JCI The Journal of Clinical Investigation

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J Clin Invest. 1983;71(4):816-831. https://doi.org/10.1172/JCI110835.

Research Article

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RAPID REGULATION OF APOLIPOPROTEIN B,E RECEPTORS, BUT NOT OF APOLIPOPROTEIN E RECEPTORS, BY INTESTINAL LIPOPROTEINS AND BILE ACIDS

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ABSTRACT Two distinct lipoprotein receptors can be expressed in the dog liver. One is the apolipoprotein (apo-) B,E receptor. This receptor binds apo-B-containing low density lipoproteins (LDL), as well as apo-E-containing lipoproteins, such as the cholesterol-induced high density lipoproteins (HDL_c). The second hepatic lipoprotein receptor is the apo-E receptor. It binds apo-E HDL_c and chylomicron remnants, but not LDL. The present studies were undertaken to determine whether short-term (acute) regulation of the two receptors can occur in response to perturbations in hepatic cholesterol metabolism. The design used three groups of experimental animals: (*a*) immature dogs (with both hepatic apo-B,E and apo-E receptors expressed), (*b*) adult dogs (with predominantly the apo-E receptor expressed and little detectable apo-B,E receptor binding activity), and (*c*) dogs treated with the bile acid sequestrant cholestyramine or those that have undergone biliary diversion (with apo-E receptors and induced apo-B,E receptors).

In the first series of experiments, changes in hepatic lipoprotein receptor expression were studied by delivering cholesterol to the liver via intestinal lymph lipoproteins. Dog lymph (5–11 mg of triglycerides/min per kg of body weight, 0.15–0.3 mg of cholesterol/min per kg) or saline were infused intravenously for 6–8 h into matched pairs of dogs. Serial liver biopsies were obtained at intervals of 1–2 h. A progressive loss of specific (calcium-dependent) binding of LDL was seen in hepatic membranes from both immature and cholestyramine-treated dogs. After 4–6 h of lymph infusion, almost no apo-B,E receptor binding could be detected. The decrease in binding of apo-E HDL_c to the same membranes was much less pronounced, and could be explained by a loss of binding of HDL_c to the apo-B,E receptor; there was little or no effect on apo-E receptor binding.

In the second series of experiments, the effects of a diminished hepatic demand for cholesterol on lipoprotein receptor expression were studied by suppressing bile acid synthesis. The bile acid taurocholate (2–3 μmol/kg per min) was infused intravenously over a 6-h interval. This resulted in a progressive loss of LDL binding to liver membranes of immature or cholestyramine-treated dogs. The infusion of taurocholate for 6 h did not significantly alter the expression of the apo-E receptor binding activity, whereas apo-B,E receptor activity was rapidly down-regulated. Preparation of a bile fistula in adult dogs markedly induced the expression of the apo-B,E receptor. In this state, the binding activity of the apo-B,E receptor could be almost totally abolished by reinfusion of taurocholate for 6 h, without profoundly affecting apo-E receptor binding. Evidence from the analysis of plasma lipoprotein patterns and tissue culture reactivity suggested that changes in assayed hepatic lipoprotein receptor activity occurred in concert with changes in plasma lipoproteins.

Dr. Angelin was a Visiting Scientist from the Department of Medicine, Karolinska Institute of Huddinge University Hospital, Huddinge, Sweden, supported in part by a fellowship from the Ernst Klenk Foundation. Dr. Raviola was supported in part by a grant from the Kaiser Foundation (111-9716). Address correspondence to Dr. Mahley.

Received for publication 2 September 1982 and in revised form 2 December 1982.

The results indicate that the two canine hepatic lipoprotein receptors differ in their metabolic regulation. The apo-B,E receptor responds rapidly to changes in hepatic requirements for cholesterol. The apo-E receptor appears to be more refractory to acute regulation. The rapidity of the changes in the activity of the apo-B,E receptor (within 2–4 h) suggests that the binding activity of this receptor may be regulated by factors independent of protein synthesis.

INTRODUCTION

The liver is a key organ in cholesterol and lipoprotein homeostasis (1, 2). It both synthesizes cholesterol and acquires cholesterol from chylomicron remnants and other lipoproteins. Hepatic cholesterol, regardless of its origin, can be resecreted into the plasma in endogenous lipoproteins or into the bile as free cholesterol or, after conversion, as bile acids. Biliary excretion is the dominant pathway for net removal of cholesterol from the body.

Present knowledge supports the concept that most excreted cholesterol and bile acids are derived from plasma lipoprotein cholesterol (for review see 1). The first step in the hepatic uptake of lipoprotein cholesterol is the binding of plasma lipoproteins to liver membrane receptors (2). Knowledge of the possible regulation of lipoprotein uptake by modulation of liver receptor activity is thus of major importance in understanding how body cholesterol and lipoproteins are metabolized. Moreover, nonreceptor-mediated uptake of lipoproteins may also be of significance in total body cholesterol metabolism (for review see 3).

Recent studies of canine liver have demonstrated the presence of two distinct types of high affinity receptors that interact with plasma lipoproteins (4, 5). One of the hepatic receptors is the low density lipoprotein (LDL) or apolipoprotein (apo-)[1] B,E receptor, which interacts with both apo-B-containing LDL and apo-E-containing high density lipoproteins (HDL), such as cholesterol-induced apo-E HDL_c. Normally, this receptor is not readily detectable, or is expressed at low levels with controlled metabolic conditions, in the livers of mature, adult dogs (>18 mo of age). However, the hepatic apo-B,E (LDL) receptors can easily be demonstrated (expressed at high levels) in immature, growing animals. Likewise, these receptors can be induced to high levels in adult dogs that have been treated with the bile acid sequestrant cholestyramine or subjected to prolonged fasting (4, 5). The apo-B,E receptor closely resembles the LDL receptor in fibroblasts (4–7). The second hepatic receptor, the apo-E receptor, is present on liver membranes of both adult and immature dogs. It differs from the apo-B,E receptor in that it binds apo-E-containing lipoproteins but does not interact with normal LDL. This receptor is presumably responsible for the high affinity uptake of chylomicron remnants by the liver (5, 8–11). It has been identified in liver membranes not only from dogs, but also from swine and man (4, 5).

Previous studies have indicated that the activity of the canine hepatic apo-B,E receptor can be regulated by long-term metabolic perturbations (4, 5). Feeding of the hypocholesterolemic drug cholestyramine or long-term fasting resulted in an increased expression of the apo-B,E receptor in adult dogs. On the other hand, feeding immature dogs a diet high in cholesterol and saturated fat led to the loss of apo-B,E receptor binding activity. In the present study, we have investigated whether the binding activity of the apo-B,E and/or the apo-E receptors in dog liver membranes can be influenced by induced short-term changes in liver cholesterol metabolism. We studied immature dogs (with both receptors initially expressed), adult dogs (with predominantly apo-E receptors), and dogs treated with cholestyramine (with apo-E receptors and induced apo-B,E receptors). These studies were carried out under two principal protocols: (*a*) infusion of lymph (resulting in an increased load of exogenous cholesterol delivered to the liver), and (*b*) infusion of bile acids (resulting in a reduced conversion of cholesterol to bile acids). The results indicate that short-term regulation of the binding activity of the apo-B,E receptor, but not of the apo-E receptor, occurs in dog liver membranes.

METHODS

Animals. Mature (>1.5 yr of age) and immature (<0.5 yr of age) foxhounds were obtained from Brink Farm (Paola, KS). The animals had continuous access to water and were fed a normal dog chow diet (Purina Dog Meal, Ralston Purina Co., St. Louis, MO). Six dogs, 6–8-mo-old, were treated with cholestyramine (Colestipol hydrochloride, kindly provided by Douglas McCarter of the Upjohn Co., Kalamazoo, MI) at a dose of 35 g (2–3 g/kg of body wt) for at least 10 d before being studied. The cholestyramine was mixed with the dog food (Kal Kan Chunky Meat Stew). In three mature foxhounds, cholecystectomy was performed, and a T-tube with an occludable balloon (Baldwin balloon occlusion T-tube, 10F, C. R. Bard International Inc., Murray Hill, NJ) was inserted into the common bile duct while the animals were under halothane anesthesia. The external limb of the T-tube was occluded and the balloon deflated, so that the normal enterohepatic circulation was maintained for 5 wk. This was done to establish postoperative stabilization. Total biliary diversion was established 5 d before the infusion studies. While the foxhounds were under local anesthesia, the external limb of the T-tube was opened, and the occlusion

[1] *Abbreviations used in this paper*: apo, apolipoprotein; apo-E HDL_c, cholesterol-induced high density lipoproteins containing only apo-E; CHAPS, 3[(3-cholamidopropyl) dimethylamonio] propanesulfonate.

balloon in the distal limb was inflated, creating a total bile fistula. Basal data on the animals used for the various infusion experiments are given in Table I.

