Hepatic Cholesterol and Bile Acid Synthesis, Low-Density Lipoprotein Receptor Function, and Plasma and Fecal Sterol Levels in Mice: Effects of Apolipoprotein E Deficiency and Probucol or Phytosterol Treatment

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We compared hepatic cholesterol metabolism in apolipoprotein (apo) E-knockout (KO) mice with their wild-type counterparts. We also investigated the effects of treatment with phytosterols or probucol on the activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase (cholesterol synthesis), cholesterol 7α-hydroxylase and sterol 27-hydroxylase (bile acid synthesis), and low-density lipoprotein (LDL) receptor function in this animal model of atherogenesis. These findings were then related to treatment-induced changes in plasma, hepatic, and fecal sterol concentrations. Mouse liver membranes have binding sites similar to LDL receptors; the receptor-mediated binding represents 80% of total binding and is LDL concentration-dependent. These binding sites have higher affinity for apo E-containing particles than apo B only-containing particles. Deletion of apo E gene was associated with several-fold increases in plasma cholesterol levels, 1.5-fold increase in hepatic cholesterol concentrations, 50% decrease in HMG-CoA reductase activity, 30% increase in cholesterol 7α-hydroxylase and 25% decrease in LDL receptor function. Treatment of apo E-KO mice with either probucol or phytosterols significantly reduced plasma cholesterol levels. Phytosterols significantly increased the activity of hepatic HMG-CoA reductase, and probucol significantly increased cholesterol 7α-hydroxylase activity. Neither treatment significantly altered hepatic LDL receptor function. Phytosterols, but not probucol, significantly increased fecal sterol excretion and decreased hepatic cholesterol concentrations. Plasma cholesterol lowering effects of phytosterols and probucol are due to different mechanisms: stimulation of cholesterol catabolism via increased bile acid synthesis by probucol and decreased cholesterol absorption by phytosterols. In the absence of apo E, hepatic LDL receptors could not be upregulated and did not contribute to the cholesterol lowering effects of either agent.

AS APOLIPOPROTEIN (apo) E plays a crucial role in the receptor-mediated uptake of lipoproteins,[1,2] subjects with apo E deficiency or defective apo E develop type III hyperlipidemia.[3,4] The major abnormal plasma lipoprotein in these subjects is cholesteryl ester-enriched very–low-density lipoprotein (VLDL) called ß-VLDL.[5] Apo E gene was the first lipoprotein transport gene to be deleted in knockout (KO) mice.[6,7] The ß-VLDL particles are major components of circulating lipoproteins in apo E-deficient (apo E-KO) mice. Furthermore, unlike wild-type mice, apo E-KO mice have a significant amount of low-density lipoprotein (LDL)-cholesterol with only a trace amount of high-density lipoprotein (HDL)-cholesterol.[6-8] This abnormal lipoprotein profile is believed to play a causal role in accelerated atherogenesis in this animal model.[6,7]

Both the pathogenesis of accelerated atherosclerosis and its prevention in apo E-KO mice have been extensively studied.[8-12] Although high plasma cholesterol concentrations and impaired antioxidant system are believed to play causal roles in accelerated atherogenesis, decreasing plasma cholesterol levels and increasing plasma antioxidant enzyme activities by probucol treatment paradoxically promotes atherogenesis in this animal model.[8-13] On the other hand, treatment with plant sterols significantly reduces plasma cholesterol and atherosclerosis in apo E-KO mice.[8-10] The fact that both probucol and phytosterols are effective hypocholesterolemic agents, but have opposite effects on atherogenesis, raises the question of how these agents affect hepatic cholesterol metabolism and cholesterol homeostasis. Despite extensive use of apo E-KO mice in studying atherogenesis, our knowledge about hepatic cholesterol metabolism in this model is poor.

The objectives of the present study were: (1) to examine several parameters of hepatic cholesterol metabolism in apo E-KO mice and relate them to plasma, hepatic, and fecal sterol concentrations; and (2) to determine how these parameters are affected by probucol and phytosterols (compounds effective in reducing plasma cholesterol levels, but with opposite effects on atherogenesis).

