Hepatic low density lipoprotein receptors, HMG-CoA reductase, and plasma lipids and apolipoproteins in high- and low-responding rhesus monkeys: effect of cholestyramine treatment

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Abstract Plasma lipids and apolipoproteins, and hepatic LDL receptor and HMG-CoA reductase activities in biopsy samples were measured in high- and low-responding rhesus monkeys maintained on a cholesterol-rich and regular diets. The effect of a 30-day cholestyramine treatment on the above parameters under both dietary conditions was also determined. On the cholesterol-rich diet the high-responders, when compared to the low-responders, had several-fold increased plasma cholesterol and apoB concentrations and significantly lower HDL apoA-I and cholesterol concentrations. Hepatic LDL receptor and HMG-CoA reductase activities were not detectable in the high-responders, while the low-responders expressed a reduced number of LDL receptors of normal affinity. Administration of cholestyramine resulted in a rapid induction of the hepatic LDL receptors in the high-responders and a small additional increase in the low-responders. Cholestyramine treatment also stimulated the expression of the hepatic HMG-CoA reductase in both groups of monkeys. These changes were accompanied by a dramatic drop in plasma cholesterol and apoB concentrations in the high-responders and, to a lesser extent, in the low-responders. Plasma HDL concentrations in the high-responders rose to levels higher than those seen in the low-responders. The affinity and receptor number were similar in both groups of monkeys on the control diet, but the low-responders had significantly higher HMG-CoA reductase activities. Administration of cholestyramine during the control diet had a small but significant additional effect on the hepatic LDL receptors of the low-responders but not of the high-responders. An additional increase in the hepatic HMG-CoA reductase activity of both groups of animals was also observed. It is concluded that the observed differences in the rate of intestinal absorption of dietary cholesterol (Bhattacharyya, A. K., and D. A. Eggen. 1981. *J. Lipid Res.* **21:** 518–524) appear to be thus far the only difference in the manner the two groups of monkeys handle cholesterol. The increased dietary input in the high-responders results in complete down-regulation of the hepatic LDL receptor and, as a consequence, accumulation of apoB and associated cholesterol in plasma. The lower input of dietary cholesterol in the low-responders allows for the expression of a measurable amount of hepatic LDL receptors to maintain nearly normal plasma concentrations of apoB and associated cholesterol. —**Dory, L., A. Bhattacharyya, J. Strong, and C. Chappuis.** Hepatic low density lipoprotein receptors, HMG-CoA reductase, and plasma lipids and apolipoproteins in high- and low-responding rhesus monkeys: effect of cholestyramine treatment. *J. Lipid Res.* 1990. **31:** 279–287.

Supplementary key words apoB • apoA-I • HDL • cholesterol • cholesterol-rich diet • liver microsomes • liver biopsy

Humans and other animal species exhibit a wide range in plasma cholesterol concentrations while consuming a diet of similar composition and cholesterol content (1). A number of animal models have been used to study this phenomenon (2). With the exception of some fully defined genetic defects, such as familial hypercholesterolemia, in which there is a lack of a functional LDL receptor (3), the underlying cause for this variability in the response is poorly understood.

Studies in this laboratory and those of others suggest that the extent of intestinal absorption of dietary cholesterol may play an important role in determining the high- or low-responder phenotype. Thus, the difference in plasma cholesterol concentration between high- and low-responding rhesus monkeys on a cholesterol-rich diet can be almost completely accounted for by the differences in the rate of absorption of dietary cholesterol by these animals (4, 5). These studies have shown that the rate of intestinal absorption of dietary cholesterol in high-responders was substantially higher than that of low-responders. Addition of plant sterol to either diet resulted

Abbreviations: apo, apolipoprotein; BSA, bovine serum albumin; VLDL, very low density lipoproteins; LDL, low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; HMG-CoA, hydroxymethylglutaryl coenzyme A; TLC, thin-layer chromatography; TSB, low cholesterol diet; TSBE, cholesterol-rich diet.

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in a significant decrease in cholesterol absorption; it became essentially the same in both groups of monkeys. The equalization of cholesterol absorption was accompanied by a reduction in plasma cholesterol concentrations to the point where the two groups did not differ statistically.

The phenomenon of increased intestinal cholesterol absorption in high-responding animals was also reported in squirrel monkeys (6) and African green monkeys (7). Although spontaneous hypercholesterolemia has been reported in two rhesus monkeys fed normal chow (8, 9), the molecular basis for this phenomenon remains unknown. Subsequent studies by Guertler and St. Clair (10) on cultured fibroblasts obtained from these animals failed to demonstrate a specific defect in the expression of the LDL receptor. A more recent study has identified a rhesus dam and two offspring with spontaneous hypercholesterolemia associated with diminished LDL receptor number of normal affinity (11).