Lipoprotein isolation. All dogs were fasted overnight before blood sampling. Canine LDL were isolated from the plasma of foxhounds fed the commercial dog chow. The apo-E HDL_c were prepared from the plasma of foxhounds fed a semisynthetic diet containing 5% cholesterol and 16% hydrogenated coconut oil (12). The LDL were isolated by ultracentrifugation (d = 1.02–1.063) in a 60-Ti rotor (Beckman Instruments, Inc., Mountain View, CA) at 59,000 rpm for 18 h and were purified by Geon-Pevikon block electrophoresis (13). The isolated LDL contained apo-B as the only detectable band on sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and migrated as a single band with β-mobility on paper electrophoresis. The apo-E HDL_c were isolated by Geon-Pevikon electrophoresis from the ultracentrifugal fraction, d = 1.006–1.02, as described (12) and mainly, or exclusively, contained apo-E as demonstrated by gel electrophoresis. The isolated lipoproteins were dialyzed extensively against 0.15 M NaCl, 0.01% EDTA, pH 7.0, before use. Canine ^{125}I-apo-E HDL_c were iodinated by the Bolton-Hunter procedure, as described previously (14). The ^{125}I-LDL were prepared according to the method of Bilheimer et al. (15). The specific activities of ^{125}I-apo-E HDL_c were generally 500–800 cpm/ng of protein, and those of ^{125}I-LDL were 250–500 cpm/ng.

Lymph collection. Lymph fistulas were created in mongrel dogs (15–20 kg) anesthetized with halothane, by using a modification of the procedure of Rajpal and Kirkpatrick (16), as previously described (17). The dogs were fed meat dog food (Kal Kan Chunky Meat Stew), and lymph was collected into 600-ml Fenwal blood packs (Baxter Travenol Laboratories, Travenol Laboratories, Inc., Deerfield, IL) containing disodium-EDTA (final concentration, 1 mg/ml). Lymph was generally collected for 2–3 d, stored at 4°C under sterile conditions, and used for infusion studies within 5 d of collection. The mean triglyceride and cholesterol concentrations of the lymph preparations used in the different experiments were 3.2±0.5 (SEM) g/dl and 92±12 mg/dl, respectively. More than 90% of the cholesterol and more than 98% of the triglyceride were present in the d < 1.02 density fraction, as demonstrated by density-gradient ultracentrifugation according to the method of Redgrave et al. (18). The major apoproteins of the d < 1.02 fraction, demonstrated by SDS-polyacrylamide gel electrophoresis, were apo-B, apo-A-I, apo-A-IV, and apo-E. The apo-B was almost exclusively of the low molecular weight form, similar to the B-48 described in man (19).

Preparation of chylomicron remnants. Dog lymph was kept refrigerated at 4°C overnight, and the top creamy layer was removed. This chylomicron-enriched fraction, raised to d = 1.02, was washed twice by underlayering beneath a saline-EDTA solution (d = 1.006) and centrifuged for 20 min at 25,000 rpm with an SW 28 rotor. Chylomicron remnants were prepared by modification of the procedure of Florén et al. (20). Postheparin plasma was obtained from an adult foxhound 10 min after an injection of heparin (150 U/kg of body wt). This postheparin plasma was frozen at −20°C until used. A 2-ml chylomicron aliquot (~100 mg of triglycerides) was added to 20 ml of 0.1 M Tris-HCl (pH 8.5) containing 5 g of fatty acid-free albumin, and the solution was dialyzed against 0.1 M Tris-HCl (pH 8.5) overnight. After 15 ml of postheparin plasma was added to the sample, the solution was incubated at 37°C for 26 h. At the end of the incubation, the solution was adjusted to a final density of 1.063 by KBr and refloated in a solution of 10 mM Hepes, pH 7.4, 1.1% NaCl, and 0.01% EDTA by centrifugation at 27,000 rpm for 3 h in an SW 28 rotor. The top layer was removed, its density adjusted to d = 1.063, and the lipoproteins were washed by discontinuous density-gradient ultracentrifugation (18). The resulting top layer (chylomicron remnants) was removed and dialyzed against saline. This procedure resulted in the hydrolysis of 98% of the chylomicron triglycerides. The chylomicron remnants were iodinated by the Bolton-Hunter method (14). The specific activity of the ^{125}I remnants was ~1,700 cpm/ng of protein.

Experimental procedure. In general, pairs of littermates (one control and one experimental animal) were studied in each experiment (Table I). Special attention was given to standardizing the preoperative care of the animals. They were housed at constant temperature and humidity with light cycling (dark 7 p.m. to 7 a.m.) for at least 4 d before the experiment (usually for several weeks). Food was given daily at 10 a.m., and the animals were deprived of food after 4 p.m. on the day before the experiment, which was started between 7 and 8 a.m. Induction of anesthesia was performed by an injection of sodium pentothal (25 mg/kg of body wt), whereafter the dogs were ventilated, and light anesthesia continued with methoxyflurane (Metofane, Pitman-Moore, Inc., Washington Crossing, NJ). Catheters were inserted into the femoral vein and artery, and an intravenous infusion of 0.15 M saline was given at a rate of 2.2–4.4 ml/min, with a Harvard Apparatus Co. (S. Natick, MA) infusion pump. In addition, a slow infusion of lactate-Ringer solution with 10% dextrose was given via a cephalic vein. The abdomen was opened, and two base-line liver biopsies (3–4 g each) were obtained. Special care was given to taking paired samples from similar anatomical locations in the two dogs.

After obtaining the liver biopsies to establish base-line values, an infusion of lymph (5–11 mg of triglycerides, 0.15–0.30 mg of cholesterol/kg of body wt per min) or taurocholate (2–3 μmol/kg per min, with a solution of ~20 mM taurocholate in 0.15 M saline) was started in the experimental animal. The saline infusion was continued at a similar rate into the control dog. Liver biopsies were obtained at intervals of 1–2 h from both dogs for 6–8 h. Continuous monitoring of arterial pH, pCO_2, and pO_2 was performed in nine experiments by using a Corning model 175 blood gas analyzer (Corning Medical, Corning Glass Works, Medford, MA), and demonstrated stable conditions throughout the experiments.

Isolation of canine liver membranes. Liver membranes were prepared according to the procedure of Kovanen et al. (21). The liver samples were immediately rinsed in ice-cold phosphate-buffered saline, and 1–2 g were suspended in 10–20 ml of 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1.0 mM $CaCl_2$, and 1.0 mM phenylmethylsulfonyl fluoride. All steps in the preparation were carried out at 4°C. The suspension was homogenized with four 15-s pulses in a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY; PT 30, setting 8), and the homogenates were centrifuged at 500 g for 5 min. The supernatants were recentrifuged at 8,000 g for 15 min. The resulting supernatants were then centrifuged at 100,000 g for 60 min, and the pellets were washed once by recentrifugation at 100,000 g for 60 min in the above buffer. In some experiments, receptors were solubilized from membrane fragments as described by Schneider et al. (22), by using either octyl-β-D-glucoside or 3[(3-cholamidopropyl) dimethylammonio] propanesulfonate (CHAPS; Pierce Chemical Co., Rockford, IL) at a final concentration of 15 mM, pH 7.5 (23).

Binding of ^{125}I-lipoproteins to liver membranes. The 100,000-g pellets, frozen at −70°C until assayed, were re-

suspended in 20 mM Tris-HCl, pH 7.5, 50 mM NaCl, and 1.0 mM $CaCl_2$ by forcing the suspension through a 22-gauge needle 10 times, followed by sonication of the suspension for 20 s in a Sonifier cell disruptor (Branson Sonic Power Co., Danbury, CT). The protein concentration of each suspension was determined according to the method of Lowry et al. (24). The binding of lipoproteins to liver membranes was assessed by determining the amount of ^{125}I-labeled lipoproteins associated with the membranes, as described by Basu et al. (25). Assays were carried out at 0°C on ice in 100 μl of incubation buffer containing 50 mM Tris-HCl, pH 7.5, 25 mM NaCl, 1.0 mM $CaCl_2$, and 20 mg/ml of bovine serum albumin. In general, each assay contained 100–200 μg of membrane protein, 5 μg/ml of LDL, or 0.3 μg/ml of apo-E HDL_c in the presence or absence of 30 mM EDTA. After incubation for 60–90 min, 75-μl aliquots were removed and layered onto 100 μl of fetal bovine serum in cellulose nitrate Airfuge tubes (Beckman Instruments). The tubes were centrifuged at 4°C in a Beckman Airfuge at 100,000 *g* for 45 min, the supernatants were removed by aspiration, and the pellets were washed once with 175 μl of fetal bovine serum at 100,000 *g* for 10 min. The pellets were separated by slicing the tube with a razor blade, and the radioactivity in the pellets was determined in a gamma counter (Gamma 4,000, Beckman Instruments). The binding of chylomicron remnants was assayed at 23°C in order to minimize the high level of nonspecific (calcium-independent) binding observed when studies were performed at 4°C (5).

