Cholestyramine-induced changes in low density lipoprotein composition and metabolism. I. Studies in the guinea pig

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Abstract In previous animal studies, bile acid sequestrant resins have been shown to increase the fractional catabolic rate (FCR) of a low density lipoprotein (LDL) tracer isolated from a normal donor animal and to increase hepatic LDL-receptor activity. In addition, in man, these resins are known to alter LDL composition such that low density lipoproteins are smaller, more dense, and have a decreased cholesterol:protein ratio. To determine whether metabolic consequences resulted from these changes in LDL composition, we fed cholestyramine chow (2% resin by weight) to guinea pigs, which lowered LDL cholesterol levels by 55%. LDL was isolated from control donors (C-LDL) and from cholestyramine-treated donors (CH-LDL). Compared to the C-LDL, the CH-LDL were smaller in size, depleted of cholesteryl ester and phospholipid, and had a marked decrease in their cholesterol:protein ratio. To determine whether the clearance of the altered CH-LDL was different from that of C-LDL, we labeled the two LDL preparations with ^{125}I or ^{131}I and simultaneously injected them into control and cholestyramine-treated guinea pigs. In 27/29 animals studied, the FCR of the CH-LDL was slower than that of C-LDL, demonstrating that the compositional changes alter the metabolism of CH-LDL. When C-LDL was used as the sole tracer in both control and treated animals, cholestyramine treatment increased the FCR by 41%; when CH-LDL was used as sole tracer, the increase in FCR on treatment was only 26%. This suggested that C-LDL was cleared more rapidly by the LDL-receptor pathway than was CH-LDL. Further support for this idea came from observations that C-LDL was degraded more readily by cultured fibroblasts and that nonenzymatic glucosylation abolished the difference in FCR between C-LDL and CH-LDL. These studies show that the effects of bile sequestration are complex and that the compositional changes produced have profound metabolic consequences. The implications of these observations for interpretation of LDL turnover studies are discussed.—**Witztum, J. L., S. G. Young, R. L. Elam, T. E. Carew, and M. Fisher.** Cholestyramine-induced changes in low density lipoprotein composition and metabolism. I. Studies in the guinea pig. *J. Lipid Res.* 1985. **26:** 92–103.

Supplementary key words altered low density lipoprotein composition and metabolism • fractional catabolic rate

Bile acid sequestrant resins are widely used in the therapy of hypercholesterolemia as they specifically lower plasma concentrations of low density lipoproteins (LDL) (1–3). In previous animal studies, they have been shown to increase the fractional catabolic rate (FCR) of normal LDL and to increase hepatic LDL-receptor activity (4–8). In addition to these effects we have previously shown that these resins, when administered to human subjects, produce marked alterations in the composition of LDL (1, 2) (S. G. Young, J. L. Witztum, T. E. Carew, R. M. Krauss, and F. T. Lindgren, unpublished results). In particular, the low density lipoproteins are more dense, smaller in size, and have a decreased cholesterol:protein ratio. The mechanisms underlying these compositional changes are unknown, nor is it known whether such changes in composition have metabolic consequences. To answer these questions, we developed an animal model in which bile sequestration would produce similar changes in LDL composition, and then determined whether metabolic consequences resulted from such changes. In this report we show that bile acid sequestrant resins effectively lower LDL levels in guinea pigs, and produce many of the alterations in LDL composition noted in humans. We also show that such altered LDL have a different FCR than LDL isolated from normal animals.

MATERIALS AND METHODS

Male Hartley guinea pigs weighing 0.5–1.0 kg were purchased from Charles River Breeding Labs, Inc.

Abbreviations: LDL, low density lipoprotein; FCR, fractional catabolic rate; HDL, high density lipoprotein; TMU, tetramethylurea.

(Wilmington, MA). They were fed fresh Wayne guinea pig pellets or Wayne guinea pig pellets containing 2% cholestyramine resin by weight (or colestipol hydrochloride) formed into standard guinea pig pellets by ICN Nutritional Biochemicals (Cleveland, OH). The cholestyramine resin was a gift from A. Alberts (Merck, Sharp and Dohme Research Laboratories, Rahway, NJ) and the colestipol-HCl was a gift from T. Vecchio (the Upjohn Company, Kalamazoo, MI). Control and treated guinea pigs consumed approximately equal amounts of food and gained weight at equivalent rates. Serial measurements in five treated animals indicated that plasma cholesterol levels reached new steady state levels 10 days after initiation of the drug, and in all subsequent studies treated animals were used for experiments only after a minimum of 10–14 days of treatment.

Lipoprotein isolation and characterization

To determine the effect of cholestyramine on plasma lipoprotein levels, separate aliquots of plasma from three control (chow-fed) and three cholestyramine-fed guinea pigs were simultaneously centrifuged in a Beckman Ti-50.3 rotor at d 1.019, 1.063, and 1.21 g/ml. In the guinea pig the LDL density range may extend into a higher density than 1.063 g/ml (9, 10); however, we chose to use a 1.019–1.063 g/ml fraction to avoid contamination with higher density fractions. After centrifugation at 40,000 rpm for 48 hr, the floating lipoproteins were pipetted from the top of each tube and adjusted to original plasma volume, and the total cholesterol content was determined enzymatically. Values for individual lipoproteins were determined by difference. The cholesterol content of the individual density fractions equalled 100 ± 5% of the total plasma cholesterol.

To isolate LDL for the compositional and metabolic studies described below, plasma from three or four control guinea pigs and four or five treated guinea pigs was pooled and LDL was isolated between densities d 1.019 and 1.063 g/ml with a "wash" at the upper and lower densities (11). Densities were checked for accuracy by measuring the density of the clear middle section of the lipoprotein spin solution, using a DMA 45 Digital Density Meter (Anton Paar, Graz, Austria). LDL preparations were dialyzed against phosphate-buffered saline, pH 7.35, containing 1 mM ethylenediaminetetraacetic acid (EDTA). Protein content of the LDL was determined by the method of Lowry et al. (12). Free and total cholesterol contents were determined enzymatically (Boehringer Mannheim Kit #124087), and mg of cholesteryl ester was calculated as 1.67 × mg of esterified cholesterol. Triglycerides were measured by an enzymatic method (Boehringer Kit #126012), and phospholipids by a micromodification of the Bartlett procedure (13). LDL apoprotein content was examined by precipitation with tetramethylurea (TMU) (14) and by SDS polyacrylamide slab gel electrophoresis, in gels of either a 4–20% gradient, or a 3–6% gradient (15). Gels were stained with Coomassie Blue, and the uptake of dye was quantified with a Transidyne 2510 scanning densitometer. To determine the size of the LDL particles, aliquots of LDL were negatively stained with 2% aqueous potassium phosphotungstate and electron micrographs of equal magnification were made. The diameters of 125 free-standing particles were measured on different photographs taken from different areas of the grid for each preparation. These results were plotted as a frequency distribution.

For studies of LDL metabolism, LDL samples were iodinated with ^{125}I (Amersham Co., Chicago, IL) or ^{131}I (ICN Chemical and Radioisotope Division, Irvine, CA) using a modification of the iodine monochloride method as previously described (16). Specific activities for the iodinated LDL preparations varied from 100 to 300 cpm/ng protein and the trichloroacetic acid (TCA) precipitability of LDL radioactivity was greater than 98% for all preparations used in cell culture, and in vivo experiments.