The present studies were undertaken to examine more closely some of the metabolic consequences of the differential rate of dietary cholesterol absorption between the high- and low-responding rhesus monkeys. It was hypothesized that, in the high-responders, the higher rate of dietary cholesterol absorption (and delivery to the liver) leads to a greater degree of repression of the hepatic LDL receptor and endogenous cholesterol biosynthesis than in low-responders. This, in turn, leads to the accumulation of cholesterol-rich lipoproteins in plasma, normally removed by the LDL receptor. Administration of cholestyramine should minimize the differences in the plasma cholesterol phenotype of the two groups of monkeys through a reduction in the regulatory pool size of hepatic cholesterol and induction of the LDL receptor expression (12, 13). Preliminary reports on portions of this study have appeared (14, 15).

MATERIALS AND METHODS

Animals

Four high- and five low-responding rhesus monkeys (*Macacca mulatta*) used in this study were adult males, 7.5–10 years of age and weighing between 7 and 11 kg. They were identified as high- or low-responders from a group of 80 young adult monkeys fed a high cholesterol diet, as previously described (16). Liver biopsies were obtained at indicated times (see Fig. 1) by abdominal surgery in a surgical suite at LSU Medical Center. Pieces of liver, approximately 3–4 g, were excised at various points at each biopsy. Animals were allowed to recover a minimum of 4 months, but usually 7 to 10 months before the next biopsy. The appearance of the liver tissue was normal at the time of biopsy, with minimal scarring from the previous procedure.

Diets

The high (TSBE) and low (TSB) cholesterol diets provided fat at 38% and protein at 18% of total calories and cholesterol at levels of 0.35 or 0.02 mg/kcal, respectively. The two diets were identical except for the addition of dried egg yolk powder and crystalline cholesterol to the low cholesterol diet to obtain the high-cholesterol diet. The composition of the diets was previously reported (4).

Cholestyramine was mixed with the diet (2.5 g/100 g diet) as required. The animals were fed once daily an amount (110–180 g) sufficient to maintain body weight.

Plasma lipids and apolipoproteins

Plasma cholesterol and triglycerides were measured using the Technicon Auto Analyzer II (Technicon Instruments Corp., Tarrytown, NY). Plasma HDL cholesterol was determined enzymatically (cholesterol reagent kit, Ciba-Corning, Gilford Systems, Oberlin, OH) in the supernatant obtained after precipitating VLDL and LDL with heparin–$MnCl_2$ (Bio-Rad, ECS Division, Anaheim, CA). For the purposes of this study, the difference between total and HDL cholesterol was designated LDL cholesterol, even though it includes VLDL and IDL cholesterol. Based on previously published studies from this laboratory, the VLDL + IDL fraction represents approximately 24% of the "LDL cholesterol" of high- and low- responders on the control diet and 25% and 49% of cholesterol in low- and high-responders, respectively, on the cholesterol-rich diet (17).

ApoA-I and apoB were determined by rocket electroimmunoassays, as previously described (17).

Liver LDL receptor and HMG-CoA reductase assays

The binding studies were carried out essentially as described by Hui, Innerarity, and Mahley (12). Approximately 1 g of coarsely minced liver tissue was immediately immersed in ice-cold buffer consisting of a 10 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl and 1 mM $CaCl_2$. The tissue was homogenized in a Polytron homogenizer (Brinkman Instruments) for 60 sec. The homogenate was sequentially centrifuged at 500 and 8000 *g* for 5 and 15 min, respectively. Microsomal fractions were obtained by centrifugation of the 8000 *g* supernatant at 100,000 *g* for 60 min and the pellets were washed once in the same buffer. Pellets were stored frozen at −80°C for 2–4 months without loss in LDL-binding activity.

A constant source of human LDL was used throughout the studies. Human LDL (d 1.03–1.063 g/ml) was isolated by sequential ultracentrifugation and iodinated according to the procedure of Bilheimer, Eisenberg, and Levy (18). Free iodine was removed by column chromatography and dialysis. On average, the LDL specific activity was 250 cpm/ng LDL protein. The LDL preparation contained a single band, corresponding to apoB-100, as determined by

sodium dodecyl sulfate polyacrylamide gel electrophoresis (19). The protein content of LDL and the microsomal fractions used for the binding studies or assays of HMG-CoA reductase was determined by the method of Lowry et al. (20).

Hepatic microsomes were incubated with increasing concentrations of LDL in the presence or absence of 30 mM EDTA at 0°C for 1 h in a total volume of 100 μl of a 50 mM Tris-HCl buffer, pH 7.5, containing 25 mM NaCl and 1 mM $CaCl_2$. At the end of the incubations, 50-μl aliquots of each assay were layered on 3% BSA in the same buffer in cellulose nitrate tubes and centrifuged in a Beckman Airfuge at 100,000 *g* for 15 min. The washed pellets were cut off and counted in a gamma counter (Gamma 4000, Beckman Instruments). Specific binding was calculated by subtracting binding in the presence of 30 mM EDTA from the total binding, determined in the absence of EDTA. Scatchard analyses were performed as described (21) and the equilibrium dissociation constants (K_D) and maximal binding (B_{max}) were determined.