The specific (calcium-dependent) binding of ^{125}I-lipoproteins to the liver membranes was determined by subtracting the amount of ^{125}I-lipoprotein bound in the presence of EDTA from the amount bound in the absence of EDTA. The maximum amount of lipoprotein bound to the membranes and the equilibrium dissociation constant (K_d) were determined from the Scatchard analysis (26), by using various concentrations of the ^{125}I-lipoprotein in the incubation medium. The values used for the molecular weight of LDL and apo-E HDL_c were 3×10^6 (20% protein) and 3.6×10^6 (15% protein), respectively (27).

Analysis of plasma and liver lipids. Total plasma cholesterol and plasma triglyceride levels were determined by using enzymatic procedures (Bio-Dynamics, Boehringer Mannheim Corp., Indianapolis, IN). Cholesterol, free and esterified, and triglycerides in liver tissue homogenates were quantified by a combination of thin-layer chromatography and gas-liquid chromatography (Hewlett-Packard Co., Palo Alto, CA; model 5880). Plasma concentrations of total bile acids were determined by using a 3α-steroid dehydrogenase assay (Sterognost-3α, Myegaard A/S, Oslo, Norway).

Plasma samples and LDL fractions were analyzed on polyacrylamide gradient gels (PAA4/30, Pharmacia Diagnostics, Div. of Pharmacia, Inc., Piscataway, NJ) and stained with Oil red O (28). Gels were run for 24 h at 12–14°C. After staining, peaks were quantitated by microdensitometry.

Cultured fibroblast binding assay. Normal human fibroblasts, obtained and maintained as previously described (14), were dissociated from stock flasks with 0.05% trypsin, 0.02% EDTA solution and plated in 35-mm petri dishes at a density of 3.5×10^4 cells per dish. 1 wk later, after the apo-B,E (LDL) receptors had been induced by a 48-h incubation of the cells with medium containing 10% lipoprotein-deficient serum, the cells were used for experiments. The binding assays were performed at 4°C as described (29), except that the cells plated in the 35-mm petri dishes (instead of 60-mm) were incubated with 1 ml of medium instead of 2 ml.

RESULTS

To determine whether an increased delivery of lipoprotein cholesterol of intestinal origin affected hepatic lipoprotein receptors, whole lymph was infused into six experimental dogs (cf Table I, dogs 2, 4, 6, 8, 10, and 12). The infusion resulted in a rapid increase in plasma triglyceride levels, from 34±5 mg/dl (mean±SEM) to 849±363 mg/dl. The rise in plasma cholesterol was more modest, from 131±14 to 151±11 mg/dl. As exemplified in Fig. 1A, an apparent steady-

TABLE I
Basal Data on Pairs of Dogs Used in Infusion Experiments

Dog No.	Experimental protocol	Sex	Age	Weight	Plasma cholesterol
	Treatment, infusion		*mo*	*g*	*mg/dl*
1	Saline	M	5	16	213
2	Lymph	M	5	16	197
3	Saline	M	3	10	112
4	Lymph	M	3	10	113
5*	Saline	M	4	13	189
6*	Lymph	M	4	12	130
7	Cholestyramine, saline	M	6	10	121
8	Cholestyramine, lymph	M	6	11	127
9	Cholestyramine, saline	F	8	21	97
10	Cholestyramine, lymph	M	8	23	104
11*	Saline	M	26	29	134
12*	Lymph	M	27	34	119
13	Saline	M	3	8	116
14	Taurocholate	M	3	8	115
15	Saline	M	4	17	188
16	Taurocholate	M	4	19	201
17	Saline	M	4	11	150
18	Taurocholate	M	4	12	151
19	Cholestyramine, saline	M	6	14	118
20	Cholestyramine, taurocholate	M	7	19	124
21	Bile fistula, taurocholate	F	34	20	85
22	Taurocholate	F	33	24	138
23	Bile fistula, taurocholate	M	22	26	95
24	Taurocholate	M	31	31	139
25	Bile fistula, taurocholate	M	22	27	67
26	Taurocholate	M	20	30	87
27	Cholestyramine, taurocholate	M	20	26	128
28	Taurocholate	M	26	31	110

* The perfusions of these two dogs were performed on separate days.

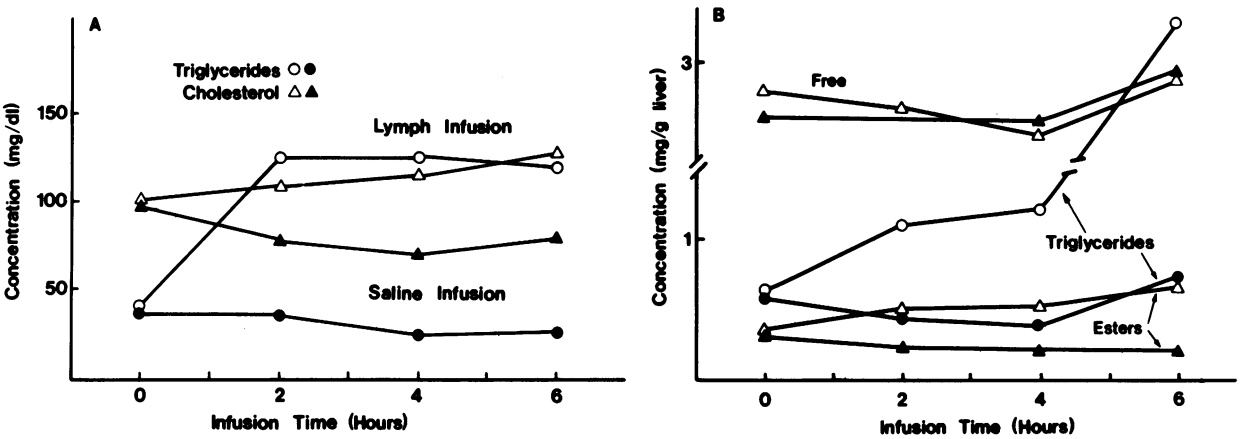

FIGURE 1 A Plasma concentrations of cholesterol and triglycerides before and during infusion of saline (closed symbols) or lymph (open symbols) in two littermate dogs (dogs 9 and 10) treated with cholestyramine (35 g/d for 3 wk). The rate of infusion of lymph corresponded to 6 mg/kg per min of triglycerides and 0.25 mg/kg per min of cholesterol. B Liver concentrations of free and esterified cholesterol (milligram of sterol per gram of tissue) and of triglycerides (milligram per gram) in the same experiment. The open symbols and closed symbols represent the data obtained during lymph or saline infusions, respectively.

state concentration of plasma lipids was reached in all animals, generally after 2–3 h of infusion. During the prolonged infusion of saline into the six paired control dogs, the plasma levels of cholesterol and triglycerides generally fell slightly (the mean cholesterol level was 141±20 mg/dl before and 111±21 mg/dl at the end of the experiment, and the mean triglyceride level was 33±4 and 28±11 mg/dl, respectively).

During lymph infusion, there was an increase in hepatic triglycerides from 0.80±0.16 to 3.24±0.43 mg/g of liver, and an increase in hepatic cholesteryl esters from 0.27±0.05 to 0.54±0.10 mg/g of liver. The level of hepatic free cholesterol remained essentially unchanged (2.15±0.28 mg/g before and 2.23±0.24 mg/g at the end of the infusion). As exemplified in Fig. 1B, the rise in the level of hepatic lipids continued throughout the lymph infusion experiment. However, the hepatic concentrations of triglycerides and free and esterified cholesterol remained fairly constant in the saline-infused control dogs.

The crude membrane fractions prepared from livers of the saline-infused immature or cholestyramine-treated dogs displayed calcium-dependent, high affinity binding of canine ^{125}I-apo-E HDL$_c$ and ^{125}I-LDL. As shown in Fig. 2, the direct binding data obtained for the ^{125}I-apo-E HDL$_c$ of a cholestyramine-treated dog during the preinfusion phase (basal control period) and after the period of saline infusion, were essentially identical. The maximum amount of apo-E HDL$_c$

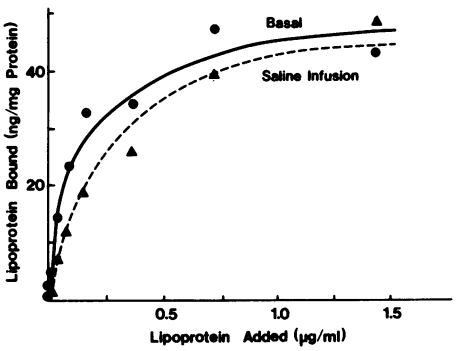

FIGURE 2 Calcium-dependent binding of ^{125}I-apo-E HDL$_c$ to liver membranes from dog 9, treated with cholestyramine (35 g/d for 3 wk), before and after infusion of saline for 6 h.