Metabolic studies of LDL

To determine whether the cholestyramine-induced changes in LDL composition altered the metabolism of LDL, LDL was isolated from control guinea pigs (C-LDL) and from cholestyramine-treated guinea pigs (CH-LDL). The protocol for these studies is shown in **Fig. 1**. In each case, LDL was derived from pools made up of samples from three to five animals, labeled with ^{125}I or ^{131}I, and equal amounts of each LDL protein was mixed, filtered through a 0.45-μm filter (Millipore Company, Bedford, MA), and injected into control and cholestyramine-treated guinea pigs. Mean weights of the two groups of animals at time of study did not differ (668 ± 133 vs. 769 ± 165, mean ± SD). Guinea pigs were lightly anesthetized with diethyl ether and the mixture of C-LDL and CH-LDL was injected via an exposed jugular vein. Blood samples were collected over the ensuing 24 hr via cardiac puncture. Whole plasma radioactivity was determined in a double-channel gamma spectrometer (Nuclear-Chicago, Des Plaines, IL). Plasma decay kinetics were analyzed using an interactive curve peeling program as previously described (17). Fractional catabolic rates (FCR) were calculated with the assumption that both C-LDL and CH-LDL are kinetically homogenous populations of particles. In some experiments the C-LDL and CH-LDL preparations were subjected to extensive nonenzymatic glucosylation of lysine residues as previously described (17). This procedure abolishes the ability of the modified lipoproteins to interact with the LDL-receptor and permits assessment of that fraction of the

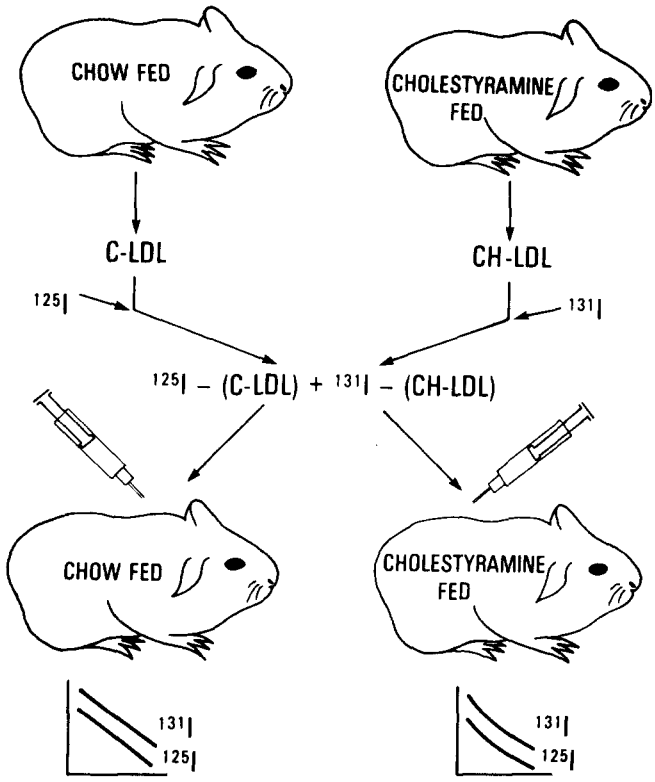

Fig. 1 Protocol for the LDL turnover studies. In experiment 1, the isotope assignment was as depicted in the figure above, while in experiments 2 and 3, the isotope assignment was reversed.

LDL clearance accounted for by the LDL-receptor-independent pathway.

Binding studies of ^{125}I-LDL to hepatic membranes

To document the effect of bile sequestrant treatment on expression of hepatic LDL-receptor activity, we performed ^{125}I-labeled LDL binding studies on partially purified hepatic plasma membranes isolated from control and treated guinea pigs. These studies were performed as we previously described for binding studies in rabbit hepatic membranes (18) and are based on the methods of Kovanen et al. (19). A 2-g sample of whole liver was homogenized in 10 ml of buffer (0.15 M NaCl, 1.0 mM $CaCl_2$, 10 mM Tris-HCl at pH 7.5, and 1 mM phenylmethylsulfonyl fluoride) on ice using a Polytron. Homogenates were filtered through nylon net (Nitex 120-μm mesh) and then membrane-enriched fractions sedimenting between 8000 g and 100,000 g were prepared as previously described (19). Membrane fractions were frozen in liquid nitrogen and stored at −70°C until use.

On the day of the experiment, frozen membranes were resuspended in a buffer of 50 mM NaCl, 1 mM $CaCl_2$, 20 mM Tris-HCl, pH 8, by flushing 10 times through a 25-gauge needle on ice. Suspensions were sonicated for two 10-sec bursts and diluted appropriately in a buffer of 20 mM NaCl, 0.63 mM $CaCl_2$, 50 mM Tris-HCl, and 20 mg/ml of bovine serum albumin. The binding assay was conducted by a modification of that described by Kita et al. (20) in a total volume of 100 μl containing 100 μg of membrane protein and indicated amounts of guinea pig ^{125}I-labeled LDL. Binding studies were carried out in the presence and absence of 6 mM EDTA. In initial experiments incubations were performed for 1 hr at 0°C in an ice-water bath. In these experiments total binding was low and it was difficult to reliably demonstrate EDTA-sensitive binding sites. In subsequent experiments, incubations were done for 1 hr at 37°C and a 6- to 7-fold increase in total ^{125}I-labeled LDL binding was noted and EDTA-sensitive binding was clearly delineated. All results reported were from incubations conducted at 37°C. In order to separate bound and free ^{125}I-labeled LDL, a 50-μl aliquot of the incubation mixture was layered over 170 μl of fetal calf serum (FCS) and centrifuged through FCS at 100,000 g in a Beckman Ti-42.2 rotor for 60 min at 4°C. The supernatant was removed by vacuum aspiration and the pellet was overlayered once with 200 μl of FCS and recentrifuged at 100,000 g for 30 min. The supernatant was aspirated, the bottom of the tube was isolated by slicing, and the membrane pellet was counted in a gamma counter. The difference between the amount of ^{125}I-labeled LDL bound in the presence and absence of excess unlabeled LDL is referred to as LDL-displaceable binding; the difference between the amount bound in the presence and absence of 6 mM EDTA is referred to as EDTA-sensitive binding.

Cell culture experiments

To determine the interaction of guinea pig LDL isolated from control and treated pigs with the LDL-receptor, a culture of guinea pig fibroblasts was established from a biopsy of abdominal skin. Uptake and degradation of guinea pig ^{125}I-labeled LDL by guinea pig fibroblasts or by human fibroblasts in culture were conducted exactly as previously described (21).

RESULTS

Effects of cholestyramine treatment on LDL levels and composition

Feeding cholestyramine resin (2% of pellets by weight) to adult guinea pigs for 10–14 days resulted in a mean decrease in plasma cholesterol of 49% (55.6 ± 13.9 mg/dl in ten control animals vs. 28.1 ± 8.7 mg/dl in nine treated animals, $P < 0.001$, mean ± SD). Plasma triglycerides were not significantly changed. As shown in **Table 1**, LDL cholesterol (d 1.019–1.063 g/ml) was reduced 55%. Although there was a reduction in

TABLE 1. Plasma lipoprotein cholesterol levels in control and cholestyramine-treated guinea pigs

	Control	Cholestyramine-Treated
	mg/dl	
Total plasma	56.3 ± 7.9	29.5 ± 6.7[a]
<1.019	3.2 ± 4.5	2.5 ± 0.8
1.019–1.063	32.0 ± 8.2	14.4 ± 2.8[b]
1.063–1.21	21.2 ± 2.3	12.7 ± 4.0[b]

Cholesterol values are mean ± SD. Three control guinea pigs and three guinea pigs fed 2% cholestyramine mixed in the food were killed after 10 days of feeding. Plasma was collected and ultracentrifugal fractions were prepared as indicated (see explanation for selection of densities in Methods section of text).