Liver HMG-CoA reductase activities were determined essentially as described by Brown, Goldstein, and Dietschy (22). Briefly, approximately 1 g of coarsely chopped liver sample was homogenized in a buffer consisting of 10 mM EDTA, pH 7.4, 0.3 M sucrose, 10 mM 2-mercaptoethanol, and 50 mM NaF using a Dounce homogenizer. The microsomal pellets were obtained by sequential ultracentrifugation, as described above. The pellets were washed and stored at −80°C for 2–4 months without change in observed activities. For the assay, aliquots of microsomal protein were preincubated in the presence or absence of *E. coli* akaline phosphatase (10 units). This preincubation was carried out in the presence of 20 mM imidazole chloride, pH 7.4, 5 mM dithiothreitol at 37°C for 60 min. The total preincubation mixture volume was 90 μl. The enzyme assay itself was started by adding 100 μl of 0.2 M potassium phosphate buffer, pH 7.4, containing 40 mM glucose-6-phosphate, 5 mM NADP, 20 mM EDTA, 10 mM DTT, and 0.7 units of glucose-6-phosphate dehydrogenase, followed immediately by addition of DL-[3-^{14}C]HMG-CoA (in a 10 μl volume) to a final concentration of 1.76 μM. The final assay volume was 200 μl and the mixture was incubated for 30 min at 37°C. The reaction was stopped by addition of 20 μl of 5 N HCl, followed by [^{3}H]mevalonic acid lactone (for recovery calculations). Mevalonolactone was extracted three times with 10 ml of ether, extracts were combined, dried, and re-dissolved in acetone. After addition of carrier mevalonolactone, the lipids were separated by TLC in acetone–benzene 1:1 (v/v), spots were visualized under ultraviolet light, scraped, and counted.

Statistical analyses

Data were analyzed for statistical significance by the *t* test of paired observations (for the same group of animals under different dietary conditions) and by the *t* test of difference between means (for comparisons between the two groups of animals).

RESULTS

The overall design of the studies is schematically shown in **Fig. 1**. Three liver biopsies (B_1–B_3) were performed on each monkey during the cholesterol-rich diet (TSBE) period, the last one (B_3) at the end of the cholestyramine treatment. Two additional biopsies (B_4 and B_5) were performed during the administration of the low-cholesterol diet (TSB), the latter at the end of the cholestyramine treatment. Plasma samples (P_1–P_7) were obtained at indicated times, and plasma lipoprotein lipids and apolipoproteins were determined. During the administration of cholestyramine on either diet, plasma samples were ob-

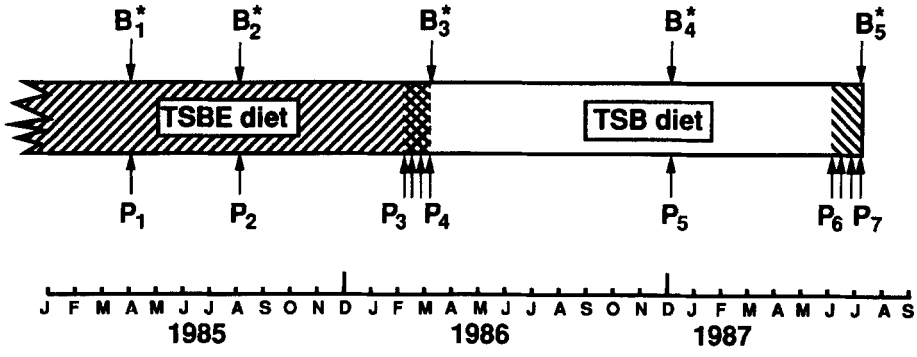

Fig. 1. Schematic design of the experimental protocol. Several liver biopsies were obtained from each animal in the study at indicated points (B*). Plasma samples were obtained at points designated as P, as well as at additional times indicated with arrows pointing up (every 10 days during cholestyramine treatment). The duration of the administration of the cholesterol-rich (TSBE) diet (hatched area) as well as that of normal (TSB) diet (clear area) is indicated. The cross-hatched area at the end of each dietary regimen represents the administration of cholestyramine. The bottom line represents the time scale of the studies.

tained at 10-day intervals to monitor more closely the changes in plasma lipids and apolipoproteins.