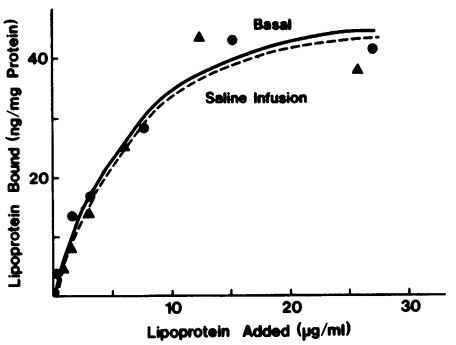

FIGURE 3 Calcium-dependent binding of ^{125}I-LDL to liver membranes from a cholestryamine-treated dog (dog 9) before and after infusion of saline for 6 h.

bound to the hepatic membranes during the preinfusion phase (basal period) was 48 ng of HDL_c protein/mg of membrane protein. The apparent equilibrium dissociation constant, as calculated from the Scatchard plot, was 0.2×10^{-9} M. There was no evident change in the binding characteristics of apo-E HDL_c at the end of the saline infusion period ($K_d = 0.2 \times 10^{-9}$ M; maximum binding (B_{max}) = 45 ng of lipoprotein protein/mg of membrane protein). The hepatic membranes from the same saline-infused dog also demonstrated saturable, calcium-dependent binding of LDL (Fig. 3). The affinity of the LDL binding was lower (apparent $K_d = 15 \times 10^{-9}$ M, both before and after saline infusion) than that of the apo-E HDL_c binding, as previously described (4, 5). The maximum amount of LDL bound was ~65 ng/mg of protein, both before and at the end of the saline infusion.

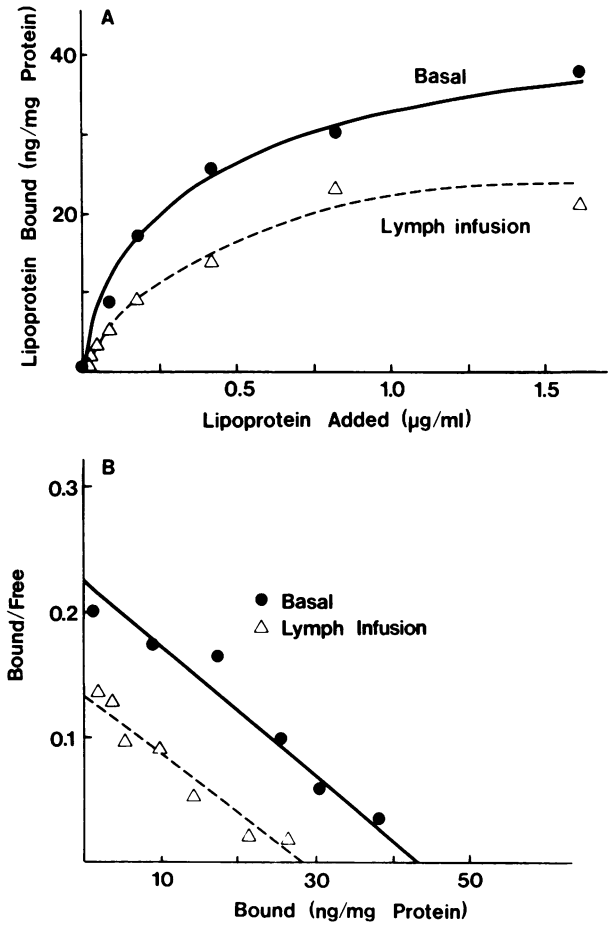

FIGURE 4 A Calcium-dependent binding of ^{125}I-apo-E HDL_c to liver membranes from dog 10, which were treated with cholestyramine (35 g/d for 3 wk) before and after infusion of lymph for 6 h. B Scatchard plot of the binding data.

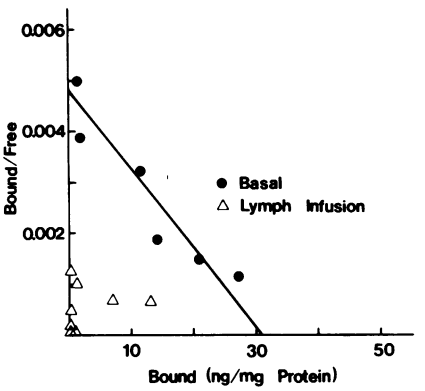

FIGURE 5 Scatchard plot of calcium-dependent binding of ^{125}I-LDL to liver membranes from a cholestyramine-treated dog (dog 10) before and after infusion of lymph for 6 h.

The hepatic membranes derived from immature or cholestyramine-treated dogs infused with lymph (Table I, dogs 2, 4, 6, 8, 10, and 12) likewise demonstrated calcium-dependent binding of apo-E HDL_c and LDL in the preinfusion basal state. However, after 6 h of lymph infusion, there was a reduction in the amount of apo-E HDL_c bound to liver membranes (Fig. 4A). The affinity of the binding did not change, but the maximum amount of apo-E HDL_c bound decreased (in this example, from 42 to 28 ng/mg of protein) (Fig. 4B). This decrease in the binding of apo-E HDL_c could theoretically be explained by a reduction of the binding activity of the apo-B,E and/or apo-E receptors. Studies of LDL binding showed that after 6 h of lymph infusion, saturable, high affinity, calcium-dependent binding of LDL to the same liver membranes was no longer demonstrable (Fig. 5). As previously described (30), binding of LDL and apo-E HDL_c to the apo-B,E receptor occurs in a proportion of 3–4:1. By using this ratio, a reduction of 8–10 ng/mg in apo-E HDL_c binding could be calculated to correspond to the loss of apo-B,E receptor binding activity in this experiment (31 ng/mg of protein). Thus, most, if not all, of the changes in apo-E HDL_c binding induced by lymph infusion could be accounted for by a near total loss of detectable apo-B,E receptor binding activity.

The prompt, dramatic decrease in apo-B,E receptor binding activity that followed the infusion of lymph lipoproteins into the immature or cholestyramine-treated dogs is illustrated in Fig. 6. The major reduction in LDL binding occurred between 2 and 4 h after the start of the lymph infusion. This rapid change in calcium-dependent LDL binding activity could be demonstrated repeatedly in both immature dogs and dogs treated with cholestyramine (Table II), whereas apo-E HDL_c binding activity was less dramatically affected by lymph infusion (Table III). The results from all the experiments, using a given concentration

of LDL or apo-E HDL_c for the binding assays, are summarized in Tables II and III. The results are expressed as the percentage of change in the activity based on the zero time (basal control) values. The mean reduction in binding of apo-E HDL_c, compared with parallel control values after 6–7 h of lymph infusion, was 28±16%. By contrast, the corresponding reduction in LDL binding was 88±8%.

To determine more clearly whether or not the apo-E receptor was significantly down-regulated by the infusion of intestinal lipoproteins, the binding of apo-E HDL_c to liver membranes from an adult dog (which expressed apo-E receptor binding activity and little detectable LDL binding) was measured during a 9-h infusion of lymph (Table I, dogs 11 and 12). Although high affinity LDL binding was not readily demonstrable (<1 ng of LDL protein bound/mg of hepatic membrane protein), calcium-dependent, saturable, high affinity binding of apo-E HDL_c was virtually unchanged by the lymph infusion. With an apparent K_d of 0.13×10^{-9} M, the maximum amount of apo-E HDL_c bound before lymph infusion was 37 ng of HDL_c protein/mg of hepatic membrane protein, and 35 ng of HDL_c protein/mg at the end of the 9 h of lymph infusion. Infusion of saline into an adult dog had no effect on the binding of apo-E HDL_c.

Chylomicron remnants are presumed to bind to both the apo-B,E and apo-E receptors. Results from the present study demonstrated that ^{125}I-labeled chylomicron remnants do in fact bind to both receptors. Even after a 6-h infusion of lymph, membranes from a cholestyramine-treated dog bound chylomicron remnants with high affinity (Fig. 7). The degree of reduction in maximum binding observed after suppression of apo-B,E receptor binding activity was in reasonable agreement with that seen for apo-E HDL_c by using membranes from the same dog (Fig. 4B). There was no change in the binding of chylomicron remnants to the liver membranes during saline infusion (data not shown).