MATERIALS AND METHODS

Animals and Diets

Four-week-old male C57BL/6J mice homozygous for deletion of apo E gene (apo E-KO) and their wild-type counterparts (C57BL/6J) along with LDL receptor-deficient mice were purchased from the Jackson Laboratory, Bar Harbor, ME. Animals were housed individually with ad libitum access to food and water in a well-ventilated animal unit with a 12:12-hour light-dark cycle. The mice were maintained on a PicoLab mouse diet[20] (Jamieson's Pet Food Distributor, Delta, British Columbia). After a 10-day adaptation period, 24 of the apo E-KO mice were divided into 3 groups of 8 mice each, matched for their plasma lipid levels and body weight. The mice were fed the same diet without supplementation (untreated) or supplemented with 1% (wt/wt) probucol (Hoechst Marion Roussel, Cincinnati, OH; probucol-treated); or 2% (wt/wt) a phytosterol mixture (phytosterol-treated) for 20 weeks. The phytosterol mixture used contained 69% β-sitosterol, 16% sitostanol,

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and 15% campesterol.[9] The study was approved by the Animal Care Committee of the University of British Columbia.

Blood Sampling

Blood was taken from either tail vein (at the initiation of the study) or at the end of the investigation from the right ventricle of the anesthetized mice. At the end of the study, the anesthetized mice were killed by cardiac puncture as previously described.[9] Plasma was separated by centrifugation and used for biochemical analyses.

Plasma, Fecal, and Hepatic Sterol Analyses

During the last 2 weeks of the experiment, fecal material was collected from each individual mouse cage and kept frozen until the analyses. Sterol contents of the liver homogenates, fecal materials, and final plasma samples were extracted and quantitated using gas capillary liquid chromatography as previously described.[14] Plasma-free cholesterol concentrations were determined by an enzymatic assay as previously described.[9]

Hepatic Enzyme (3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase, Cholesterol 7α-Hydroxylase, and Sterol 27-Hydroxylase) Activities

Hepatic microsomes and mitochondria were prepared by differential ultracentrifugation as previously described.[15] The various enzyme activities were measured using the previously published methods.[15-17] Briefly, for 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase activity, an aliquot of the microsomal preparation was incubated for 15 minutes at 37°C in a buffer containing an nicotinamide adenine dinucleotide phosphate (NADPH)-generating system and ^{3}H mevalonolactone as an internal recovery standard. The reaction was started with the addition of 30 nmol [3-^{14}C]HMG-CoA and stopped with the addition of 20 μL 6 N HCl. After lactonization at 37°C for 30 minutes, the products were separated by thin-layer chromatography and quantified by liquid scintillation counting. Microsomal cholesterol 7α-hydroxylase activity and mitochondrial sterol 27-hydroxylase were measured by isotope incorporation methods.[16,17] Briefly, ^{14}C-cholesterol was incubated with hepatic microsomes or mitochondria, and the products were extracted and applied to silica-gel plates. The amount of 7α-hydroxycholesterol or 27-hydroxycholesterol formed per milligram protein/minute was defined as the unit of the activity of enzymes, respectively. In animals with increased hepatic cholesterol concentrations (untreated apo E-KO and probucol-treated apo E-KO), hepatic cholesterol 7α-hydroxylase activity was also assayed in a reconstituted system after removal of endogenous cholesterol by acetone treatment and compared with similarly assayed microsomes from untreated wild-type animals.[23]

Hepatic LDL Receptor Function

Liver membranes were used to determine the function of LDL receptors by measuring specific binding of ^{125}I-labeled LDL from either human (LDL_H) or cholesterol-fed apo E-KO mice (LDL_M) as previously described.[18-20] LDL particles were separated by ultracentrifugation at density 1.019 to 1.063 g/L and VLDL particles at a density of 1.006 to 1.019 g/L and labeled with ^{125}I by the iodine monohydrochloride method, as previously described.[20] The LDL receptor-mediated binding was determined as the difference between total and nonspecific binding (measured in the absence and presence of 40-fold excess unlabeled lipoprotein, respectively). As negative controls, liver membranes from 4 LDL receptor-deficient mice were also analyzed for LDL receptor function.