Plasma cholesterol concentrations in the high- and low-responding monkeys for the duration of the studies are shown in **Fig. 2.** Throughout the study the high-responding monkeys had statistically significantly greater plasma cholesterol concentrations than the low-responders. The differences were most obvious during the administration of the TSBE diet (P_1 through P_3); over *threefold differences were seen. Administration of cholestyramine* significantly decreased the plasma cholesterol concentrations of the high-responders (within 30 days) and, to a lesser, but significant degree, that of the low-responders. Plasma cholesterol concentrations changed little while the animals were on the low cholesterol (TSB) diet, even during the administration of cholestyramine.

Effect of cholestyramine treatment on plasma lipids and apolipoproteins

The effect of cholestyramine treatment during the TSBE and TSB diets on various plasma variables is shown in **Fig. 3**. Administration of cholestyramine while on the cholesterol-rich diet resulted in a number of significant changes in the lipoprotein profile of both high- and low-responders (Fig. 3a–e). It resulted in a parallel and precipitous drop in LDL cholesterol and plasma apoB concentrations (85% decrease in both) in high-responders. Most of the changes occurred in the first 10 days of the treatment. Qualitatively similar changes occurred in the low-responders: a 48% decrease in LDL cholesterol and a 49% decrease in plasma apoB were observed. Conversely, cholestyramine treatment resulted in a significant, nearly twofold increase in plasma apoA-I and a 2.6-fold increase in HDL cholesterol concentrations in the high-responding individuals. Cholestyramine treatment had no effect on these variables in low-responders. By the end of the cholestyramine treatment, plasma apoB and apoA-I as well as LDL and HDL cholesterol concentrations were statistically indistinguishable in the two groups of monkeys. These changes were also reflected in the LDL/HDL cholesterol ratios of the high-responders, which fell from about 14 to less than 1 (see Fig. 3e). This ratio also decreased somewhat in the low-responders, but the change failed to reach statistical significance.

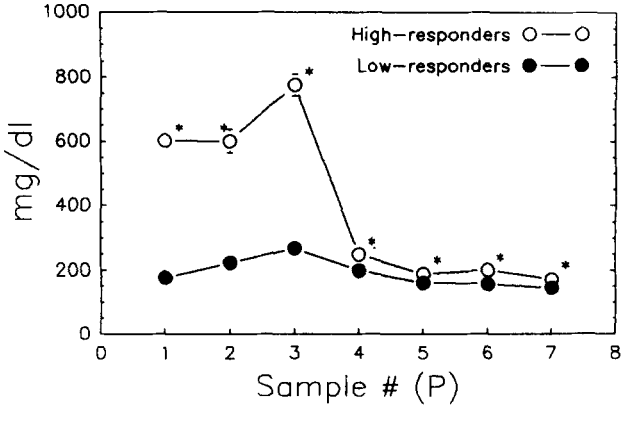

Fig. 2. Plasma cholesterol concentrations in high- (○) and low-responding (●) monkeys throughout the study. Plasma sample numbers (P) correspond to those indicated in Fig. 1; *, value significantly different from the corresponding low-responder value (at $P<0.05$).

At the conclusion of the cholestyramine treatment, both groups of animals were taken off the cholesterol-rich diet and put on the control low-cholesterol (TSB) diet. They were maintained on this diet for 15 months (see Fig. 1) before a second administration of cholestyramine was initiated. The results from this phase of the study are shown in Fig. 3f–j. At the beginning of the treatment none of the variables measured, except plasma LDL cholesterol, were statistically different in the two groups of monkeys. Cholestyramine treatment of high-responders during the control diet resulted in only a transient decrease in plasma LDL cholesterol, but lost significance by the end of the treatment (although it amounted to 25%); and a small (17.5%), but significant decrease in apoB. Both LDL cholesterol and plasma apoB were further significantly depressed by cholestyramine treatment in low-responders (both by 14%). The treatment had no effect on plasma apoA-I or HDL cholesterol levels in either group of animals. The LDL/HDL cholesterol ratios remained similar in both groups of animals throughout the treatment.

Hepatic LDL receptor activities

In order to investigate the possible mechanism responsible for the rapid lowering of plasma LDL by cholestyramine treatment of the high-, and to a lesser extent, low-responders while on the TSBE diet, the activity of the hepatic LDL receptors was assayed at the end of the treatment and compared to those seen in the same animals 7 months earlier, while on the cholesterol-rich diet alone. Typical binding curves for individual high- and low-responding monkeys on a cholesterol-rich diet before and after cholestyramine treatment are shown in **Fig. 4**. Note that, in the absence of cholestyramine, specific LDL binding was not detectable in hepatic microsomes obtained from a high-responding individual (Fig. 4C), while it was present in the low-responding monkey (4A). Cholestyramine treatment resulted in an induction of measurable LDL binding activity in the high-responder and increased binding in the low-responder (4D and B).