The results thus far obtained indicated that the binding activity of the hepatic apo-B,E receptor, but not the apo-E receptor, was rapidly reduced by a plasma infusion of intestinal lipoprotein cholesterol and triglycerides. These observations were further tested by considering the effects of bile acid infusion on the regulation of the hepatic lipoprotein receptors. The biosynthesis of bile acids is regulated by the amount of bile acids returning to the liver (negative feedback) (31). Suppression of bile acid formation would therefore considerably reduce the hepatic demand for cholesterol. Consequently, we investigated whether canine hepatic lipoprotein receptors were affected by the infusion of exogenous taurocholate, the major endogenous bile acid in the dog (32–34).

When taurocholate was infused at a rate of 3.2 μmol/kg per min for 6 h into an immature dog, the calcium-dependent binding of apo-E HDL_c was reduced by ~50%, whereas the affinity of the binding remained unchanged (Fig. 8A). Before bile acid infusion, membranes from the immature dog bound large amounts of LDL with high affinity (K_d = 17

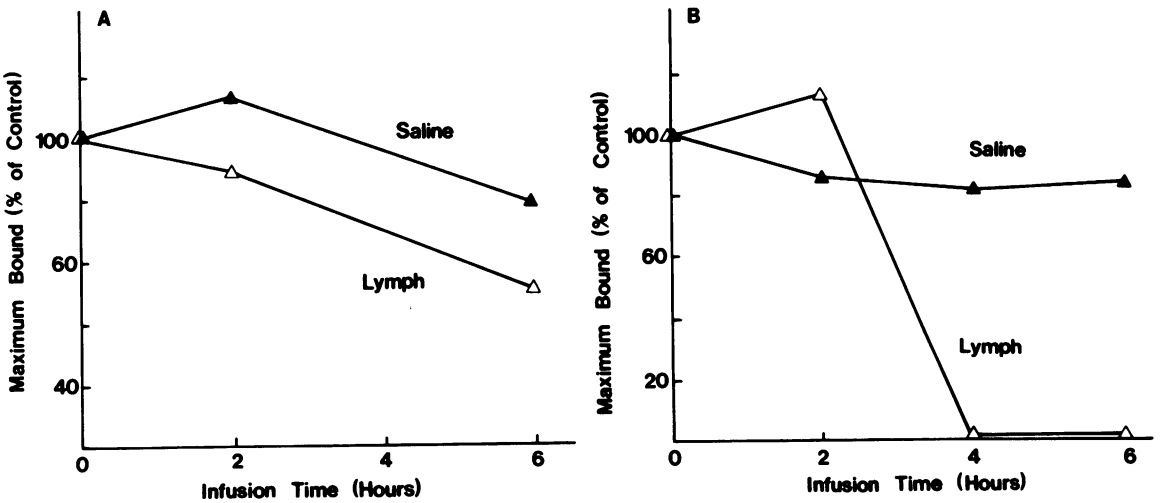

FIGURE 6 Binding of apo-E HDL_c (A) and LDL (B) to liver membranes from cholestyramine-treated dogs infused with saline or lymph for 6 h. The data shown are the maximum amounts of apo-E HDL_c or LDL bound (calculated from the Scatchard plots) expressed as a percentage of the basal values. The results are from a pair of littermate dogs (dogs 9 and 10) treated with cholestyramine (35 g/d for 3 wk) before the experiment.

TABLE II

*Specific (Calcium-dependent) Binding of ^{125}I-LDL to Liver Membranes from Immature or Cholestyramine-treated Dogs Infused with Saline or Lymph**

		Binding activity							
		Infusion time							
		h							
Dog	Infusion	0	1	2	3	4	5	6	7
		% (of initial)							
Immature dogs									
1	Saline	100 (12.7)‡	85	84	186	—	81	—	81
2	Lymph	100 (11.9)	88	42	17	—	<1	—	<1
3	Saline	100 (17.4)	—	63	—	82	—	51	—
4	Lymph	100 (17.0)	—	18	—	<1	—	<1	—
5	Saline	100 (23.4)	—	—	57	—	91	—	—
6	Lymph	100 (58.2)	—	—	38	—	23	—	—
Cholestyramine-treated dogs									
7	Saline	100 (17.4)	—	106	—	92	—	64	—
8	Lymph	100 (10.8)	—	79	—	46	—	<1	—
9	Saline	100 (10.5)	—	90	—	52	—	67	—
10	Lymph	100 (9.8)	—	105	—	11	—	24	—

* The assay of LDL binding to hepatic membranes was performed as described in Methods by using a constant concentration of membrane protein (100–200 μg) and labeled lipoprotein (4.0–5.3 μg/ml) within each experiment. Data are given as a percentage of the initial (basal control) value.

‡ Absolute values for the binding of LDL (nanograms of lipoprotein protein per milligram of membrane protein) appear in parentheses.

TABLE III

*Specific (Calcium-dependent) Binding of ^{125}I-Apo-E HDL$_c$ to Liver Membranes from Immature or Cholestyramine-treated Dogs Infused with Saline or Lymph**

		Binding activity							
		Infusion time							
		h							
Dog	Infusion	0	1	2	3	4	5	6	7
		% (of initial)							
Immature dogs									
1	Saline	100 (12.1)‡	141	112	162	—	150	—	80
2	Lymph	100 (9.3)	84	86	38	—	35	—	24
3	Saline	100 (9.3)	—	102	—	70	—	40	—
4	Lymph	100 (12.0)	—	81	—	—	—	48	—
5	Saline	100 (21.0)	—	—	56	—	79	—	—
6	Lymph	100 (36.7)	—	—	44	—	42	—	—
Cholestyramine-treated dogs									
7	Saline	100 (15.7)	—	78	—	69	—	72	—
8	Lymph	100 (15.2)	—	113	—	44	—	57	—
9	Saline	100 (26.7)	—	112	—	94	—	95	—
10	Lymph	100 (27.0)	—	104	—	63	—	79	—

* The assay of apo-E HDL$_c$ binding to hepatic membranes was performed as described in Methods by using a constant concentration of membrane protein (100–200 μg) and labeled lipoprotein (0.24–0.73 μg/ml) within each experiment. Data are given as a percentage of the initial (basal control) value.

‡ Absolute values for the binding of apo-E HDL$_c$ (nanogram of lipoprotein protein per milligram of membrane protein) appear in parentheses.

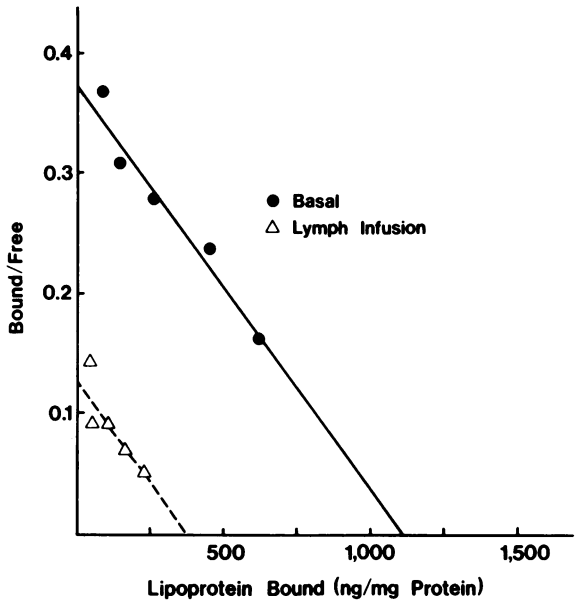

FIGURE 7 Scatchard plot of calcium-dependent binding of chylomicron remnants to liver membranes from a cholestyramine-treated dog (dog 10) before and after infusion of lymph for 6 h. Binding studies were performed at 23°C with ^{125}I-labeled dog chylomicron remnants.

$\times 10^{-9}$ M) in the presence of calcium (Fig. 8B). After 6 h of taurocholate infusion, this binding was almost totally abolished (Fig. 8B). The change in maximum binding of apo-E HDL_c could thus be explained by the disappearance of apo-B,E receptor binding activity, whereas the binding activity of the apo-E receptor appeared to be relatively unaffected. In agreement with the results obtained in the lymph infusion studies, the binding of chylomicron remnants was reduced to the same extent as was that of apo-E HDL_c (Fig. 8C). There was no change in the binding activity of any of the three lipoproteins (apo-E HDL_c, LDL, and chylomicron remnants) during saline infusion in the parallel control dog (data not shown).

A rapid reduction in LDL binding activity after taurocholate infusion was confirmed in three immature dogs, as well as in a cholestyramine-treated dog (Table IV). The changes in apo-E HDL_c binding were much less pronounced (Table V). The binding activities of the LDL and apo-E HDL_c were compared by using a LDL concentration of 4.2–7.3 μg/ml and an apo-E HDL_c concentration of 0.2–0.6 μg/ml in the binding assays. The mean relative reduction at 6 h, compared with the matched saline-infused controls, was 69±16% for LDL binding, and only 20±15% for apo-E HDL_c binding.

