean ± standard error of the mean of the three separate samples.

Table 1. Plasma lipid and lipoprotein concentrations in normal subjects, in a patient with familial hypercholesterolemia (FH), before and after portacaval shunt surgery, and in a patient with abetalipoproteinemia (Abeta).

Subjects	Number	Cholesterol (mg/dl)				Triglycerides (mg/dl)
		Total	VLDL	LDL	HDL	
Normal	1088	189 ± 1.2*	16 ± 0.3	123 ± 1.1	50 ± 0.4	87 ± 1.3
FH	1					
Before		1034	47	957	29	200
After		743	24	691	23	156
Abeta	1	43	1	4	38	20

*Values are means ± standard error of the mean for 1088 different individuals.

was labeled with ^{125}I by the iodine monochloride method (*14, 15*) and specific activities ranged from 2.7×10^9 to 4.6×10^9 becquerels (Bq) per milligram of LDL protein (1 Bq = 1 disintegration per second).

Lipoproteins for apoE binding were rich in apoE but devoid of apoB; they were obtained by plasmapheresis of 500 ml of plasma 2 hours postprandially from an abetalipoproteinemia patient with an $E_{3/3}$ phenotype. After preparative ultracentrifugation of the plasma lipoproteins at 1.21 g/ml at 4°C for 48 hours, the lipoproteins underwent preparative electrophoresis (Geon-Pevicon) (*16*). Fractions that were rich in apoE when analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and radioimmunoassay were dialyzed and used for binding studies. ApoE was isolated from an E_3 homozygote and purified as described (*3*). Purified $apoE_3$, labeled by the iodine monochloride method (*14*) as modified for lipoproteins (*15*), was reassociated with the apoE-rich lipoproteins (calculated mass ratio of $apoE_3$ to particle, 1:1) during a 30-minute incubation at 37°C in phosphate-buffered saline (*p*H 7.4). The labeled lipoprotein particles were then isolated by ultracentrifugation for 36 hours at 4°C (density, 1.21 g/ml). More than 90 percent of the ^{125}I-labeled apoE remained associated with the lipoprotein fraction. Lipoprotein concentrations were determined by the method of Lowry *et al.* (*17*), with bovine serum albumin as standard.

Hepatic membrane binding of ^{125}I-labeled lipoproteins was assessed as described (*10*). Membrane protein (100 to 200 μg) was then added to a buffer containing 50 m*M* NaCl and 20 m*M* tris-HCl (*p*H 7.5), to which was added 1 m*M* $CaCl_2$. The ^{125}I-labeled lipoproteins were added at the indicated concentration with or without unlabeled lipoproteins to obtain a final total assay volume of 0.1 ml. Incubations were carried out at 37°C for 30 minutes. Bound ^{125}I-lipoproteins were separated from free ligand as described earlier (*10*). Specifically bound ^{125}I-lipoprotein was defined as the difference in the amount of ^{125}I-lipoprotein quantitated in samples incubated with and without excess unlabeled lipoproteins.

Concentrations of plasma lipids, lipoproteins, apoB, and apoE from the normal and dyslipidemic subjects are summarized in Tables 1 and 2. Before shunt surgery, the total and LDL cholesterol concentrations of the FH subject were five- to eightfold greater than normal, reflecting a massive increase in plasma apoB-containing lipoproteins. After surgery, the total, very low density lipoprotein (VLDL) and LDL cholesterol concentrations of the FH subject decreased by 22 to 48 percent but were still much higher than normal. In contrast, in the patient with abetalipoproteinemia, virtually all of the plasma cholesterol was carried in the high density lipoproteins (HDL), reflecting an absence of lipoprotein particles containing apoB. These results are consistent with the characteristic changes in apoB-containing lipoproteins in the two disorders and confirm the effect of portacaval shunt on the plasma concentration of apoB-containing lipoprotein particles in FH (*18*).

Hepatic membranes from normal and dyslipidemic patients competitively bound ^{125}I-LDL and ^{125}I-apoE–containing particles (Fig. 1). The binding of ^{125}I-LDL to FH hepatic membranes lacking the usual fibroblast LDL receptor was less than 50 percent of the binding to normal membranes (Fig. 1, left panel) as reported earlier (*10*). In contrast, the hepatic membranes from the abetalipoproteinemia patient bound twice as much ^{125}I-LDL as did normal membranes and almost five times as much as membranes isolated from FH patients.

Table 2. Concentrations of apolipoprotein B and E in normal subjects, in a patient with familial hypercholesterolemia (FH) before and after portacaval shunt surgery, and in a patient with abetalipoproteinemia (Abeta). ApoB was quantitated by radial immunodiffusion and apoE by radioimmunoassay.

Subjects	Number	Apolipoprotein concentration (mg/dl)	
		apoB	apoE
Normal	50	85 ± 2.7*	5.7 ± 0.2*
FH	1		
Before		422	3.5
After		227	4.7
Abeta	1	0	8.2

*Values represent the mean ± standard error of the mean detected in individual normolipidemic subjects.

Despite the decreased binding by hepatic membranes from the FH patient the apoE-containing lipoprotein particles bound normally to these membranes (Fig. 1, right panel). The hepatic membranes from the abetalipoproteinemia patient, however, competitively bound only 56 percent of the normal amount of apoE. Since the coefficient of variation on replicate samples was less than 7 percent for normal, FH, and abetalipoproteinemia hepatic membranes, these differences are substantial. These data indicate that the hepatic membrane recognition of LDL and apoE-containing particles are different and do not vary coordinately in individuals with strikingly different plasma concentrations of apoB-containing lipoprotein particles.