Binding studies were performed on all liver samples obtained from animals on both diets, without and with cholestyramine administration. The results, shown in the form of averaged Scatchard analyses, are summarized in **Fig. 5**. In addition, average B_{max} and K_D values were ob-

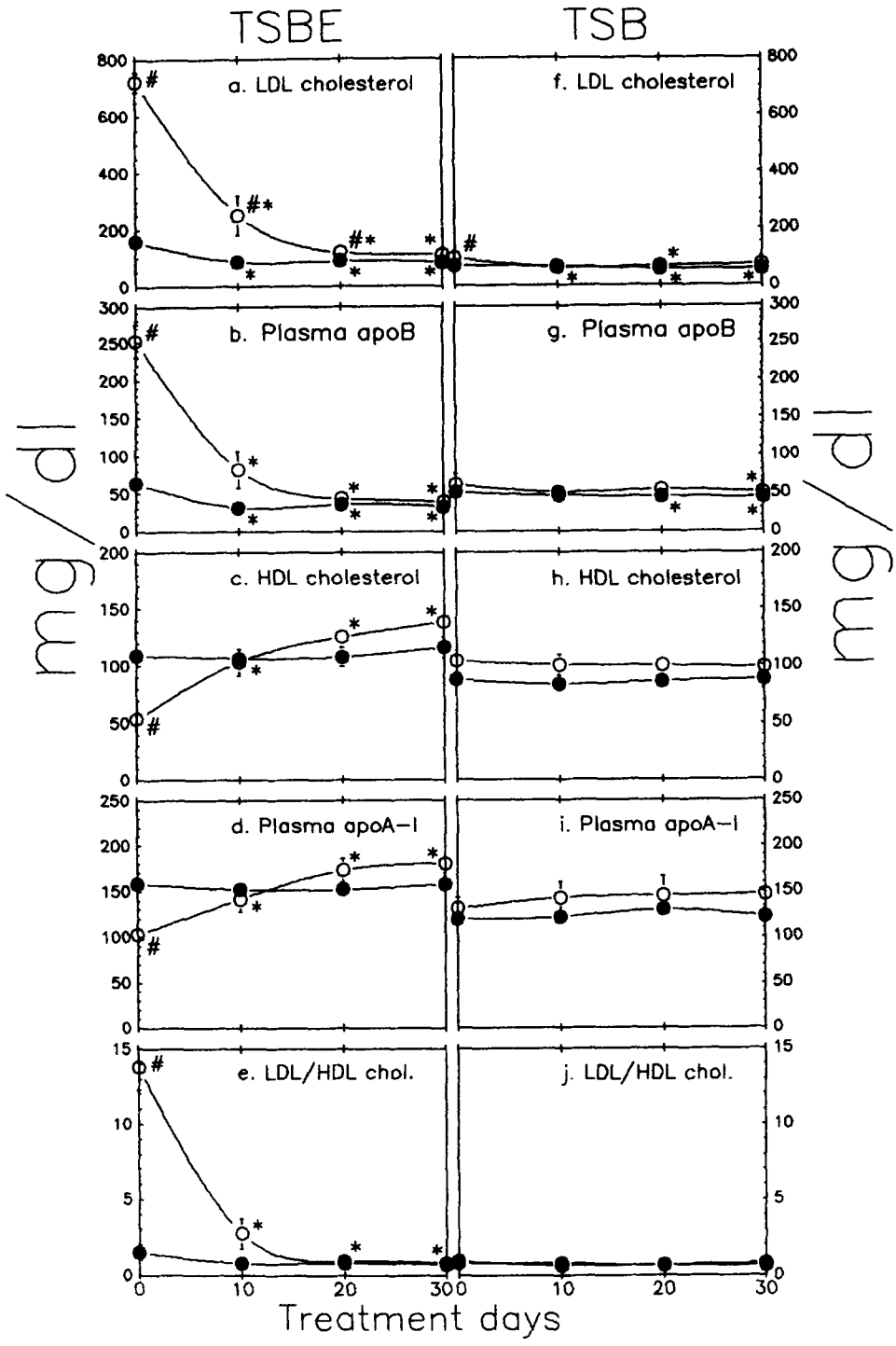

Fig. 3. The effect of cholestyramine treatment on plasma lipid and apolipoprotein concentrations in high- (○) and low- (●) responders, while on the high cholesterol (TSBE) or control (TSB) diet. Points represent the mean ± SEM of five low-responding and four (TSBE) and three (TSB) high-responding rhesus. (The size of the symbol may obscure the SEM.) The 0 and 30 day points on the left (TSBE) panel correspond to P_3 and P_4, respectively, and the 0 and 30 day points on the right (TSB) panel correspond to P_6 and P_7, respectively, as shown in Fig. 1; #, value, in high-responders, significantly different (at $P<0.05$) from the corresponding low-responder value; •, value in either group of animals significantly different (at $P<0.05$) from the 0 day treatment value of the same group.