The marked reduction in apo-B,E receptor binding to LDL observed in the membrane binding assay of the cholestyramine-treated dog (Table I, dog 20) was also observed in the solubilized liver membrane binding assay for the same dog (Table VI). The LDL binding was reduced from 303 to 19 ng/mg of solubilized liver membrane protein by taurocholate infusion (3 μmol/kg per min). These results, obtained with the solubilized receptor assay, suggested that the decrease in apo-B,E receptor activity, as determined by the membrane binding assay, was not due simply to a masking of receptor binding sites on the membranes.

In order to characterize further the possible role of bile acids as regulators of hepatic membrane apo-B,E receptor binding activity, total bile fistulae were created in three adult dogs. While the dogs were under general anesthesia, a liver biopsy (basal) was obtained, and an occludable T-tube was inserted into the common bile duct after cholecystectomy (as described in Methods). 5 wk after the operation, the distal limb of the T-tube was occluded, thus creating a total bile fistula. After biliary drainage for 5 d, each dog was anesthetized, a base-line liver biopsy was obtained (referred to as a bile fistula sample), and an infusion of taurocholate (3 μmol/kg per min) was given for 6 h (Table I, dogs 21, 23, and 25).

The interruption of the enterohepatic circulation of the bile acids by the fistula induced apo-B,E receptor binding activity, as evidenced by high affinity, calcium-dependent binding of LDL to liver membranes (Table VII). There was also a clear increase in the binding of apo-E HDL_c, most of which could be accounted for by the binding of HDL_c to the apo-B,E receptors. However, some increase in the binding activity of the apo-E receptor cannot be excluded. The reinfusion of taurocholate, which presumably suppressed the bile acid biosynthesis induced by the bile fistula, resulted in a marked reduction of LDL binding and a return of apo-E HDL_c binding to initial levels (Table VII). Similar changes were observed with solubilized membrane receptors (Table VIII). Thus, stimulation of bile acid formation was associated with the induction of apo-B,E receptor binding activity, and suppression of bile acid biosynthesis resulted in rapid reduction in this activity.

It could be argued that the suppressive action of taurocholate was due to a less specific action of the bile acid on hepatic plasma membranes, e.g., the bile acids exerting a detergent effect on the cell surface receptors. However, such an effect would have had to be directed only against the apo-B,E receptors, because binding to the apo-E receptor was still observed. In order to test this possibility, we studied the effect of taurocholate on LDL binding activity in a cultured fibroblast system. When taurocholate, at concentrations of up to 1.5 mM, was incubated for 16 h with confluent human fibroblasts grown in lipoprotein-de-

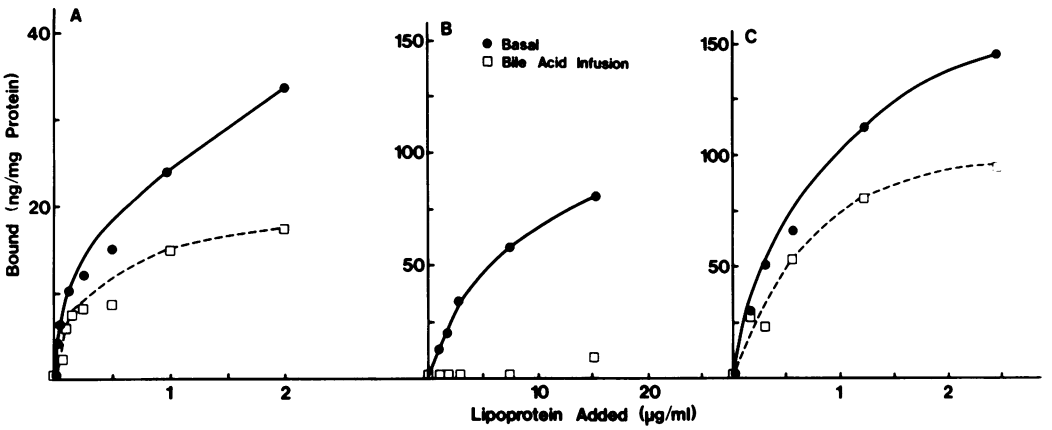

FIGURE 8 A Calcium-dependent binding of apo-E HDL_c to liver membranes of an immature dog (dog 18) before and after infusion of taurocholate 3.2 μmol/kg per min, for 6 h. B Calcium-dependent binding of LDL to the same liver membranes. C Calcium-dependent binding of chylomicron remnants to the same liver membranes.

ficient serum, there was no effect on the direct binding of ^{125}I-LDL (data not shown). This corresponds to a much higher concentration of taurocholate in the medium than was obtained in the infusion studies (mean plasma bile acid concentration after 6 h of infusion, 0.22±0.05 mM, n = 6). In addition, plasma membranes from the adrenals of taurocholate-infused dogs (dogs 20, 27, and 28) still bound large amounts of LDL with high affinity (K_d = 25, 18, and 27 × 10^{-9}; B_{max} = 380, 767, and 877 ng of lipoprotein/mg of membrane protein, respectively), even though the liver membranes obtained simultaneously showed no demonstrable binding of LDL. The data for the adrenal membrane binding of LDL closely agreed with those reported previously for normal dogs (35) and for a saline-infused dog (dog 19: K_d = 12 × 10^{-9}, B_{max} = 855 ng/mg of protein). The binding data obtained with adrenal membranes suggested that hepatic and adrenal apo-B,E receptors are regulated independently. As would be anticipated, taurocholate infusion resulted in a profound down-regulation of the hepatic apo-B,E receptors, without an obvious effect on the adrenal apo-B,E receptor binding activity.

Finally, the relative insensitivity of the apo-E receptor to acute regulation by bile acid infusion was studied in adult dogs expressing predominantly the hepatic apo-E receptor. Liver membranes from dog 22 bound apo-E HDL_c with an apparent K_d of 0.3 × 10^{-9} M both before and after 6 h of taurocholate infusion. The maximum amount of apo-E HDL_c bound was 23 ng/mg of protein before the infusion and 22 ng/mg of protein at the end of the infusion. Similar results were obtained when binding to solubilized membrane receptors was determined in two additional adult dogs (Table IX).

Thus, the binding activity of the apo-B,E receptors in dog liver membranes appears to be subject to rapid regulation in response to changes in the hepatic de-

TABLE IV
*Specific (Calcium-dependent) Binding of ^{125}I-LDL to Liver Membranes from Immature or Cholestyramine-treated Dogs Infused with Saline or Taurocholate**

		Binding activity			
		Infusion time			
		h			
Dog	Infusion	0	2	4	6
		% (of initial)			
Immature dogs					
13	Saline	100 (5.6)‡	105	81	59
14	Taurocholate	100 (5.7)	37	11	7
15	Saline	100 (8.2)	99	110	74
16	Taurocholate	100 (10.3)	68	33	53
17	Saline	100 (25.1)	100	106	89
18	Taurocholate	100 (29.8)	100	49	37
Cholestyramine-treated dogs					
19	Saline	100 (31.8)	101	80	89
20	Taurocholate	100 (21.0)	32	<1	<1

* The assay of LDL was performed as described in Methods by using a constant concentration of membrane protein (100–200 μg) and labeled lipoprotein (4.2–7.3 μg/ml) within each experiment. Data are presented as a percentage of the initial (basal control) value.

‡ The absolute values for the binding of LDL (nanograms of lipoprotein protein per milligram of membrane protein) appear in parentheses.

TABLE V

*Specific (Calcium-dependent) Binding of ^{125}I-Apo-E HDL$_c$ to Liver Membranes from Immature or Cholestyramine-treated Dogs Infused with Saline or Taurocholate**

Dog	Infusion	Binding activity, Infusion time 0 h	2 h	4 h	6 h
			% (of initial)		
Immature dogs					
13	Saline	100 (12.2)‡	104	83	61
14	Taurocholate	100 (16.2)	58	68	65
15	Saline	100 (10.1)	97	87	71
16	Taurocholate	100 (11.8)	62	42	72
17	Saline	100 (11.4)	87	105	115
18	Taurocholate	100 (8.9)	51	100	82
Cholestyramine-treated dogs					
19	Saline	100 (19.5)	106	92	96
20	Taurocholate	100 (23.1)	42	58	38

* The assay of the binding of apo-E HDL$_c$ was performed as described in Methods by using a constant concentration of membrane protein (100–200 μg) and labeled lipoprotein (0.22–0.63 μg/ml) within each experiment. Data are given as a percentage of the initial (basal control) value.