Statistical Analyses

A single factor analysis of variance (ANOVA) followed by the application of the Tukey test was used to detect statistically significant intergroup differences. Results are expressed as mean ± SEM.

RESULTS

Effects of Deletion of apo E Gene on Hepatic Cholesterol Metabolism in C57BL/6J Mice

We compared several aspects of hepatic cholesterol metabolism in apo E-KO mice and their wild-type counterparts, C57BL/6J, both groups fed PicoLab mouse diet. Lack of apo E gene was associated with: (1) more than 10 times higher mean plasma total cholesterol concentrations (24.5 ± 4.8 *v* 2.0 ± 0.04, mmol/L, $P < .01$); (2) one half of HMG-CoA reductase activity (68 ± 14 *v* 133 ± 14, pmol/mg protein/min, $P < .05$); (3) 1.5 times higher hepatic cholesterol content (30 ± 4 *v* 21 ± 1, umol/mg protein, $P < .05$); (4) approximately 30% increase in cholesterol 7α-hydroxylase activity (36 ± 2 *v* 28 ± 3, pmol/mg protein/min, $P < .05$); and (5) a not statistically significant lower LDL receptor function as measured by specific binding of ^{125}I-LDL_H to liver membranes (161 ± 27 *v* 217 ± 20, ng/mg protein).

Table 1. Effects of 20-Week Treatment With Either Probucol or Phytosterols on Plasma and Hepatic Sterol Levels in apo E-KO Mice

Analytes	Untreated (n)	Probucol-Treated (n)	Phytosterol-Treated (n)
Plasma			
Total cholesterol (mmol/L)	24.5 ± 4.8 (8)	6.6 ± 2.3% (8)	15.3 ± 1.6* (8)
Free cholesterol (mmol/L)	11.2 ± 2.6 (8)	3.2 ± 0.5* (8)	8.8 ± 2.1 (8)
Desmosterol (μmol/L)	Not detected (8)	Not detected (8)	0.4 ± 0.1 (8)
Campesterol (μmol/L)	637 ± 264 (8)	101 ± 49* (8)	563 ± 156 (8)
Sitosterol (μmol/L)	147 ± 75 (8)	28 ± 11* (8)	166 ± 78 (8)
Total sterol (mmol/L)	25.1 ± 5.4 (8)	6.8 ± 2.4* (8)	16.5 ± 1.8* (8)
Hepatic			
Total cholesterol (μmol/mg/protein)	30.0 ± 3.6 (8)	28.6 ± 2.6 (7)	13.9 ± 1.6* (5)

NOTE. Free cholesterol concentrations were measured by an enzymatic method and the rest of analytes by gas chromatography method. Values are mean ± SEM.

*$P < .05$ compared with untreated.

Table 2. Fecal Sterol Compositions in apo E-KO Mice

Sterol	Untreated (n)	Probucol-Treated (n)	Phytosterol-Treated (n)
Cholesterol	1.6 ± 0.1 (8)	1.7 ± 0.1 (8)	2.3 ± 0.3* (8)
Coprostanol	0.5 ± 0.1 (8)	0.7 ± 0.1 (8)	0.3 ± 0.0 (8)
Lanosterol	0.1 ± 0.0 (8)	0.1 ± 0.0 (8)	0.2 ± 0.0* (8)
Sitosterol	1.8 ± 0.1 (8)	1.3 ± 0.1 (8)	33.9 ± 4.4* (8)
Total sterols	7.0 ± 0.2 (8)	6.0 ± 0.3 (8)	58.3 ± 7.4* (8)
Noncholesterol sterols	5.4 ± 0.2 (8)	4.2 ± 0.3 (8)	56.0 ± 7.1* (8)

NOTE. μ/g dry feces; mean ± SEM.
*$P < .05$ compared with untreated.