[a]Different from control at $P < 0.01$ by Student's t-test.

[b]$P < 0.05$.

the d 1.063–1.21 g/ml fraction as well, most likely this reflected a reduction in LDL since the guinea pig has very little HDL (22) and LDL (lipoproteins containing almost exclusively apoB) is known to extend into the d 1.063–1.100 g/ml fraction (9, 10). We also found that feeding of colestipol-HCl (2% of food by weight) was equally effective, leading to a 59% drop in plasma cholesterol (49.2 ± 6.7 vs. 20.0 ± 4.2 mg/dl, $P < 0.001$, four controls vs. six treated animals). It is important to note that these differences were determined in guinea pigs that weighed approximately 700–800 g. In younger, lighter guinea pigs these differences were not as marked, due to a greater variability in plasma cholesterol levels of control animals.

We have previously shown that LDL particles (d 1.019–1.063 g/ml) isolated from humans on colestipol-HCl therapy are smaller in size and have a decreased cholesterol:protein ratio. To determine whether a similar reduction in LDL particle size occurred in the guinea pigs treated with a bile sequestrant, we examined by electron microscopy negatively stained preparations of LDL isolated from control donors (C-LDL) and cholestyramine-fed donors (CH-LDL). In three separate preparations we observed that the mean particle size of the CH-LDL was smaller than that of C-LDL (**Fig. 2**). In addition, there appeared to be a selective decrease in larger LDL particles, so that the CH-LDL distribution was skewed toward smaller particles. In the example given in Fig. 2, the mean particle diameter decreased from 216 Å to 198 Å. This 9% decrease in mean CH-LDL particle diameter, however, corresponds to a 22% decrease in mean particle volume. Obviously, for many of the particles, the change would be even greater. From compositional data presented in **Table 2**, it can be appreciated that the CH-LDL was depleted in cholesteryl ester and phospholipids, but not in free cholesterol or triglyceride. In the typical LDL obtained from the treated guinea pigs (column B, Table 2) the cholesteryl ester to protein ratio had decreased 34% from control, and in the LDL obtained from animals with the most marked response to cholestyramine (column C, Table 2), the fall was 44%. These decreases in the ratio of core to surface material are consistent with the changes in particle diameter noted above. The decrease in the phospholipid to protein ratio and an increase in the molar ratio of free cholesterol/phospholipid from 0.64 to 2.0 [similar to the ratio found in the LDL of cholesterol-fed guinea pigs (22)] suggests that profound changes occurred in the organization of the surface layer as well. In many preparations, however, the decrease in the phospholipid:protein ratio was not so marked as the example shown in Table 2. It is also of interest to note that the cholesterol:protein ratio of the VLDL from the treated animals was also considerably reduced compared to that of controls (1.41 vs. 0.53).

In addition to the structural changes noted above, we also sought to determine whether alterations in apoprotein content of LDL had occurred. When examined by TMU precipitation techniques, apoB made up 86% and 93% of the apoprotein content of C-LDL and CH-LDL, respectively. We also examined apoprotein content by polyacrylamide slab gel electrophoresis using a 4–20% gradient. Using densitometric scanning, apo B constituted 86% and 91.3% of the apoprotein content of LDL in the C-LDL and CH-LDL, respectively

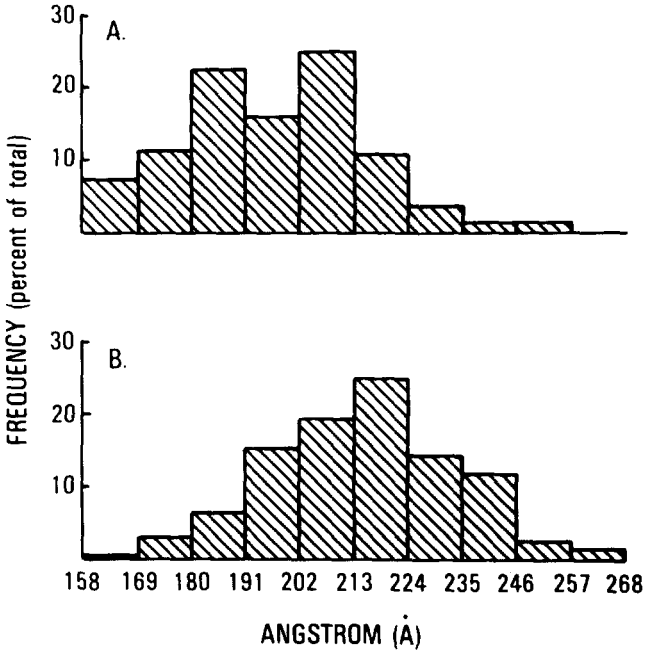

Fig. 2 Frequency distribution of LDL particle size for LDL isolated from control guinea pig (C-LDL), panel B, and for LDL isolated from cholestyramine-treated guinea pig (CH-LDL), panel A. LDL was prepared and electron micrographs were taken as described in Methods. Diameters of 125 free-standing particles were measured from different photographs taken from different areas of the grid and plotted as frequency distribution. Mean diameter of C-LDL is 215.5 ± 18.1 Å and of CH-LDL 198.5 ± 20.1 Å ($P < 0.001$). Distribution of particle size for the control LDL is similar to that previously reported for guinea pig LDL (11).

(Fig. 3, gels A and B). When apoprotein content of these two preparations was examined on a 3–6% gradient in order to separate apoB subspecies, in both preparations we noted an apoB species of approximately 500,000 molecular weight. In addition, in some but not all preparations of CH-LDL, we observed a significant component of a "little apoB" of approximately 300,000 molecular weight. In order to determine whether changes in apoproteins other than apoB had occurred, we ran heavily overloaded gels using a 4–20% gradient (Fig. 3, gels C and D). There were very small amounts of a 43–44,000 molecular weight apoprotein and traces of apoproteins with molecular weights less than 15,000. However, within the limits of this technique, no consistent differences were observed between the two LDL preparations.

Induction of hepatic LDL-receptor activity

Bile sequestrant therapy in dogs and rabbits (5–8) leads to an increase in specific binding sites for LDL on isolated hepatic membranes. To determine whether this was also true in the guinea pig, we performed binding studies of guinea pig LDL to partially purified hepatic membranes isolated from control and cholestyramine-treated animals. Initial binding experiments performed at 0°C yielded low levels of total LDL binding and it was difficult to demonstrate specific EDTA-sensitive binding sites. However, when binding experiments were done at 37°C, there was a 6- to 7-fold increase in the amount of total LDL bound and EDTA-sensitive binding was clearly delineated. Under these conditions, LDL-displaceable binding to membranes isolated from cholestyramine-treated animal was considerably increased compared to control (**Fig. 4**, left panel). This increase was accounted for by increases in both EDTA-insensitive sites as well as EDTA-sensitive sites (Fig. 4, right panel). Thus, in confirmation of previous reports (5–8), EDTA-sensitive binding, which is presumed to represent classic LDL-receptor binding (18, 20), was increased in guinea pigs by cholestyramine therapy. However, there was an increase in EDTA-insensitive sites as well. This was confirmed in other experiments by demonstrating increased binding of ^{125}I-labeled methyl LDL to hepatic membranes from cholestyramine-treated guinea pigs (data not shown). The physiologic significance, if any, of the increase in LDL-displaceable but EDTA-insensitive binding is not known.