‡ The absolute values for the binding of apo-E HDL$_c$ (nanograms of lipoprotein protein per milligram of membrane protein) appear in parentheses.

mand for cholesterol. An important question is whether these changes in hepatic membrane binding of LDL observed in vitro are reflected by corresponding changes in LDL catabolism in vivo. If receptor-mediated hepatic uptake of canine LDL is of importance to the overall catabolism of this lipoprotein, we would expect to observe an increased plasma concentration of LDL during lymph or bile acid infusion, due to the reduction in apo-B,E receptor activity. Such studies are hampered by the presence of lymph lipoproteins in the plasma after lymph infusion; however, the bile acid infusion experiments provided an opportunity to test this hypothesis. Although total plasma cholesterol was reduced in cholestyramine-treated and bile-diverted animals (Table I), there were no consistent changes in total plasma lipids during infusion. Direct measurement of the changes in LDL concentrations proved to be impossible because of the very low levels of canine plasma LDL and because of the several steps necessary to purify canine LDL (ultracentrifugation and preparative electrophoresis).

However, it was possible to access indirectly the changes in plasma lipoproteins by comparing the abilities of the plasma lipoproteins ($d < 1.21$ fraction) to compete with LDL for binding to the apo-B,E receptors of fibroblasts before and during bile acid infusion. It was observed that the $d < 1.21$ lipoprotein fraction of the taurocholate-infused, cholestyramine-treated dog (dog 20) had a greater ability, based on cholesterol content of the $d < 1.21$ fraction, to bind to the apo-B,E receptors of human fibroblasts than did the corresponding $d < 1.21$ fraction obtained from the saline-infused, cholestyramine-treated control dog (dog 19). Thus, 50% displacement of ^{125}I-LDL binding occurred at a cholesterol concentration of 5 μg/ml in the bile acid-infused dog after 6 h, vs. 50% displacement of LDL at 20 μg/ml in the saline-infused control after 6 h. As shown in Fig. 9A, more detailed studies demonstrated a progressive increase with time in the bind-

TABLE VI

*Specific (Calcium-dependent) Binding of ^{125}I-Apo-E HDL$_c$ and ^{125}I-LDL to Solubilized Liver Membrane Receptors before and during Infusion with Taurocholate, 3 μmol/kg per min**

	Binding of Apo-E HDL$_c$	Binding of LDL
	ng/mg	
Basal	269	303
Taurocholate, 4 h	102	19

* The solubilization of membrane receptors was performed by using octyl-β-D-glucoside (dog 20). The binding activity of the solubilized membrane was determined by incubating 50 μg of solubilized protein with 0.9 μg/ml of ^{125}I-apo-E HDL$_c$ or 30 μg/ml of ^{125}I-LDL at 0°C for 90 min in the absence and presence of 30 mM EDTA.

TABLE VII

*Specific (Calcium-dependent) Binding of Apo-E HDL$_c$ and LDL to Liver Membranes from an Adult Dog (No. 21) before and after Creation of a Total Bile Fistula and after Reinfusion of Taurocholate for 6 h**

	Binding of Apo-E HDL$_c$		Binding of LDL	
	Apparent K_d	Maximum bound	Apparent K_d	Maximum bound
	$M \times 10^9$	ng/mg	$M \times 10^9$	ng/mg
Basal	0.10	18	ND‡	<1
Bile fistula (5 d)	0.18	46	13	48
Reinfusion of taurocholate	0.14	22	ND	<1

* Data obtained from Scatchard analysis of binding curves (26).

‡ ND, high affinity binding not demonstrable.

TABLE VIII

Specific (Calcium-dependent) Binding of Apo-E HDL_c and LDL to Solubilized Receptors of Liver Membranes from Dogs 23 and 25 before and after Total Biliary Diversion, and after Perfusion with Taurocholate for 6 h[*]

	Binding of Apo-E HDL_c		Binding of LDL	
	Dog 23	Dog 25	Dog 23	Dog 25
	ng/mg protein			
Basal	47	—	4	—
Bile fistula	358	73	71	124
Reinfusion of taurocholate	60	12	<1	29

[*] Liver membrane protein (~10 μg of protein) solubilized with CHAPS was incubated with apo-E HDL_c (0.58 and 0.47 μg/ml for dogs 23 and 25, respectively) and LDL (9.0 and 16.2 μg/ml, respectively) for 90 min at 0°C in the absence and presence of 30 mM EDTA. No basal sample was available from dog 25.

ing activity of the $d < 1.21$ plasma lipoprotein fraction of a bile fistula dog (dog 23) during taurocholate infusion. A 50% displacement of the ^{125}I-LDL occurred at a cholesterol concentration of 18 μg/ml for the $d < 1.21$ fraction before the infusion of taurocholate and at a cholesterol concentration of 2 μg/ml after 6 h of taurocholate infusion. There was also a small increase in binding to the fibroblast receptor of the $d < 1.21$ fraction of the taurocholate-infused adult dog (Fig. 9B). Furthermore, gradient gel electrophoresis of the plasma of the taurocholate-infused, bile fistula dog (dog 23) demonstrated a relative increase in the lipid staining of the LDL. As shown in Fig. 10, microdensitometric scanning of the gel patterns revealed a significant change in LDL concentration, comparing the plasma sample taken immediately before the bile acid infusion with that obtained after 4 h of infusion. No change in the plasma lipoprotein distribution was observed in the taurocholate-infused adult dog (dog 24).

DISCUSSION

The present studies were performed to determine whether rapid regulation of the binding activity of hepatic lipoprotein receptors occurs in response to acute changes in hepatic cholesterol demand. The results show a marked difference in the metabolic regulation of the two receptors previously demonstrated on hepatic membranes, the apo-B,E receptor and the apo-E receptor. In response to two completely different perturbations of hepatic cholesterol metabolism, an excessive load of exogenous cholesterol in the form of lymph lipoproteins and an induced feedback inhibition of bile acid biosynthesis by the infusion of exogenous bile acids, there was a dramatic decrease in the binding activity of the apo-B,E receptors on canine hepatic membranes. On the other hand, the binding activity of the apo-E receptors on the same membranes was not significantly affected. These results from short-term (acute) experiments are in accordance with prior observations from long-term experiments in which evidence was found for metabolic regulation of apo-B,E, but not for apo-E receptor binding activity (2, 4, 5).

The significance of the hepatic, receptor-mediated uptake of apo-E-containing lipoproteins has been shown previously (4, 5, 8–11), and is further illustrated in the present studies. The importance of apo-E in the catabolism of chylomicron remnants is evident from observations of patients with type III hyperlipoproteinemia; such subjects have a specific variant form of apo-E (E2) (36–38). This isoform does not bind normally to lipoprotein receptors (39–41) and, consequently, these subjects accumulate chylomicron remnants (β-very low density lipoproteins [β-VLDL]) in their plasma despite normal amounts of apo-B in the remnants (42). The importance of the apo-E receptor in the binding and subsequent uptake of chylomicron remnants by the liver can be inferred from at least two lines of evidence in the present study. First, the changes in the binding of chylomicron remnants to hepatic membranes followed very closely the pattern of binding of apo-E HDL_c to the membranes, but not that of LDL (Figs. 4B, 5, 7, and 8). This implies that chylomicron remnants can bind to both apo-B,E and apo-E receptors. Second, in spite of a continued infusion of lymph chylomicrons over a time period when LDL binding was almost totally abolished (cf Fig. 6B), there was no evidence of a progressive increase in

TABLE IX

Specific (Calcium-dependent) Binding of Apo-E HDL_c to Solubilized Receptors of Liver Membranes from Dogs 24 and 26 before and after Infusion of Taurocholate for 6 h[*]

	Binding of Apo-E HDL_c	
	Dog 24	Dog 26
	ng/mg protein	
Basal	81	35
Taurocholate	100	20

[*] Liver membrane protein (10 μg of protein) solubilized with CHAPS was incubated with apo-E HDL_c (0.58 and 0.47 μg/ml for dogs 24 and 26, respectively) for 90 min at 0°C in the absence and presence of 30 mM EDTA. The binding of LDL (at concentrations of 9.0 and 16.2 μg/ml for dogs 24 and 26, respectively) was <1 ng/mg of protein in both dogs.

plasma lipids, and hepatic lipids continued to increase (cf Fig. 1). This suggests that the apo-E receptor binding activity of canine hepatic membranes may handle a considerable load of lipoproteins of dietary origin. Accordingly, the adult dog, which predominantly expressed apo-E receptor binding activity, did not show a reduced capacity for clearing lymph chylomicrons. If we assume that all of the cholesterol of the infused lymph was taken up by the liver, it can be estimated that the apo-E receptor-mediated uptake cleared at least 1.5 g of cholesterol during a typical 6-h experiment.