Effects of Probucol or Phytosterols on Plasma and Hepatic Sterol Levels in apo E-KO Mice

Data on plasma and hepatic sterol concentrations in apo E-KO mice are summarized in Table 1. Treatment with probucol and phytosterols resulted in 72% (25 *v* 7 mmol/L) and 40% (25 *v* 15 mmol/L) reductions ($P < .05$), respectively, in plasma total cholesterol levels in apo E-KO mice, as compared with untreated apo E-KO mice. Although both treatments resulted in reduction in plasma-free cholesterol levels in apo E-KO mice, only the change in plasma-free cholesterol in probucol-treated animals reached statistical significance ($P < .05$). Hepatic cholesterol concentrations were significantly reduced ($P < .05$) in phytosterol-, but not in probucol-treated animals (Table 1). Only trace amounts of plant sterols were detected in all liver specimens.

Fecal Sterol Concentrations and Composition in apo E-KO Mice

Compared with untreated apo E-KO mice, there was no change in fecal cholesterol concentration in the probucol-treated mice, but a significant increase (+44%, $P < .05$) in the phytosterol-treated group (Table 2). Fecal concentrations of several other sterols in apo E-KO mice are also shown in Table 2. Compared with untreated apo E-KO mice, phytosterols increased lanosterol 2-fold ($P < .05$). As expected, the dietary supplementation with phytosterols caused a marked increase in sitosterol and total sterol excretion. In addition, cholestanol, coprocampestanol, coprositostanol, campesterol, sitostanol, stigmasterol, and lanosterol were detected in small amounts in the fecal extracts (data not shown). Changes in fecal sterol concentrations in probucol-treated apo E-KO mice were not statistically significant. Probucol and its metabolites were also detected in fecal materials from the probucol-treated animals.

Hepatic Enzyme Activities

The effects of probucol and phytosterols on the hepatic enzymes involved in cholesterol biosynthesis (HMG-CoA reductase) and catabolism (cholesterol 7α-hydroxylase, and sterol 27-hydroxylase) are shown in Table 3. Phytosterols markedly increased the activity of HMG-CoA reductase (+184% compared with untreated apo E-KO mice, $P < .05$), but only moderately increased cholesterol 7α-hydroxylase (18%, not statistically significant), while probucol significantly increased the activity of hepatic cholesterol 7α-hydroxylase activity (+45%, $P < .05$). Changes in hepatic mitochondrial sterol 27-hydroxylase activities by either treatment were not statistically significant.

Hepatic LDL Receptor Function

Figure 1 represents optimal conditions for the measurements of LDL receptor function in mouse hepatic membranes. Mouse liver membranes have binding sites that are similar to LDL receptors, where excess unlabeled apo B- or apo E-containing lipoproteins can effectively compete with ^{125}I-labeled LDL for binding to these sites. The receptor-mediated binding of radiolabeled LDL_M to untreated wild-type mouse hepatic membranes was determined as the difference between total and nonspecific binding (measured in the absence and presence of excess unlabeled LDL, respectively). It represented approximately 80% of total binding, was ^{125}I-LDL_M concentration-dependent, reached a maximum with 25 μg/mL ^{125}I-LDL_M (Fig 1A), and increased linearly with increasing membrane concentrations up to 0.5 mg/mL (Fig 1B). The specificity and affinity of mouse hepatic LDL receptors for various lipoproteins are presented in Fig 2. The specificity of the LDL receptor for lipoprotein ligands is indicated by the ability of unlabelled lipoprotein to significantly inhibit binding of ^{125}I-LDL. The

Table 3. Effects of Probucol and Phytosterols on the Activities of Enzymes of Hepatic Cholesterol Metabolism in apo E-KO Mice

Enzyme	Untreated (n)	Probucol-Treated (n)	Phytosterol-Treated (n)
HMG-CoA Red (pmol/mg protein/min)	68.4 ± 14.0 (8)	147.0 ± 31.6 (8)	194.2 ± 41.1* (8)
Cholesterol 7α-Hyd (pmol/mg protein/min)	36.5 ± 1.7 (7)	52.9 ± 6.6* (7)	43.1 ± 1.8 (7)
Sterol 27-Hyd (pmol/mg protein/min)	18.4 ± 1.4 (8)	23.8 ± 3.3 (6)	19.0 ± 2.5 (8)

NOTE. Values are mean ± SEM.
Abbreviations: Red, reductase; Hyd, hydroxylase.
*$P < .05$ compared with untreated.