The potential regulation of these receptors was determined by assessing the binding of LDL and apoE lipoprotein particles to hepatic membranes from an FH patient before and after portacaval shunt surgery (Fig. 2). In this individual who had no fibroblast LDL receptors, the portacaval shunt increased ^{125}I-LDL binding by 47 percent (Fig. 2, left panel). However, even after portacaval shunt surgery, the degree of LDL binding was still less than normal. The apoE lipoprotein particle binding was normal before shunt surgery and increased by 47 percent after shunt surgery (Fig. 2, right panel). Therefore, the apoE lipoprotein binding to FH hepatic membranes appeared to be regulated upward with portacaval anastomosis.

In vitro study of fibroblasts from patients with homozygous FH has led to an understanding of the role of the cholesterol-rich apoB-containing lipoproteins in the delivery of lipids to nonhepatic tissues (*2*). The elucidation of the LDL receptor pathway has stimulated interest in evaluating other possible apolipoprotein-cell interactions that may be important in the lipid transport system. Since the liver is a major organ in lipid and lipoprotein metabolism, apolipoprotein receptors of potential importance in normal and dyslipoproteinemic subjects have been investigated. Evaluation of lipoprotein metabolism in vivo (*3, 4, 6, 19*), tissue uptake of lipoproteins (*20,*

21), isolated hepatic membrane receptor studies (*5–7, 10, 22, 23*), and lipoprotein metabolism in cultured hepatic cells (*24*) point to an elaborate apolipoprotein recognition system in mammalian liver. The LDL receptor on canine hepatic membranes and the human fibroblast LDL receptor undergo rapid regulation by perturbations in cholesterol and bile acid metabolism (*5–7, 19*). Specific apoE recognition sites, however, did not appear to undergo similar modulation (*7, 19*). In addition, a pathway in liver that is distinct from the LDL and apoE receptor and that may have a broad specificity for lipoprotein recognition has been proposed (*10, 24, 25*). The mammalian liver, therefore, contains numerous pathways by which lipids and lipoproteins may be metabolized.

The genetic and physiologic roles of these different hepatic receptors were investigated in human subjects by studying isolated hepatic membranes from normal individuals, from a patient with FH who lacked the fibroblast LDL receptor, and from a patient with abetalipoproteinemia who had no circulating LDL lipoprotein particles. Despite the absence of the usual fibroblast LDL receptor, low levels of residual LDL-specific binding were observed in hepatic membranes from the FH patient (Fig. 1) (*10*). Of particular importance, however, was the observation that apoE binding was normal. The residual apoB binding to these membranes may reflect binding to receptors with broad specificity or a cross-recognition with the apoE receptor; this possibility is supported by the 47 percent increase in both apoE and apoB binding to membranes obtained from the FH patient after portacaval shunt surgery (Fig. 2). The apoE binding, however, was not only genetically distinct from LDL binding but was also regulated upward after portacaval shunt surgery.

Hepatic membrane studies in abetalipoproteinemia also support the concept of distinct LDL and apoE receptors in humans. The absence of plasma apoB lipoprotein particles in this disorder was associated with a reciprocal increase in LDL receptor expression and a reduction in the expression of the apoE receptors on isolated hepatic membranes (Fig. 1). Blum *et al.* (*26*) and Innerarity *et al.* (*27*) proposed that the increased apoE lipid-rich particles in abetalipoproteinemia lead to normalized lipid transport through an apoE-mediated uptake process. Our study supports the concept that the lipid-rich apoE particles are recognized by human hepatic membranes independent of an LDL receptor. Not only is the regulation of the LDL and apoE receptors independent, the apoE receptor pathway may in some way lead to normalization of cholesterol homeostasis in abetalipoproteinemia (*26–30*).

These combined results indicate that hepatic receptors for LDL and apoE are present in humans. The LDL receptor, as was reported in earlier mammalian studies, undergoes regulation. However, the hepatic apoE receptor in humans, unlike that in other species, can also be modulated. Therefore, human hepatic receptors for LDL and apoE-containing lipoproteins are physiologically and genetically distinct.

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31. We thank R. Ronan for the isolation and purification of apoE and M. Meng for evaluating lipoprotein subfractions by sodium dodecyl sulfate gel electrophoresis.

17 August 1984; accepted 3 November 1984

Immunoprecipitation of Insulin Receptors from Cultured Human Lymphocytes (IM-9 Cells) by Antibodies to pp60^{src}

Abstract. *The family of tyrosine-specific protein kinases includes proteins encoded by retroviral oncogenes as well as receptors for insulin and several growth factors. Antibodies to pp60^{src}, the protein encoded by the* src *oncogene of Rous sarcoma virus (RSV), can specifically immunoprecipitate affinity-labeled insulin receptors from cultured human lymphocytes (IM-9 cells). This precipitation is specifically inhibited by the* src *gene product purified from RSV-transformed rat cells. These observations provide evidence that there is structural homology between the insulin receptors and pp60^{src}.*

The product of the v-*erb* B oncogene of avian erythroblastosis virus is a truncated form of the epidermal growth factor (EGF) receptor (*1*). The v-*erb* B gene is a member of a family of retroviral oncogenes encoding tyrosine-specific protein kinases with structural homology to pp60^{src} (*2*), the protein encoded by the *src* oncogene of Rous sarcoma virus (RSV). Similarly, the EGF receptor and those for insulin, insulin-like growth factor I (IGF I), and platelet-derived growth factor belong to a family of cell surface receptors with ligand-stimulated, tyrosine-specific protein kinase activity (*3*). These observations have led to the hypothesis that the genes for this family of cell surface receptors may be proto-oncogenes corresponding to the retroviral oncogenes in the *src* family. We now show that antisera containing antibodies to pp60^{src} can immunoprecipitate affinity-labeled insulin receptors. Moreover, this precipitation can be competitively inhibited by the protein encoded by the *src* gene. These observations are consist-