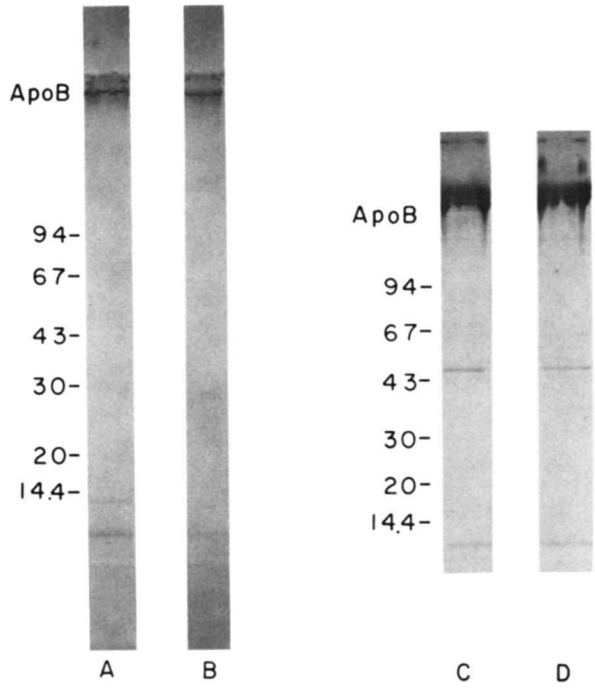

Fig. 3 Photographs of SDS-polyacrylamide gel electrophoresis on a 4–20% gradient of apoLDL isolated from control and cholestyramine-treated guinea pigs. In panels A and B, 40 μg of apoLDL was applied, while >80 μg was applied in panels C and D. ApoLDL from control LDL is shown in panels A and C, and apoLDL isolated from cholestyramine-treated guinea pigs is shown in B and D. The positions of standard molecular weight markers (× 10^{-3}) as well as apoB are indicated.

TABLE 2. Chemical composition of LDL isolated from control (C-LDL) and cholestyramine-treated (CH-LDL) guinea pigs

	% of Total Weight		
	C-LDL	CH-LDL	
	A	B	C
Cholesteryl ester	48.3	42.0	41.4
Free cholesterol	5.9	10.0	7.4
TG	6.8	12.5	11.6
PL	18.2	7.8	8.5
Protein	20.8	27.8	31.1
Ratios			
Lipid/protein (w/w)	3.80	2.61	2.22
TG/protein (w/w)	0.33	0.45	0.37
Total cholesterol/protein (w/w)	1.67	1.27	1.03
Esterified chol/protein (w/w)	1.39	0.91	0.79
Free chol/protein (w/w)	0.28	0.36	0.24
Esterified chol/free chol (mol/mol)	4.89	3.37	2.50
PL/protein (w/w)	0.87	0.28	0.27
Free chol/PL (mol/mol)	0.65	2.56	1.74

Composition of LDL obtained from control and cholestyramine-fed guinea pigs. Four control guinea pigs were fed standard pellets and six were fed cholestyramine pellets. Plasma cholesterol was determined on each animal, plasma was pooled, and LDL was isolated between densities 1.019 and 1.063 g/ml. Mean plasma cholesterol for C-LDL (column A) was 49 ± 7 mg/dl. The CH-LDL was divided into two pools: column B (four animals) for which plasma cholesterol was 35 ± 2 mg/dl and column C (two animals) for which plasma cholesterol was 13 ± 1 mg/dl. The latter represented a greater than average change and consequently LDL from these animals was analyzed separately. The composition of C-LDL is similar to that previously reported (22).

Alterations in metabolic behavior of LDL

To determine whether the changes in lipoprotein composition caused changes in the lipoprotein's metabolic behavior, we compared the turnover of C-LDL

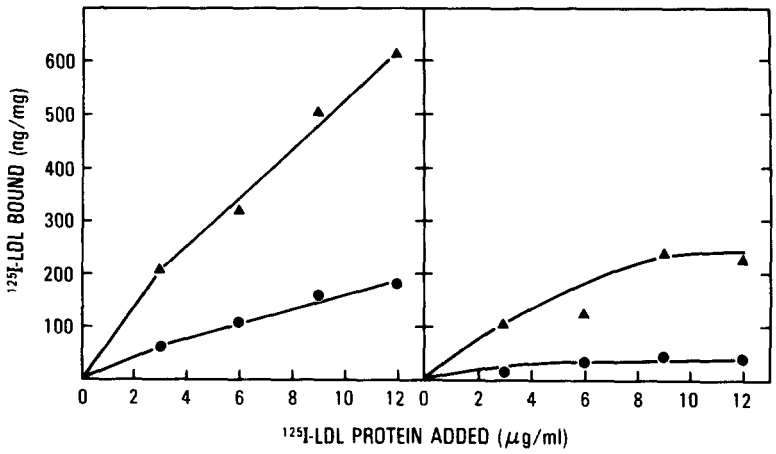

Fig. 4 Binding of ^{125}I-labeled guinea pig LDL to hepatic membranes from control and cholestyramine-treated guinea pigs. Partially purified hepatic membranes (8000–100,000 g fraction) were prepared from guinea pigs fed control pellets (●) or 2% cholestyramine pellets (▲). ^{125}I-labeled guinea pig LDL was incubated with membranes for 1 hr at 37°C and bound LDL was separated from free by ultracentrifugation. Binding was determined in the presence and absence of an excess of unlabeled guinea pig LDL (1 mg/ml) and in the presence and absence of 6 mM EDTA. Left panel shows the amount of LDL-displaceable binding to membranes from control and cholestyramine animals (i.e., total binding minus binding in presence of excess unlabeled LDL). Right panel shows the amount of EDTA-sensitive binding (i.e., total binding minus binding in the presence of 6 mM EDTA).

and CH-LDL preparations injected simultaneously into guinea pigs. Fig. 1 depicts the protocol for these studies. We studied the turnover of three different pairs of labeled C-LDL and CH-LDL preparations, injected into a total of 19 animals, 10 controls and 9 cholestyramine-treated. To rule out an isotope effect as the cause of any difference in turnover, we labeled C-LDL with ^{125}I and CH-LDL with ^{131}I in experiment 1, and in experiments 2 and 3 the isotope assignment was reversed.

The plasma decay curves for one pair of LDL preparations injected into a control and cholestyramine-fed guinea pig are shown in **Fig. 5**. The FCR for the C-LDL was greater in the treated pig than in the control pig (0.166 vs. 0.103 pools hr^{-1}). The FCR for the CH-LDL was also greater in the treated pig than in the control (0.124 vs. 0.078). However, note that in each animal, treated or control, the CH-LDL was metabolized more slowly than C-LDL.

The FCR in all three experiments, for control and cholestyramine-treated guinea pigs, are shown in **Table 3**, while **Table 4** gives a statistical treatment of data from all three experiments. In 17 of the 19 animals in which the double-labeled studies were performed, the CH-LDL had a slower turnover than the C-LDL with a mean decrease of 19% [ΔFCR ÷ (FCR) of C-LDL] × 100 and a range of 3 to 47%. In Table 4, the horizontal values compare the turnover of C-LDL and CH-LDL in the same animal. In the control animals, the FCR of the CH-LDL was approximately 14% slower than that of the C-LDL, while in the cholestyramine-treated pigs CH-LDL was 23% slower than C-LDL.