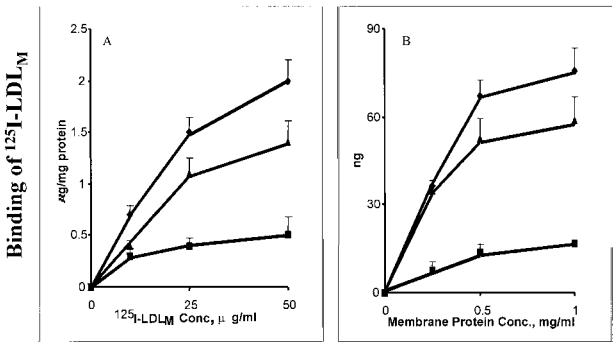

Fig 1. Determination of optimal conditions for the measurements of LDL receptor function in hepatic membranes from untreated C57BL/6J wild-type mice. Total (◆), nonspecific (■) and LDL receptor-mediated (▲) binding of ^{125}I-labeled mouse LDL (LDL_M from cholesterol-fed apo E-KO mice) were measured in the absence of unlabeled LDL_M, presence of 40-fold excess unlabeled LDL_M, and as the difference between total and nonspecific binding, respectively. LDL receptor-mediated binding of ^{125}I-LDL_M was measured after a 1-hour incubation at 0°C of 40-μg liver membrane protein with increasing concentrations of ^{125}I-LDL_M in a total volume of 80 μL (A), or with 25 μg/mL ^{125}I-LDL_M and increasing concentrations of liver membrane protein (B). Each data point (mean ± SEM) is from measurements of 3 to 4 animals.

affinity of the receptor for a ligand is indicated by the maximum amount of ligand bound per membrane unit after 1 hour of incubation (ie, the higher binding of a ligand per unit membrane protein, the higher the affinity of the membrane receptor for this ligand). Unlabeled LDL_M at a concentration of 1 mg/mL or higher inhibited the binding of ^{125}I-LDL_M to liver membranes more than 80% (Fig 2A). Other apo B- and/or apo E-containing lipoproteins (LDL_H and $VLDL_H$ or $VLDL_M$) can also specifically and efficiently compete with ^{125}I-LDL_M for binding to the mouse liver membranes (Fig 2B). Thus, measurements of specific ^{125}I-LDL binding to hepatic membranes (difference between total and nonspecific binding) reflect the binding function of the LDL receptor (apo B, E receptor). However, these apo E/B specific binding sites showed different affinities for various lipoprotein particles (Fig 2C). These mouse LDL receptors have a higher affinity for lipoproteins containing apo E, such as VLDL, than only apo B-containing LDL, regardless of the source of the lipoprotein particles (ie, mouse or human). Furthermore, they have higher affinity for mouse LDL and VLDL than human LDL and VLDL (Fig 2C). It should be noted that binding activity does not necessarily imply uptake of lipoprotein particles.

Figure 3 shows total and receptor-mediated binding of human radiolabeled LDL to hepatic membranes from wild-type, apo E-KO, and LDL receptor-KO mice. Receptor-mediated LDL binding to liver membranes from apo E-KO animals tended to be lower, but was not statistically significant from wild-type controls. However, most of the LDL specific binding was eliminated in the membranes from LDL receptor-KO animals. The effects of treatment with probucol and phytosterols on receptor-mediated LDL binding are illustrated in Figure 4. As previously shown (Fig 2C), mouse hepatic LDL receptors have a higher affinity for mouse LDL than human LDL. How-

