# Hepatic mRNA levels for the LDL receptor and HMG-CoA reductase show coordinate regulation in vivo

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**Abstract** A sensitive solution hybridization assay using autologous cRNA probes was developed with the aim to study the simultaneous regulation of hepatic mRNA levels, on a quantitative basis, for the LDL receptor (LDLr), HMG-CoA reductase, and cholesterol 7α-hydroxylase (Cho-7-hx) in C57BL/6J mice. With the purpose to suppress and stimulate transcript levels respectively, animals received established high fat diets, cholesterol-enriched diets, and a diet supplemented with mevinolin and colestipol. One hundred nineteen animals were investigated in six separate experiments. In spite of an eightfold increase in hepatic cholesterol induced by a high fat diet, the LDLr and the HMG-CoA reductase mRNA levels were only reduced to 60–70% and 25–50% of control values, respectively. When the data from all animals were analyzed, a strong positive correlation was obtained between the mRNA levels for the LDLr and HMG-CoA reductase ($r = 0.79$, $P < 0.001$). A significant relation remained when control animals only were analyzed ($n = 42$, $r = 0.59$, $P < 0.001$). Cho-7-hx mRNA showed a regulatory pattern that differed from that of the LDLr and HMG-CoA reductase; feeding cholesterol at 1.7% and 5% but not at 0.4% elevated the mRNA levels for Cho-7-hx while the LDLr and HMG-CoA reductase mRNA levels were maximally suppressed already at 0.4% of dietary cholesterol. The results show that the mRNA levels for the LDLr and HMG-CoA reductase are regulated in parallel in the liver in vivo during various metabolic perturbations as well as at normal physiologic conditions. In agreement with previous results, cholesterol 7α-hydroxylase exhibits a different regulatory response.—**Rudling, M.** Hepatic mRNA levels for the LDL receptor and HMG-CoA reductase show coordinate regulation in vivo. *J. Lipid Res.* 1992. **33:** 493–501.

**Supplementary key words** solution hybridization • cholesterol • high fat • feeding • mevinolin • colestipol • apoE • apoB

The plasma cholesterol level in various species including humans is determined by the net balance between the input of cholesterol into plasma (synthesis) and the removal rate from plasma, the latter in part dependent on the number of LDL receptors (LDLr). The liver is a key organ in control of this balance not only because it harbors the major part of the LDL receptors in the body, but also because it is responsible for a large part of the cholesterol being synthesized within the body where HMG-CoA reductase is rate limiting. Furthermore, the liver is the only organ that can excrete significant amounts of cholesterol from the body predominantly after being converted into bile acids—an enzymatic pathway governed by the rate-limiting enzyme cholesterol 7α-hydroxylase, (Cho-7-hx). The regulation of HMG-CoA reductase is extremely high and rapid due to regulation at the transcriptional, translational, as well as at the protein level (1). The LDLr is believed to be regulated at the transcriptional level; however recent reports suggest that the LDL receptor may also be regulated at a posttranscriptional level in vitro and in vivo (2, 3). As regards the Cho-7-hx, current data so far indicate that this enzyme shows regulation at the level of transcription (4, 5). The LDL receptor and HMG-CoA reductase activities show coordinate regulation in cultured cells (6). Studies on the simultaneous regulation of LDL receptor and HMG-CoA reductase activity in the liver in vivo are sparse and previous evidence has indicated that the LDL receptor activity in vivo may be regulated independently of that for HMG-CoA reductase in the liver (7).

At the RNA level, in vitro, the LDLr and HMG-CoA reductase show coordinate regulation (1). However, no quantitative studies have addressed the question whether such regulation also exists in the liver in vivo. In a recent report (8), using a semiquantitative techni-

Abbreviations: LDL, low density lipoproteins; VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; LDLr, low density lipoprotein receptor; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; Cho-7-hx, cholesterol 7α-hydroxylase; FPLC, fast protein liquid chromatography.

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que, it was concluded that HMG-CoA reductase is not regulated at the transcriptional level in the liver of rats fed a diet containing 2% cholesterol.

Since quantitative data are not available concerning the simultaneous regulation of hepatic mRNA levels for the LDLr and HMG-CoA reductase, or Cho-7-hx, it is of importance to establish how they are regulated at the mRNA level across a broad range of metabolic perturbations commonly used in this field of research. To best answer this question, a solution hybridization titration assay, previously documented as highly accurate with a sensitivity of approximately one molecule of mRNA per cell (9), was used for the quantitative measurement of transcript levels. To suppress transcript levels, C57 BL/6J mice were fed high fat diets that had previously been used to induce hypercholesterolemia and arteriosclerosis in this mouse strain (10, 11). To maximally induce the above transcript levels, cholesterol depletion was induced by combined treatment with colestipol and mevinolin.