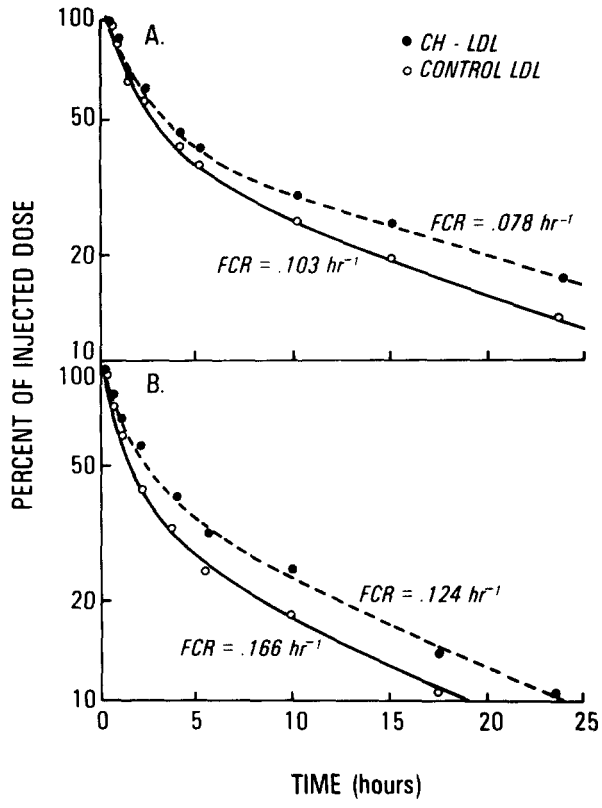

Fig. 5 Plasma decay curves of LDL isolated from control animals (control-LDL) and cholestyramine-treated animals (CH-LDL) injected into a control guinea pig (panel A) and a cholestyramine-treated guinea pig (panel B). In both guinea pigs, the same mixture of C-LDL and CH-LDL was injected and the resultant plasma decay curves were analyzed by curve peeling techniques as described in the Methods section.

TABLE 3. Comparison of FCR of C-LDL and CH-LDL injected into control and treated guinea pigs

	C-LDL	CH-LDL
Experiment 1		
Control guinea pig (n = 3)	0.122 ± 0.015	0.116 ± 0.012
Treated guinea pig (n = 3)	0.146 ± 0.014	0.138 ± 0.009
Experiment 2		
Control guinea pig (n = 3)	0.113 ± 0.008	0.086 ± 0.004
Treated guinea pig (n = 6)	0.159 ± 0.014	0.110 ± 0.012
Experiment 3		
Control guinea pig (n = 4)	0.099 ± 0.003	0.086 ± 0.003

All values for FCR are in pools per hour and are mean ± SD of indicated number of experiments. In each experiment, LDL derived from control animals (C-LDL) and cholestyramine-treated animals (CH-LDL) were labeled with ^{125}I and ^{131}I, mixed, and injected into control or cholestyramine-treated animals. In experiment 1, CH-LDL was labeled with ^{131}I and C-LDL with ^{125}I; in experiments 2 and 3, the isotope assignment was reversed.

The vertical columns compare the turnover of a single tracer injected into control and treated animals. Using the C-LDL as the sole tracer in both control and treated animals, cholestyramine therapy increased the FCR of LDL 41%; using CH-LDL as the tracer, the increase was only 26%.

Clearance of C-LDL and CH-LDL by receptor-dependent and receptor-independent pathways

The data presented above suggested that compared to CH-LDL the more rapid clearance of C-LDL was due to enhanced uptake by LDL-receptor-mediated pathways. To test this hypothesis further, we isolated LDL from two human subjects during a control period (C-LDL) and again 1 week later after they had consumed colestipol-HCl (CH-LDL). [We have shown previously that the turnover of human and guinea pig LDL is similar in the guinea pig (17).] Each pair of C-LDL and CH-LDL preparations was labeled and injected into cholestyramine-treated guinea pigs. As with C-LDL and CH-LDL isolated from guinea pigs, the human C-LDL tracers were cleared more rapidly than CH-LDL in each of seven animals tested (**Table 5**, experiments 1 and 2). The C-LDL and CH-LDL tracers were then glucosylated (Glc-LDL), a modification we demonstrated previously that abolishes the ability of LDL to interact with the LDL-receptor (17). Thus, the clearance of Glc-LDL occurs exclusively by LDL-receptor-independent pathways (23). When Glc-(C-LDL) and Glc-(CH-LDL) were simultaneously injected into treated guinea pigs, their clearance rates were much slower and equal (Table 5), i.e., the receptor-independent clearance of C-LDL and of CH-LDL was similar. To document the observation that this phenomenon also occurred with homologous guinea pig LDL, the above protocol was repeated with LDL isolated from control and cholestyramine-treated guinea pigs (Table 5, experiment 3). Similar results were again found when the homologous tracers were injected. In experiments 1, 2, and 3, LDL-receptor activity accounted for 76, 69, and 77%, respectively, of the clearance of the C-LDL. In turn, LDL-receptor activity accounted for only 60, 61, and 71%, respectively, of the clearance of CH-LDL. These data strongly suggest that the faster clearance of C-LDL, compared to CH-LDL, was due solely to enhanced LDL-receptor-mediated clearance.

Interaction of control and treated LDL with fibroblasts and hepatic membranes

The in vivo experiments strongly suggested that C-LDL had an increased "affinity" for the LDL-receptor compared to CH-LDL. To test this possibility further, ^{125}I-labeled LDL was prepared from pools of isolated LDL derived from control and treated animals, and their rates of degradation by guinea pig fibroblasts were determined. Four separate pools of C-LDL and

TABLE 4. Analysis of combined FCR data pooled from all three experiments

	C-LDL	CH-LDL	
Control guinea pig (n = 10)	0.110 ± 0.006	0.095 ± 0.006	$P < 0.01$
Treated guinea pig (n = 9)	0.155 ± 0.010	0.120 ± 0.009	$P < 0.01$
	$P < 0.001$	$P < 0.05$	

This table combines the FCR data (in pools per hr, mean ± SEM) for the three experiments presented in Table 3. LDL derived from control guinea pigs (C-LDL) or cholestyramine-treated guinea pigs (CH-LDL) were labeled with ^{125}I or ^{131}I and simultaneously injected into control guinea pigs or cholestyramine-treated guinea pigs. Details of experiments are given in the text and in the footnote to Table 3. Data comparing the FCR of C-LDL versus CH-LDL in a single animal, e.g., the horizontal comparisons above, were analyzed by a paired Student's *t*-test. Data comparing the FCR of a single label in a control or treated guinea pig, e.g., the vertical comparisons, were analyzed by nonpaired Student's *t*-test. *P* values were determined using two-tailed tables. The values for C-LDL in the control animals were not significantly different from the CH-LDL in treated animals ($P = 0.95$).

TABLE 5. Turnover of C-LDL and CH-LDL (before and after glucosylation) in cholestyramine-treated guinea pigs

Type of LDL preparation	C-LDL	CH-LDL
Experiment 1		
Nonglucosylated (n = 3)	0.105 ± 0.011	0.081 ± 0.008[a]
Glucosylated (n = 3)	0.025 ± 0.003	0.032 ± 0.001
Experiment 2		
Nonglucosylated (n = 4)	0.189 ± 0.017	0.153 ± 0.021[a]
Glucosylated (n = 3)	0.059 ± 0.012	0.059 ± 0.010
Experiment 3		
Nonglucosylated (n = 3)	0.137 ± 0.020	0.116 ± 0.021[a]
Glucosylated (n = 3)	0.031 ± 0.005	0.032 ± 0.005

All values for FCR are in pools per hour, mean ± SD of indicated number of experiments. (n) indicates number of animals injected with each pair of LDL tracers. In experiment 1, LDL was isolated from the same human subject during a control period (C-LDL) and after 1 week of colestipol therapy (CH-LDL). A second individual was the donor for experiment 2; while in experiment 3, the LDL preparations were isolated from control and cholestyramine-treated guinea pigs. Each pair of C-LDL and CH-LDL was labeled with ^{125}I and ^{131}I. An aliquot of each labeled preparation was glucosylated (by incubation with glucose and $NaCNBH_3$) while another aliquot was mock-incubated in the absence of glucose (17). Each pair (C-LDL and CH-LDL) of nonglucosylated and glucosylated preparations was then injected into a cholestyramine-treated guinea pig. In each of the 10 animals injected with nonglucosylated preparations of C-LDL and CH-LDL, the clearance of the C-LDL exceeded that of CH-LDL. In turn, glucosylation abolished this difference.