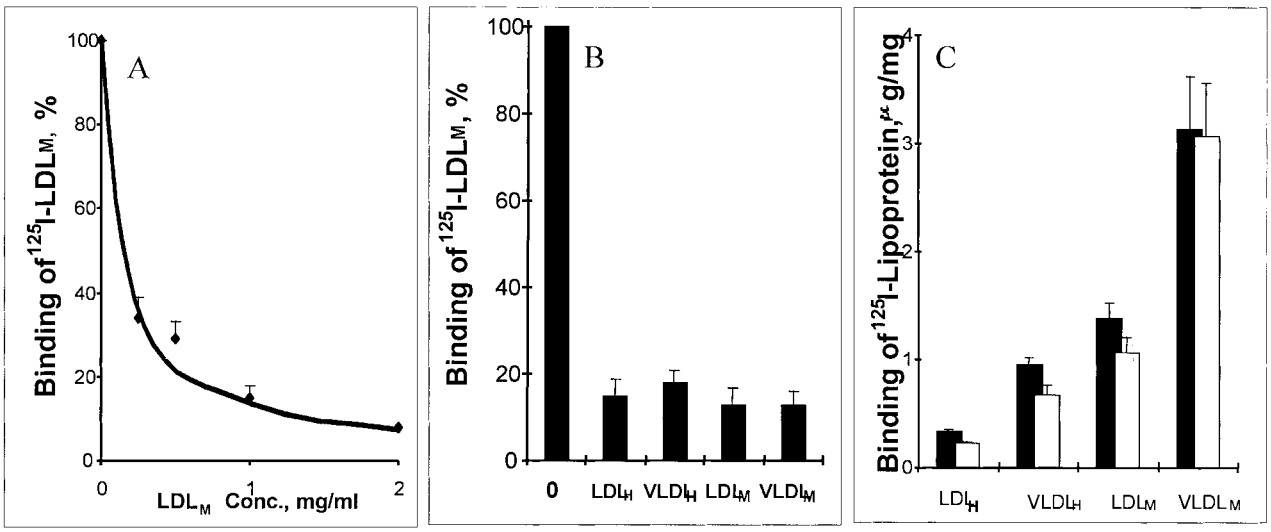

Fig 2. Specificity and affinity of LDL receptors for various lipoproteins in hepatic membranes from wild-type mice. Each data point or bar represents the mean ± SEM from 3 to 4 animals. (A) Inhibition of binding of ^{125}I-LDL_M to hepatic mouse membranes by increasing concentrations of unlabeled LDL_M ($P < .01$ for all tested levels, maximum reached at about 1 mg/mL). (B) Specificity of hepatic mouse LDL receptors for binding various lipoproteins. Binding of ^{125}I-LDL_M to mouse hepatic membranes was significantly inhibited ($P < .01$) by 40-fold excess of unlabeled apo E-/apo B-containing lipoproteins from humans (LDL_H and $VLDL_H$) and cholesterol-fed apo E-KO mice (LDL_M and $VLDL_M$). (C) Different affinity of mouse hepatic LDL receptors for ^{125}I-labeled lipoproteins from humans (LDL_H and $VLDL_H$) and cholesterol-fed apo E-KO mice (LDL_M and $VLDL_M$). Total binding of mouse ^{125}I-labeled lipoproteins (solid bars) were measured in the absence of unlabeled lipoproteins and LDL receptor-mediated binding (open bars) as the difference between total and nonspecific binding. Nonspecific binding (not shown) was measured in the presence of 40-fold excess unlabeled lipoproteins. The affinity of the mouse LDL receptors (ie, relative binding per milligram protein after 1 hour of incubation) is higher ($P < .01$) for ligands for the mouse (compared with human) source. For each source, it is higher ($P < .01$) for apo E–containing VLDL compared with LDL that contains only apo B.