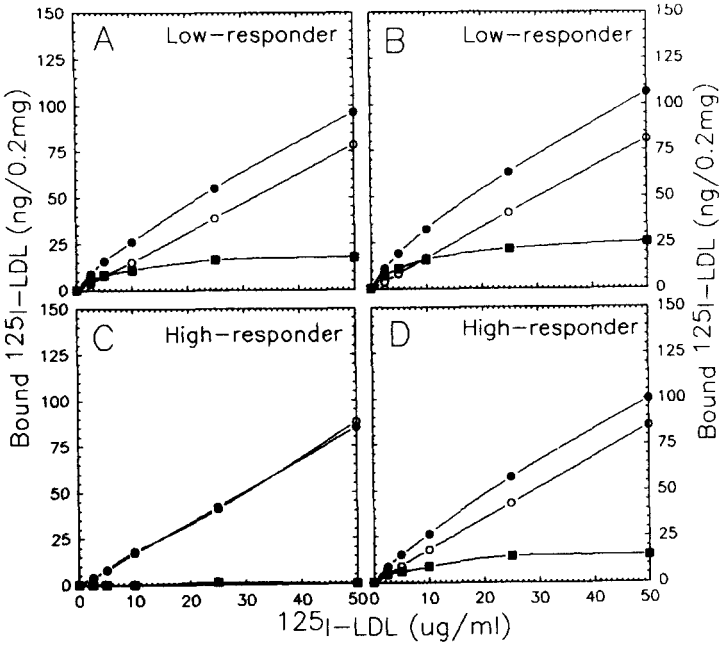

Fig. 4. Binding of ^{125}I-labeled LDL to liver microsomes from a low responder (Rh # 7432) on the TSBE diet prior to and after cholestyramine treatment is shown in panels A and B, respectively. Binding of ^{125}I-labeled LDL to liver microsomes from a high responder (Rh # 7400) on the TSBE diet prior to and after cholestyramine treatment is shown in panels C and D, respectively. In each panel total (●), nonspecific (○), and net (■) binding are shown. Nonspecific binding was determined in the presence of 30 mM EDTA and was subtracted from total binding to obtain net (specific) binding. Details of the binding studies are described in the Methods.

tained from individual Scatchard curves and are shown in **Table 1**.

As already indicated in Fig. 4, none of the high-responding monkeys expressed detectable LDL binding activity while on the TSBE diet, while low-responders did (Fig. 5A). Administration of cholestyramine for 30 days induced a measurable amount of LDL binding in the high-responders and increased LDL binding in low-responders (Fig. 5B). The affinity and number of receptors in high-responders expressed in response to the cholestyramine treatment were similar to those of the low-responders without cholestyramine treatment ($\sim 1.5 \times 10^{-9}$ M and 100 ng/mg microsomal protein, respectively). Both high- and low-responders exhibited significant and similar amounts of specific LDL binding on the TSB diet (K_D = 1.0–1.6 × 10^{-9} M, B_{max} = 140 ng/mg microsomal protein). The apparent affinity for LDL and the number of receptors were similar in both groups of animals. Cholestyramine treatment during the TSB diet had little additional effect on the affinity or number of receptors in