[a]FCR of C-LDL different from CH-LDL at $P < 0.03$ by paired Student's t-test.

CH-LDL were prepared and studied. Fig. 6 depicts one experiment in which a clear increase in LDL specific degradation was noted when the C-LDL was the tracer. Scatchard analysis of this experiment indicated that the increased degradation was accounted for by an apparent increase in "affinity" for the receptor, rather than by a change in number of "binding sites" on the LDL. A similar result was found in a second pair of control and CH-LDL preparations examined, but a third pair showed only minimal differences in the same direction, and a fourth pair showed no difference. Thus, in general these results are supportive of the in vivo data, but were not as consistent. Although the reason for this lack of reproducibility is unknown, we suspect that the fibroblast system lacks the sensitivity to detect subtle differences consistently in LDL preparations that are seen in vivo. For example, in guinea pigs we can consistently detect a 5–25% inhibition of LDL clearance in vivo when as few as 2–5% of lysine residues of LDL are glucosylated, yet we cannot consistently detect this difference in terms of uptake and degradation in cultured fibroblasts (24). We also examined the binding of C-LDL and CH-LDL to hepatic membranes isolated from control and cholestyramine-treated guinea pigs, but could not detect any consistent difference.

DISCUSSION

In this report, we document the fact that bile acid-sequestrant therapy in guinea pigs significantly lowers LDL levels, as it does in humans. The reduction in steady state levels of LDL is accompanied by profound changes in LDL composition, characterized by a decrease in the average particle size, a decrease in the cholesteryl ester and phospholipid content, and a decrease in the cholesterol:protein ratio, suggesting an

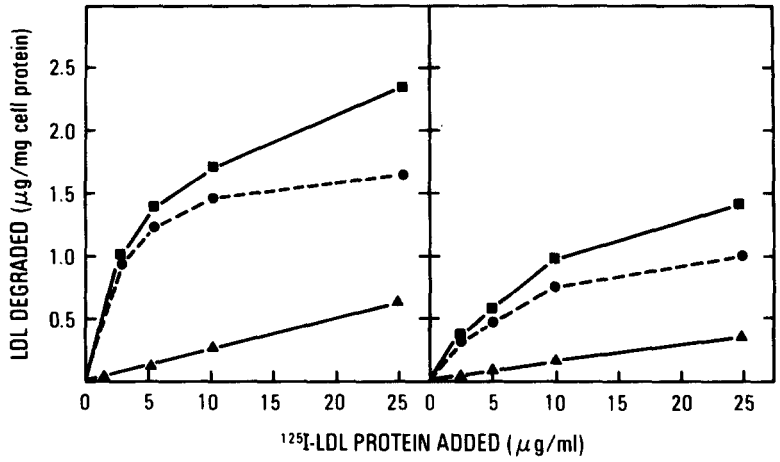

Fig. 6 Degradation of LDL isolated from control (left panel) or cholestyramine-treated (right panel) guinea pigs by guinea pig fibroblasts in culture. LDL was isolated from control or treated animals, iodinated, and then incubated for 20 hr with guinea pig fibroblasts that had LDL receptors induced by preincubation with lipoprotein-deficient serum. LDL degradation was determined in the absence (■) or presence (▲) of an excess of unlabeled control (left panel) or cholestyramine-treated (right panel) guinea pig LDL. (●– – –●) displays the calculated difference between degradation in the presence and absence of unlabeled LDL.

increase in the ratio of surface to core components. In general, these changes are similar to those noted for LDL isolated from humans on bile acid-sequestrant therapy (1–3) (S. G. Young, J. L. Witztum, T. E. Carew, R. M. Krauss, and F. T. Lindgren, unpublished results). No consistent alterations in the apoprotein content of the LDL isolated from cholestyramine-treated animals was observed, but small though important changes may have occurred that were beyond the resolution of the techniques used.

Feeding cholestyramine to guinea pigs altered their metabolism of LDL so the FCR in treated animals increased 41% compared to control animals when a single control LDL tracer was injected into both. As others have reported, feeding a bile sequestrant to dogs (5) and rabbits (4) increased the FCR of a single injected control LDL tracer. This increase in FCR appears to be mediated by an increase in hepatic LDL-receptor activity (5–8). Our experiments also indicate an increase in EDTA-sensitive binding sites in hepatic membranes prepared from cholestyramine-treated guinea pigs (Fig. 4). In addition, when hepatic binding experiments were conducted at 37°C, we also noted increases in LDL-displaceable, but EDTA-insensitive, binding. The physiologic significance, if any, of the latter observation remains to be determined.

The studies reported here demonstrate that, in addition to any effect that cholestyramine had on increasing hepatic LDL-receptor activity, it also produced profound alterations in the composition of LDL, which independently affected the metabolism of LDL. In 27 of 29 animals studies (Tables 3 and 5), the LDL derived from cholestyramine-treated animals or human subjects (CH-LDL) had a slower FCR than LDL derived from control animals or human subjects (C-LDL), and the difference in clearance rate between the two preparations was *greater* in cholestyramine-treated animals than in controls (Table 4). In addition, while the increase in FCR produced by bile sequestration using the C-LDL as the tracer was 41%, the increase using CH-LDL was only 26% (Table 4). These data suggest that the slower clearance of the CH-LDL is due to decreased "affinity" of the CH-LDL for the LDL-receptor. The in vivo experiments with glucosylated LDL shown in Table 5 strongly support this interpretation. Whether this difference is due to differences in all of the LDL particles in C-LDL and CH-LDL, or due to different subpopulations within the LDL is not currently known.

In previous studies of the effects of bile sequestration (or ileal bypass) on LDL metabolism in dogs and rabbits (4, 5, 7, 25), a single preparation of LDL obtained from homologous (or even heterologous) normal controls was injected into control and treated animals. Increases in FCR of 28% (dogs) to as much as 120% (rabbits) were found. In our study, using C-LDL as the tracer in both control and treated animals, the FCR was much faster (+41%) in the cholestyramine-treated guinea pigs, which agrees with the previous results in dogs and rabbits. One might have assumed, therefore, that this increase in FCR explains the decrease in LDL levels, and using the change in FCR to calculate changes in absolute clearance rates would confirm this. However, one might argue that, at steady state in treated animals, the CH-LDL actually found is markedly different from LDL present under control conditions, and using C-LDL as the tracer in both control and treated animals does not reflect the true situation in treated animals. In this report we clearly demonstrate that CH-LDL has a different FCR than C-LDL. Thus, at steady state in the treated animal, even though cholestyramine therapy increases the potential for receptor-mediated clearance of LDL via induction of hepatic receptors, the changes in LDL composition partially or almost completely negate this effect. Thus, when comparing the FCR of the C-LDL in the control animals (0.110 ± 0.006 hr^{-1}) with the FCR of CH-LDL in treated animals (0.120 ± 0.009 hr^{-1}), the small increase in FCR in the treated animals was not statistically different. Most importantly, the magnitude of the increase in FCR cannot account for the decrease in plasma LDL concentration. Furthermore, in preliminary studies in dogs [in which autologous canine C-LDL (purified by Pevikon block electrophoresis) was injected into control dogs, and then after cholestyramine treatment autologous CH-LDL was reisolated and reinjected into the same treated dogs], we could not demonstrate an increase in FCR, despite a greater than 50% reduction in the treated dogs' LDL cholesterol levels (data not shown).