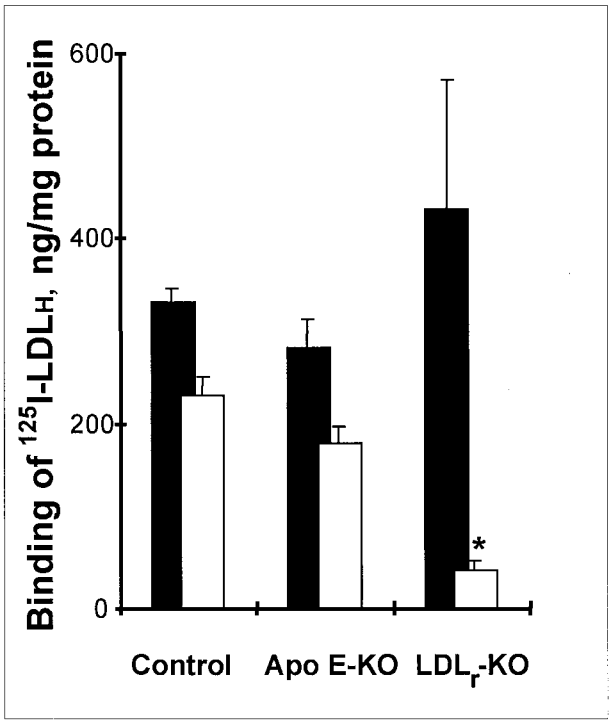

Fig 3. Hepatic LDL receptor function in untreated C57BL/6J wild-type (control), apo E-KO, and LDL receptor-KO mice. The bars represent mean ± SEM from at least 4 animals per group. LDL receptor-mediated binding of ^{125}I-LDL$_M$ to hepatic membranes (open bars) is measured as the difference between total binding (solid bars) and nonspecific binding (not shown, measured in the presence of 40-fold excess of unlabeled LDL). * $P < .01$ (significantly lower than values for C57BL/6J wild-type and apo E-KO mice).

ever, whether LDL receptor function was measured with labeled mouse LDL (Fig 4A) or human LDL (Fig 4B), probucol slightly decreased LDL receptor function (change not statistically significant), and phytosterols had no effect.

DISCUSSION

Compared with their wild-type counterparts, apo E-KO mice have higher plasma and hepatic cholesterol concentrations, reduced hepatic HMG-CoA reductase activity and LDL receptor function, and higher hepatic microsomal cholesterol 7α-hydroxylase activity. This indicates that the absence of apo E results in higher plasma cholesterol concentrations and hepatic cholesterol content and is associated with downregulation of hepatic HMG-CoA reductase activity and LDL receptor function. Hepatic sterol concentrations represent the net balance of uptake (LDL receptor- and non-LDL receptor-mediated), HMG-CoA reductase-controlled biosynthesis, and secretion (with and without prior conversion to bile acids). One can speculate that the elevated levels of plasma cholesterol in apo E-KO mice may be associated with greater nonreceptor-mediated hepatic sterol uptake and/or reduced secretion. However, additional investigation is needed to further define mechanisms for the hepatic sterol accumulation in apo E-KO mice.

We also have shown that apo E-KO mice were responsive to plasma cholesterol-lowering effects of both probucol and phytosterols. Our previous studies showed that probucol treatment causes a greater than 70%, 50%, and greater than 85% decreases in mean pooled VLDL-, LDL-, and HDL-cholesterol levels, while phytosterols decrease only VLDL- and LDL-cholesterol levels, over 30% and 20%, respectively, and increase (14%) HDL-cholesterol levels.[8] However, the cholesterol-lowering by these 2 compounds is associated with opposite effects on the development of atherosclerotic lesions in this particular animal model.[8,9] In contrast to phytosterols, probucol treatment results in an up to 3-fold increase in aortic lesion size (1.1 ± 0.1 *v* 0.4 ± 0.1 mm^2) in male apo E-KO mice.[8] On the other hand, neither probucol nor phytosterols has proatherogenic effects in wild-type C57BL/6J mice.[8] While the mechanism of cholesterol-lowering effect of probucol is not well defined,[21-23] phytosterols primarily inhibit cholesterol absorption.[24-26]