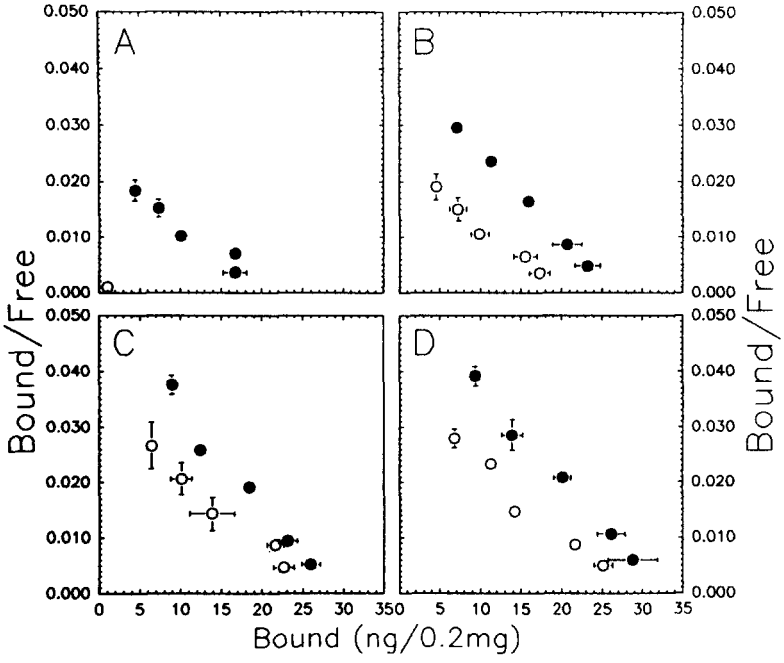

Fig. 5. Summary of the Scatchard analyses of the binding studies of human LDL to high-responder (○) and low-responder (●) rhesus liver microsomal fractions obtained from animals during the administration of TSBE diet without cholestyramine (panel A) and with cholestyramine (panel B). Panels C and D represent the summary of the analyses of samples obtained during the TSB diet in the absence and presence of cholestyramine treatment, respectively. Five low-responders were used throughout, and four and three high-responders were used while on the TSBE and TSB diet, respectively. *Bound* and *bound/free* values from individual binding curves (carried out in duplicate) were calculated and averaged at each ^{125}I-labeled LDL concentration. The error bars represent ± SEM and were plotted for each dimension, but in some cases are obscured by the size of the symbol.

TABLE 1. Average K_D and B_{max} values (± SEM) in the presence or absence of cholestyramine treatment during the TSBE and TSB diet administration periods

	TSBE Diet				TSB Diet			
	− Cholestyramine		+ Cholestyramine		− Cholestyramine		+ Cholestyramine	
Animals	K_D[a]	B_{max}[b]	K_D	B_{max}	K_D	B_{max}	K_D	B_{max}
High-responders	nd[c]	nd	1.5 ± 0.2[d]	103 ± 7[d]	1.6 ± 0.3	142 ± 2	1.2 ± 0.1	130 ± 16
Low-responders	1.5 ± 0.1[e]	101 ± 9[e]	1.1 ± 0.1	134 ± 11	1.0 ± 0.1	145 ± 7	1.0 ± 0.1	165 ± 15[d]

Values were obtained from individual Scatchard analyses. Five low-responders were used throughout the study, while four and three high-responders were used during the TSBE and TSB diet periods, respectively.
[a]Amount × 10^{-9} M.
[b]Given as ng/mg microsomal protein.
[c]Not detectable.
[d]Significantly different from pre-treatment value (within the same group).
[e]Significantly different from corresponding high-responder value ($P < 0.05$).

the high-responders. An additional small but significant increase in receptor number was observed in the low-responders, correlating with the additional decrease in plasma apoB concentration in these animals.

Hepatic HMG-CoA reductase

In order to determine the relationship between hepatic LDL receptor activity and cholesterol biogenesis, hepatic HMG-CoA reductase activities were also assayed on liver biopsy samples obtained during the TSBE as well as TSB diet periods before and after treatment with cholestyramine. In general, rhesus HMG-CoA reductase activities over the course of these studies were low. Assays in the absence of alkaline phosphatase did not, in any instance, result in measurable activities. For this reason only total reductase activities (dephosphorylated) are reported.

As shown in **Table 2**, HMG-CoA reductase was not detectable in the samples obtained from either group of animals while on the TSBE diet before cholestyramine treatment. Cholestyramine treatment induced the enzyme to a similar extent in both groups of animals (18 and 22 pmol · mg^{-1}/min in high- and low-responders, respectively). On the TSB diet the low-responders exhibited higher levels of this enzyme, when compared to the high-responders (18 vs 12 pmol · mg^{-1}/min). This difference was maintained upon subsequent treatment with cholestyramine, which induced a 75% and a 60% increase in the enzyme activity in the high- and low-responders, respectively.

DISCUSSION

The extent of intestinal absorption of dietary cholesterol has previously been identified in this laboratory as one of the major factors determining the rhesus monkey phenotype with respect to plasma cholesterol concentration (4, 5). High-responding animals have a consistently higher rate of dietary cholesterol absorption and lower overall rates of endogenous cholesterol synthesis than low-responding ones. Addition of plant sterols (2%) to the cholesterol-rich diet erased the observed differences in the rates of cholesterol absorption and equalized plasma cholesterol concentrations (5).

The present studies provide additional insight into the metabolic consequences of differential dietary cholesterol absorption. Specifically, we have now shown that high-responding monkeys, while on the cholesterol-rich diet, exhibited no detectable hepatic LDL (apoB,E) receptors. This is in direct contrast to the low-responders, which on the same diet retained a significant amount of hepatic LDL receptors (70% of those found while on the low-

TABLE 2. Rhesus liver HMG-CoA reductase activities found in the absence or presence of cholestyramine treatement

	TSBE Diet		TSB Diet	
	Cholestyramine		Cholestyramine	
Animals	−	+	−	+
	pmol · mg^{-1} · min^{-1}			
High-responders	nd[a]	18 ± 3[b]	12 ± 1	21 ± 1[b]
Low-responders	nd	22 ± 2[b]	18 ± 2	29 ± 2[b]

[a]Not detectable.
[b]Significantly different ($P < 0.05$) from pre-treatment value (within the same group).

cholesterol diet) of normal affinity. Endogenous hepatic cholesterol biosynthesis was negligible in both groups of monkeys on the cholesterol-rich diet. The expression of the hepatic LDL receptor during administration of the control (TSB) diet was identical, both in terms of numbers (B_{max}) and affinity (K_D), in the two groups of animals.