Since the increased clearance of LDL cannot explain the decreased LDL concentration, one could argue that decreases in production are responsible. As shown in **Table 6**, using C-LDL as tracer in the control state and CH-LDL as tracer in the treated state, the calculated LDL-apoB production rate decreased 29%, accounting for a large part of the net decrease in LDL levels. How then do you reconcile the fact that hepatic LDL-receptor activity is increased with the data from Table 6 indicating that LDL levels are decreased because of decreased LDL production? This could occur if the increased hepatic LDL-receptor activity caused more small, cholesterol-enriched VLDL (or IDL) to exit the plasma compartment before being converted to LDL (26), i.e., enhanced LDL-receptor activity increased the "shunt" pathway. Note however, that even though LDL levels could be reduced by such a mechanism, if an LDL tracer were injected into a cholestyramine-treated guinea pig, it would still be cleared more rapidly because LDL-receptors are induced.

TABLE 6. Estimate of LDL turnover parameters in control and cholestyramine-treated guinea pigs

	Control	Treated	% Change
LDL-cholesterol pool size (mg)	9.61 ± 1.91	4.84 ± 1.04[a]	−50
LDL-cholesterol flux (mg hr^{-1})	1.06 ± 0.31	0.57 ± 0.15[a]	−46
Fractional catabolic rate (pools hr^{-1})	0.110 ± 0.02	0.120 ± 0.03	+9
LDL-B pool size (mg)	5.76 ± 1.14	3.82 ± 0.82[a]	−34
LDL-B production rate (mg hr^{-1})	0.64 ± 0.19	0.45 ± 0.12[b]	−29

Data were calculated for the C-LDL tracer in control animals and CH-LDL tracer in treated animals. LDL cholesterol pool size was calculated assuming LDL-chol values of 0.32 mg/ml for control guinea pigs and 0.14 mg/ml in treated pigs. LDL-apoB pool sizes were calculated using ratios of 1.67 and 1.27 for cholesterol:protein ratio of C-LDL and CH-LDL, respectively (see Table 2). These calculations also assume all LDL-cholesterol leaves plasma in association with LDL-protein, and that all LDL-protein is apoB, and that all particles within the LDL density range are kinetically homogeneous (see Discussion).

[a]Different from control, $P < 0.001$.

[b]Different from control, $P < 0.02$.

The interpretation of the data presented above, and in Table 6, depends on the assumption that LDL is kinetically homogeneous and therefore one can calculate production rates from knowledge of pool size and FCR. However, as demonstrated in this report, this assumption may not be true. The physical properties of LDL particles found in the d 1.019–1.063 g/ml fraction of control and cholestyramine-treated guinea pigs are different, as is their metabolism. In the control state there is a predominance of larger, more buoyant, and more cholesterol-enriched particles than in the treated state. The data could also be explained by postulating that the large LDL particles found in the control state have an enhanced "affinity" for the LDL-receptor. Therefore, in situations where hepatic LDL-receptor activity is enhanced (e.g., cholestyramine-feeding), these large cholesterol-enriched particles are preferentially removed, leaving residual smaller cholesteryl ester-depleted particles which have relatively decreased affinity for the LDL-receptor. By this argument, when LDL (d 1.019–1.063 g/ml) is isolated from cholestyramine-treated animals in a steady-state, larger LDL particles would be underrepresented and lead to an apparent decrease in FCR when iodinated and injected into recipient animals. Elsewhere we clearly demonstrate in humans that colestipol treatment selectively depletes large LDL particles from plasma, while there is little change in mass of small LDL particles (S. G. Young, J. L. Witztum, T. E. Carew, R. M. Krauss, and F. T. Lindgren, unpublished results). Utilizing the data from this study and assuming that LDL can be divided into as few as two populations of particles (e.g., large and small) each having different fractional clearance rates from plasma, it is possible to construct kinetic models that adequately explain the observed plasma decay kinetics and pool sizes in control and treated guinea pigs, without involving any decrease in LDL production rate. Thus, as a working hypothesis, we propose that bile sequestrant therapy induces hepatic LDL-receptor activity, which in turn selectively removes large, more buoyant, cholesteryl ester-enriched LDL particles from plasma, leaving behind smaller, denser, and cholesteryl ester-depleted particles. Therefore, plasma LDL-cholesterol levels fall primarily because of the rapid catabolism of a subfraction of larger, less dense particles within the LDL density range. We cannot at present clearly distinguish this possibility from that outlined above; namely, that all the LDL particles in the C-LDL and CH-LDL are kinetically homogenous (albeit that CH-LDL and C-LDL have different FCR's) and therefore LDL production is decreased in the treated animals. However, preliminary data in our laboratory suggest that large, less dense LDL (d 1.025–1.040 g/ml) have a more rapid clearance than smaller, more dense LDL (d 1.050–1.063 g/ml), a finding in support of the kinetic heterogeneity of particles found within the traditional LDL density range. Finally, it is also possible that *both* of the above mechanisms are operative. Thus, in response to bile sequestration, hepatic LDL-receptors are induced. This leads to enhanced removal from plasma of small VLDL and IDL particles, thereby decreasing LDL production. Of particles reaching LDL density, large LDL particles are preferentially removed, leaving behind small LDL.

The changes in LDL composition in the treated animals are similar to those observed in humans treated with bile acid sequestrants (1–3) (S. G. Young, J. L. Witztum, and T. E. Carew, unpublished results). Recently we have demonstrated that similar composition-induced changes in LDL metabolism also occur in humans (S. G. Young, J. L. Witztum, and T. E. Carew, unpublished results). In humans all kinetic studies have used a protocol in which C-LDL was used as tracer during the control period and CH-LDL was reisolated for use as tracer during the bile sequestrant treatment period. Langer and Levy (27) reported that therapy increased the FCR of LDL 42% and Shepherd et al. (3) reported an increase of 29%. However, in the latter

study, a narrow cut of LDL (d 1.030–1.050 g/ml) was used that may not be representative of CH-LDL since it may not have included the bulk of "heavier" LDL found in many treated patients. In contrast, Sigurdsson and Kjartansson (28) (using LDL of d 1.019–1.063 g/ml) reported that bile sequestrants do not increase the FCR of LDL, but lower LDL levels by decreasing production of LDL. Furthermore, in a preliminary analysis of ongoing studies on the effects of colestipol HCl on lipoprotein kinetics in humans, we have found an apparent heterogeneous effect on LDL clearance (S. G. Young, Y. A. Kesaniemi, J. L. Witztum, unpublished observations). These differing observations may be related to different degrees to which LDL composition has been changed in the treated state. However, the studies reported here suggest that all such studies in humans are likely to underestimate the true extent of induction of hepatic LDL-receptors.

One interpretation of the present data is that particles found in the typical LDL density range (d 1.019–d 1.063 g/ml) are not kinetically homogeneous. In fact, Goebel, Garnick, and Berman (29) have previously suggested this possibility based on analysis of LDL kinetic data in human subjects. If this is true, it implies that one cannot reliably calculate LDL production rates as is currently done, since the underlying assumption of such calculations is the kinetic homogeneity of particles within the LDL density range. Our studies clearly demonstrate that bile sequestrants have complex effects on lipoprotein structure and lipoprotein metabolism. Since similar changes in LDL composition are produced by other dietary or drug-induced manipulations, these effects will have to be considered in future kinetic studies of LDL metabolism.