It would be interesting to consider whether the rapid loss of apo-B,E receptor binding activity observed during lymph infusion is of importance under more physiological conditions. Feeding a diet rich in cholesterol and saturated fat to immature dogs has been shown to repress hepatic apo-B,E receptor binding activity almost completely within 30 d (5). No information is presently available with regard to the effect of shorter periods of cholesterol feeding in the dog. However, hepatic LDL binding activity was reduced by ~50% as soon as 2 d after the initiation of a cholesterol-rich diet in the rabbit (43). It is reasonable to speculate that the ingestion of meals rich in cholesterol may affect the hepatic uptake of LDL by rapidly influencing the apo-B,E receptor. Differences in the extent of the regulation of apo-B,E receptors, as well as in the expression of apo-E receptors, among various species may thus provide an explanation to the known variability

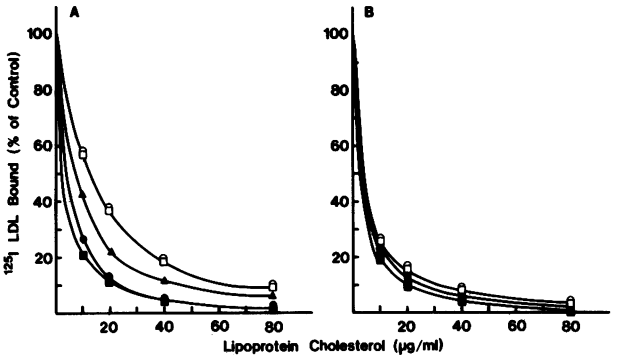

FIGURE 9 A Comparison of the ability of the $d < 1.21$ fractions from a bile fistula dog (dog 23) infused with taurocholate (3 μmol/kg per min) to compete with human ^{125}I-LDL for binding to normal human fibroblasts. Plasma was obtained 30 min before (○) and at the start of the infusion (□), and then at 2 (▲), 4 (●), and 6 (■) h after infusion. B Comparison of the ability of the corresponding plasma fractions from a taurocholate-infused adult dog (dog 24) to compete with human ^{125}I-LDL for binding to normal human fibroblasts. Studies were performed at 4°C for 2 h with 2 μg/ml of ^{125}I-LDL and the indicated concentrations of $d < 1.21$ lipoprotein fraction added on the basis of total cholesterol.

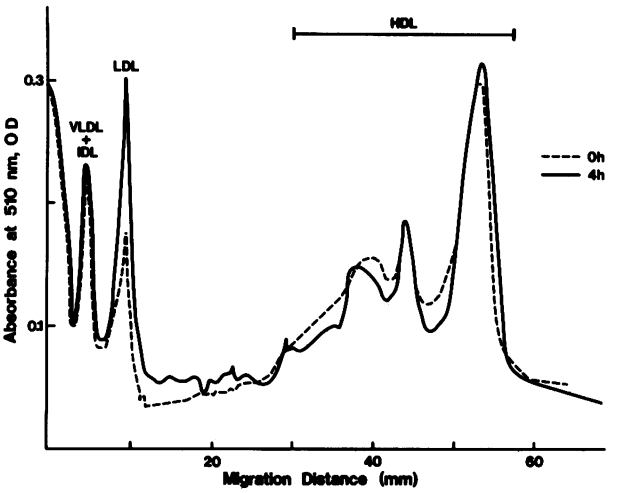

FIGURE 10 Microdensitometry scans of gradient gels (4–30% acrylamide) obtained after lipid staining of the plasma from a taurocholate-infused, bile fistula dog before the start of the infusion (- - -) and after 4 h of infusion (——). The regions of the scan labeled as LDL and HDL were identified by comparison with purified canine LDL and HDL (d = 1.063–1.21). The large particles that appear at the top of the gel represent VLDL and intermediate density lipoproteins (IDL).

in the response to such diets. Further work is clearly needed to evaluate this possibility.

In agreement with previous studies (4–6), the present work demonstrates that an increased expression of hepatic apo-B,E receptors could be induced by conditions that stimulate the biosynthesis of bile acids, i.e., cholestyramine treatment and biliary diversion. These results indicate that the hepatic requirement for cholesterol to be used in the biosynthesis of bile acids is an important regulator of apo-B,E receptor activity in the liver. The total interruption of the enterohepatic circulation of bile acids, which results in maximal induction of bile acid biosynthesis (31), was linked to a rapid and dramatic induction (within 5 d) of apo-B,E receptor expression on hepatic membranes. The binding activity of these receptors was almost totally abolished when enhanced bile acid biosynthesis was suppressed by infusing bile acids into the circulation. A similar marked and rapid decrease in apo-B,E receptor binding was achieved by infusing bile acids into cholestyramine-treated dogs, as well as into immature dogs (Table IV). The infusion rate chosen was high enough to suppress bile acid biosynthesis almost totally, but still well below the maximum excretory capacity of the liver (32–34).

The association between hepatic LDL uptake (apo-B,E receptor activity) and bile acid metabolism has considerable relevance to some recent observations in man. Cholestyramine treatment has been shown to

reduce plasma LDL levels in individuals with heterozygous familial hypercholesterolemia by increasing the receptor-mediated elimination of LDL (44). However, plasma LDL levels in patients with homozygous (receptor-negative) familial hypercholesterolemia are not lowered even by treatment with a total bile fistula (45). In addition, suppression of bile acid biosynthesis in man, which occurs during treatment with the primary bile acid chenodeoxycholic acid (46, 47), results in a slight, but significant, increase in plasma LDL levels (48, 49).[2] Chenodeoxycholic acid treatment in man is associated with decreased hepatic cholesterol synthesis (50), a reduced production rate of VLDL (51) (the precursor of human plasma LDL), and possibly a reduction in LDL catabolism. The data in the present study suggest that the increase in plasma LDL levels and a possible reduction in LDL catabolism may be related to the rapid and dramatic down-regulation of hepatic apo-B,E receptor activity after bile acid treatment.

A particularly significant finding in the present work was the observation that the reduction in apo-B,E receptor binding activity on dog liver membranes occurred so rapidly (i.e., within 2–4 h), in response to both lymph and bile acid infusion. The decrease in binding activity was not only detected with the membrane binding assay, but also with the solubilized membrane preparations. The reduction was apparently limited to the liver, as the binding of LDL to adrenal membranes was not changed by bile acid infusion. While the regulation of the hepatic apo-B,E (LDL) receptors appears to be controlled by the cellular need for cholesterol, these results indicate that different mechanisms may operate to control receptor expression in various tissues. For example, in fibroblasts, preincubation with LDL for ~24 h is necessary to obtain substantial down-regulation of receptor binding activity. The decrease in LDL binding activity in fibroblasts is the consequence of reduced synthesis, and thus of a decrease in the apparent number of receptor molecules (52). However, in some situations there may be a more rapid regulation of the activity of the fibroblast receptor (53). The rapidity of the down-regulation of hepatic apo-B,E receptor activity (within 2–4 h) suggests that a mechanism independent of protein synthesis may be involved in modulating the expression of the hepatic apo-B,E receptors.

The mechanism responsible for the rapid loss of apo-B,E receptor binding activity on hepatic membranes cannot be determined from the present work; however, several possibilities should be considered. One interesting possibility is that the hepatic receptor is subject to rapid recycling, as has been demonstrated for the fibroblast receptor (54). In cultured human fibroblasts, the LDL (apo-B,E) receptors recycle every 12–15 min, and inhibition of this process can lead to a rapid loss of cell surface binding activity without a change in the total number of receptors. For example, in one study the treatment of fibroblasts with the ionophore monensin, which prevents receptor recycling, resulted in a loss of 75% of the surface binding activity of LDL within 15 min, and of 90% within 60 min (55). In the present study of hepatic apo-B,E receptors, intracellular structural or conformational modification, or accelerated degradation of the recycling receptors, which would prevent expression of the apo-B,E receptors, could explain the rapid down-regulation of these receptors. To test these possibilities, experiments using a suitable in vitro culture system would be necessary. Whatever the mechanism, however, the present study presents clear evidence that the apo-B,E binding activity can be rapidly modulated and that the two hepatic receptors, the apo-B,E and the apo-E receptors, behave differently in response to induced changes in hepatic cholesterol metabolism.

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ACKNOWLEDGMENTS

We thank Ms. Evelyn Kavaler and Mr. Edwin Ludwig for their excellent technical assistance, Ms. Patsy Babbitt for lipid analysis, and Mr. Peter Lindquist for assisting in animal surgery. We thank Dr. David Burke for performing the gradient gel electrophoresis, and Dr. Ernest Friedlander for his help in assays involving receptor solubilization. We appreciate the editorial assistance of Mr. Russell Levine and the manuscript preparation by Mr. Richard Wolfe. Also, we thank Ms. Gwen Watson for graphics illustration.

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