Recent studies suggest that bile sequestrants (colestipol or cholestyramine) lower plasma cholesterol levels by induction of the LDL receptors (12, 23–25). We have therefore used cholestyramine administration to assess the ability of these animals to induce their hepatic LDL receptors. The present data clearly indicate no deficiencies in the ability of high-responders to regulate the expression of the LDL receptors; administration of cholestyramine during the cholesterol-rich diet induced the expression of the hepatic LDL receptors in the high-responders to levels similar to those seen in the low-responders. The induction of these receptors was accompanied by a dramatic change in the apolipoprotein- and lipoprotein profiles. The levels of apoB-containing lipoproteins and cholesterol associated with them fell dramatically within 20 days of treatment. These changes were accompanied by an equally dramatic rise in plasma apoA-I and HDL cholesterol concentrations. As a result, the LDL/HDL cholesterol ratio became indistinguishable between the two groups of animals. These observations are entirely consistent with the observed induction of the hepatic LDL receptors, but the mechanism responsible for the correction of HDL concentrations in the high-responders remains unknown.

Administration of cholestyramine during the normal (TSB) diet had a smaller effect. It induced an additional small but significant number of hepatic LDL receptors in the low responders, but appeared to have no effect on those of the high-responders. A larger sample number in future studies may reveal that cholestyramine can be effective in high-responders on a basal diet as well. Cholestyramine treatment stimulated HMG-CoA reductase activities by 75% and 60% in the high- and low-responders, respectively. The lack of significant changes in the hepatic LDL receptors in the high-responders correlated with the rather small changes in their plasma concentrations of apoB and LDL cholesterol. Cholestyramine treatment under these conditions had no effect on plasma apoA-I and HDL cholesterol concentrations in either group of monkeys.

The present studies have thus failed to reveal additional fundamental deficiencies in the manner in which these two groups of monkeys handle cholesterol. The observed differences in the rate of absorption of dietary cholesterol remain, thus far, the only and sufficient distinction between the two groups. Thus, the available data suggest that the increased input of dietary cholesterol in the form of intestinally derived lipoproteins in the high-responding individuals overloads the hepatic storage capacity to the point where a complete down-regulation of HMG-CoA reductase and the LDL receptors occur. This, in turn, leads to the accumulation of the apoB-containing lipoproteins in the plasma. Low-responders, on the other hand, maintain the expression of the LDL receptors, albeit at lower levels, in the face of decreased input of intestinally derived cholesterol. They are thus able to maintain significantly lower plasma concentrations of apoB and cholesterol. The removal of chylomicron remnants, the primary carriers of dietary cholesterol, is presumably unimpeded in either group of monkeys, as the receptor responsible for the removal of these particles is refractive to metabolic regulation (26, 27). Treatment of the high- and low-responding monkeys with cholestyramine stimulates, through depletion of the hepatic cholesterol pool, the induction of the LDL receptor in both groups to a similar extent, and the two groups of monkeys become virtually indistinguishable with respect to their plasma apolipoprotein and lipid profile.

Clearly, factors other than input of exogenously derived cholesterol can affect the hepatic regulatory cholesterol pool size. In addition to responses that limit hepatic cholesterol content (such as down-regulation of LDL receptors and/or HMG-CoA reductase), mechanisms that increase cholesterol excretion (and bile acid formation) or secretion (lipoprotein synthesis and secretion) could potentially play an important role in determining the responder phenotype. Thus, for instance, differences in the intestinal absorption of dietary cholesterol were deemed insufficient to account for the differences in plasma cholesterol concentrations in African green monkeys (7).

The possibility that cholestyramine administration may, in addition to the depletion of hepatic supply of cholesterol, also directly inhibit the absorption of dietary cholesterol was not ruled out in the present studies. Available data suggest that mixing cholestyramine with the diet prior to administration has no effect on cholesterol absorption in humans (28). Regardless of the effect cholestyramine might have on cholesterol absorption, the present data clearly demonstrate the normal ability of the high responders to regulate *1*) the degree of the hepatic LDL receptor expression and *2*) the extent of endogenous cholesterol biosynthesis. The latter has also been demonstrated under in vivo conditions in previous studies in this laboratory (5).

The present studies suggest that, in addition to other loci, the extent of intestinal cholesterol absorption may be an important factor in determining plasma cholesterol concentrations in the human population and clearly point out the potent effect of cholestyramine treatment. In light of these results, a thorough evaluation of cholestyramine

treatment of human hypercholesterolemias under strictly controlled conditions deserves further attention.

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