Treatment of apo E-KO mice with phytosterols significantly increased HMG-CoA reductase activity and was associated with detection of an increased amount of plasma desmosterol (a precursor of cholesterol). This finding is consistent with decreased intestinal cholesterol absorption leading to increased hepatic HMG-CoA reductase activity and cholesterol biosynthesis; phytosterols, which are known to inhibit cholesterol absorption, significantly upregulate the activity of this rate-controlling enzyme of cholesterol biosynthesis. In contrast, treatment with probucol resulted in a significant increase in the activity of the bile acid synthetic enzyme, cholesterol 7α-hydroxylase. Thus, although mean HMG-CoA reductase activity tended to increase, hepatic cholesterol concentration in the probucol-treated mice was not increased. This can be explained by both low uptake (due to slightly decreased LDL receptor function) and more rapid conversion of cholesterol to bile acids via both the classic and alternative bile acid synthetic pathways. Upregulated cholesterol 7α-hydroxylase was associated with significantly lower plasma cholesterol levels in our study. This finding is consistent with a previously suggested relationship between cholesterol 7α-hydroxylase and plasma cholesterol concentrations.[27]

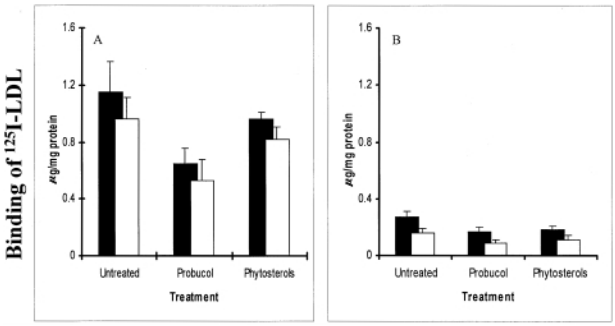

Fig 4. Hepatic LDL receptor function in apo E-KO mice after treatment with probucol and phytosterols. The bars represent mean ± SEM from at least 5 animals per group. The ^{125}I-labeled LDL used was separated from either blood of cholesterol-fed apo E-KO mice (A) or human blood (B). LDL receptor-mediated binding (open bars) is measured as the difference between total binding (solid bars, measured in the absence of unlabeled LDL) and nonspecific binding (not shown, measured in the presence of 40-fold excess of unlabeled LDL).

Phytosterols, but not probucol, significantly increased fecal cholesterol excretion compared to untreated apo E-KO. Because the diet contained only a trace amount (285 ppm) of cholesterol, practically all the fecal cholesterol was of endogenous origin. Thus, decrease in cholesterol reabsorption by phytosterols was associated with lower hepatic cholesterol content (less than half of that in either of the other groups) and with a further increase in HMG-CoA reductase activity. It is of interest that others reported no differences between apo E-KO mice and their wild-type counterparts in biliary secretion of cholesterol.[28] Increased biliary cholesterol secretion[29] coupled to decreased intestinal cholesterol reabsorption resulted in the lower cholesterol content observed in the phytosterol-treated animals. It should be noted that the coordinated regulation of HMG-CoA reductase and LDL receptor function that is normally observed[30] was not found in probucol- and phytosterol-treated apo E-KO mice. Increased HMG-CoA reductase activity was not associated with upregulated hepatic LDL receptor function in the absence of apo E. Moreover, our data may indicate a nonreceptor pathway for uptake of plasma lipoproteins by hepatocytes, which may lead to modification of HMG-CoA reductase activity. We believe that these are interesting observations and merit further investigation.

In conclusion, this study shows that deletion of apo E gene causes significant alterations in cholesterol metabolism. Apo E-KO mouse hepatic membranes contain specific binding sites for both human and mouse LDL and VLDL particles. Cholesterol-lowering effects of probucol are accompanied by a significant increase in hepatic cholesterol 7α-hydroxylase activity and to a lesser extent in that of hepatic sterol 27-hydroxylase in apo E-KO mice. Phytosterols, but not probucol, significantly increase fecal cholesterol excretion, resulting in decreased cholesterol absorption. This leads to lower plasma cholesterol levels and a marked increase in hepatic HMG-CoA reductase activity, without upregulation of LDL receptors most likely due to a deficiency of apo E.

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