The recently completed LRC-CPPT Trial demonstrated that reduction of LDL levels by use of cholestyramine reduced the subsequent risk for coronary artery disease despite only modest cholesterol lowering (30). The positive benefits of cholestyramine therapy may relate in part to the selective removal of larger, more cholesterol-enriched LDL. Nevertheless, the metabolic consequences of the accumulation of smaller, more dense LDL is unknown. Since LDL particles of differing size may have different effects on the atherogenic process (31, 32), it will be important to determine whether the smaller particles produced by bile sequestrant therapy have differing tissue sites of catabolism and/or ability to penetrate the arterial wall.

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REFERENCES

1. Witztum, J. L., G. Schonfeld, and S. W. Weidman. 1976. The effects of colestipol on the metabolism of very low density lipoproteins in man. *J. Lab. Clin. Med.* **88:** 1008–1018.
2. Witztum, J. L., G. Schonfeld, S. W. Weidman, W. E. Giese, and M. A. Dillingham. 1979. Bile sequestrant therapy alters the composition of low density and high density lipoproteins. *Metabolism* **28:** 221–229.
3. Shepherd, J., C. J. Packard, S. Bicker, T. D. V. Lawrie, and H. G. Morgan. 1980. Cholestyramine promotes receptor-mediated low-density-lipoprotein catabolism. *N. Engl. J. Med.* **302:** 1219–1222.
4. Slater, H. R., C. J. Packard, S. Bicker, and J. Shepherd. 1980. Effects of cholestyramine on receptor-mediated plasma clearance and tissue uptake of human low density lipoproteins in the rabbit. *J. Biol. Chem.* **265:** 10210–10213.
5. Kovanen, P., D. W. Bilheimer, J. L. Goldstein, J. J. Jaramillo, and M. Brown. 1981. Regulatory role for hepatic low density lipoprotein receptors in vivo in the dog. *Proc. Natl. Acad. Sci. USA.* **78:** 1194–1198.
6. Hui, D. Y., T. L. Innerarity, and W. Mahley. 1981. Lipoprotein binding to canine hepatic membranes. *J. Biol. Chem.* **256:** 5646–5655.
7. Chao, Y. S., T. T. Yamin, and A. W. Alberts. 1982. Effects of cholestyramine on low density lipoprotein binding sites on liver membranes from rabbits with endogenous hypercholesterolemia induced by a wheat starch-casein diet. *J. Biol. Chem.* **257:** 3623–3627.
8. Angelin, B., C. A. Raviola, T. L. Innerarity, and R. W. Mahley. 1983. Regulation of hepatic lipoprotein receptors in the dog. *J. Clin. Invest.* **71:** 816–831.
9. Chapman, M. J., G. L. Mills, and J. H. Ledford. 1975. The distribution and partial characterization of the serum apolipoproteins in the guinea pig. *Biochem. J.* **149:** 423–436.
10. Chapman, M. J., and G. L. Mills. 1977. Characterization of the serum lipoproteins and their apoproteins in hypercholesterolaemic guinea pigs. *Biochem. J.* **167:** 9–21.
11. Hatch, F. T., and R. S. Lees. 1968. Practical methods for plasma lipoprotein analysis. *Adv. Lipid Res.* **6:** 1–68.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
13. Barlett, G. 1958. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
14. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
15. Kane, J. P., D. A. Hardman, and H. Paulus. 1980. Hetero-

geneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
16. Sniderman, A. D., T. E. Carew, and D. Steinberg. 1975. Turnover and tissue distribution of ^{125}I-labeled low density lipoprotein in swine and dogs. *J. Lipid Res.* **16:** 293–299.
17. Witztum, J. L., E. M. Mahoney, M. J. Branks, M. Fisher, R. Elam, and D. Steinberg. 1982. Nonenzymatic glucosylation of low density lipoprotein alters its biologic activity. *Diabetes.* **31:** 283–291.
18. Pittman, R. C., T. E. Carew, A. D. Attie, J. L. Witztum, Y. Watanabe, and D. Steinberg. 1982. Receptor-dependent and receptor-independent degradation of low density lipoprotein in normal rabbits and in receptor-deficient mutant rabbits. *J. Biol. Chem.* **257:** 7994–8000.
19. Kovanen, P. T., M. S. Brown, and J. L. Goldstein. 1979. Increased binding of low density lipoprotein to liver membranes from rats treated with 17 alpha-ethinyl estradiol. *J. Biol. Chem.* **254:** 11367–11373.
20. Kita, T., M. S. Brown, Y. Watanabe, and J. L. Goldstein. 1981. Deficiency of low density lipoprotein receptors in liver and adrenal gland of the WHHL rabbit, an animal model of familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **78:** 2268–2272.
21. Drevon, C. A., A. D. Attie, S. H. Pangburn, and D. Steinberg. 1981. Metabolism of homologous and heterologous lipoproteins by cultured rat and human skin fibroblasts. *J. Lipid Res.* **22:** 37–46.
22. Sardet, C., H. Hansma, and R. Ostwald. 1972. Characterization of guinea pig plasma lipoproteins: the appearance of new lipoproteins in response to dietary cholesterol. *J. Lipid Res.* **13:** 624–639.
23. Steinbrecher, U. P., J. L. Witztum, Y. A. Kesäniemi, and R. L. Elam. 1983. Comparison of glucosylated low density lipoprotein with methylated or cyclohexanedione-treated low density lipoprotein in the measurement of receptor-independent low density lipoprotein catabolism. *J. Clin. Invest.* **71:** 960–964.
24. Steinbrecher, U. P., and J. L. Witztum. 1984. Glucosylation of LDL to an extent comparable to that seen in diabetes slows their metabolism. *Diabetes.* **33:** 130–134.
25. Trezzi, E., G. Maione, U. Fox, and A. L. Catapano. 1983. Effect of partial ileal bypass on plasma clearance and binding of lipoproteins to liver membranes in the rabbit. *Atherosclerosis.* **46:** 269–273.
26. Kita, T., M. S. Brown, D. W. Bilheimer, and J. L. Goldstein. 1982. Delayed clearance of very low density and intermediate density lipoproteins with enhanced conversion to low density lipoproteins in WHHL rabbits. *Proc. Natl. Acad. Sci. USA.* **79:** 5693–5697.
27. Levy, R., and T. Langer. 1972. Hypolipidemic drugs and lipoprotein metabolism. *Adv. Exp. Med. Biol.* **26:** 155–163.
28. Sigurdsson, G., and H. Kjartansson. 1979. Abstracts of V International Symposium on Atherosclerosis. 460.
29. Goebel, R., M. Garnick, and M. Berman. 1976. A new model for low density apolipoprotein kinetics: evidence for two labeled moieties. *Circulation.* **54 (Suppl. 2):** II-4.
30. Lipid Research Clinics Program. 1984. The Lipid Research Clinics Coronary Primary Prevention Trial Results. I. Reduction in incidence of coronary heart disease. *J. Am. Med. Assoc.* **251:** 351–364.
31. Teng, B., G. R. Thompson, A. D. Sniderman, T. M. Forte, R. M. Krauss, and P. O. Kwiterovich. 1983. Composition and distribution of low density lipoprotein fractions in hyperapobetalipoproteinemia, normolipidemia, and familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **80:** 6662–6666.
32. Stender, S., and D. B. Zilversmit. 1981. Transfer of plasma lipoprotein components and of plasma proteins into aortas of cholesterol-fed rabbits. *Arteriosclerosis.* **1:** 38–49.