It is shown that the assay can faithfully quantitate mRNA for the LDLr, HMG-CoA reductase and Cho-7-hx, all present in low abundance in mouse liver. Even during extreme dietary fat loads, the hepatic down-regulatory responses of the LDLr and HMG-CoA reductase mRNA levels were remarkably small. However, under all conditions tested, hepatic mRNA levels for the LDLr and HMG-CoA reductase showed coordinate regulation, while Cho-7-hx did not.

## MATERIALS AND METHODS

### Materials

[$^{35}$S]α-CTP and [$^{32}$P]α-CTP were purchased from Dupont-NEN products. Mevinolin (lactone form) was kindly provided by Alfred Alberts of Merck Sharp & Dohme. Colestipol (Colestid™) was from Upjohn, Kalamazoo, MI. Glass fiber filters (Whatman GF/C) were from Whatman, Maidstone, England. Cholesterol was obtained from ICN Biochemicals (Cat. no 101380) Cleveland, OH. Mazola™ Corn Oil was from Best Foods, CPC International, Englewood, Cliffs, NJ. Other materials were from the indicated sources.

### Animals

Male C57BL/6J mice (7–8 weeks) were purchased from Jackson Laboratories, Bar Harbor, ME. All mice were exposed to 12 h of light (6 AM to 6 PM) and 12 h of darkness (6 PM to 6 AM). The animals had free access to water and chow.

### Animal diets

Diet challenges were performed for the indicated number of days. Standard mouse chow and Thomas-Hartroft diet (TD 78 399) were obtained from Teklad, Madison, WI. Two high-fat diets (referred to by their cholesterol content); "high-fat diet 1.7%" and "high-fat diet 0.6%" were prepared by grinding the Thomas Hartroft diet with standard mouse chow in a weight ratio of 1 to 2 parts and 1 to 8 parts, respectively. The resulting two high-fat diets contain, by weight, 10% cocoa butter, 10% casein, 0.7% sodium cholate, 1.7% cholesterol, and 3.3% cocoa butter, 3.3% casein, 0.2% sodium cholate, 0.6% cholesterol, respectively.

Animals treated with mevinolin + colestipol received standard mouse chow supplemented with 0.25% mevinolin and 2% colestipol. When the effects of increased cholesterol content only in the diet were investigated, the diets were prepared by mixing corn oil, into which cholesterol had been dissolved, with control mouse chow so that the final diets contained, by weight, 0.4%, 1.7%, and 5% cholesterol. The content of corn oil in these cholesterol-enriched diets was 10%. Control chow was supplemented with 10% pure corn oil only.

All mice were killed between 2 and 4 PM. Animals were anesthetized with diethylether and blood was collected into EDTA-containing tubes upon cardiac puncture. After cervical dislocation, the abdomen was opened and the liver was removed. Food content was always present in the stomach indicating that the animals were not fasting at the time of killing. A tissue sample from the liver was rapidly frozen in liquid nitrogen for later cholesterol determination. The remaining liver tissue was immediately homogenized in 4 M guanidinium thiocyanate (Fluka Chimie AG, Switzerland, Cat. no 50990) containing 1% fresh mercaptoethanol and 0.5% sarcosin-laurate, N-lauroylsarcosine (Sigma). Total hepatic RNA was isolated by ultracentrifugation of guanidinium thiocyanate homogenates on cesium chloride (BRL, Gaithersburg, MD; Cat. no 5542UB). The RNA pellets were dissolved in diethylpyrocarbonate (Sigma)-treated water (DEP-$H_2O$), extracted with phenol–chloroform, and chloroform and were thereafter precipitated with ethanol. The RNA was redissolved in DEP-$H_2O$ and quantitated assuming 1 OD unit = 37 μg of RNA/ml. The RNA was stored in aliquots at –70°C prior to assay. Hepatic lipids were extracted according to the method of Folch, Lees, and Sloane Stanley (12). Aliquots of the organic phase were evaporated under a stream of nitrogen in the presence of 7.5 mg Triton-X 100 (Sigma). Total plasma and hepatic cholesterol were determined by a commercially available enzymatic method, Singlevial™, Boehringer Mannheim Diagnostics, Cat. no 236691.

### Solution hybridization analysis of mRNA

All mRNA levels were quantitated using a solution hybridization titration assay essentially as previously described by Mathews, Norstedt, and Palmiter (9).

Complementary RNA probes (cRNA) were synthesized in the presence of [$^{35}$S]CTP by the use of reagents and protocols from Promega Biotech, Madison, WI. Unincorporated NTPs were separated by precipitation with ethanol in the presence of ammonium acetate, after the addition of 10 μg of RNase-free tRNA (Sigma, cat no. R7125) as carrier. The specific activities of probes ranged from 9 to $25 \times 10^3$ cpm/$10^{-15}$ mole. The [$^{35}$S]cRNA probe was hybridized ($15–25 \times 10^3$ cpm/incubation) overnight at 68°C to three concentrations of hepatic RNA in duplicate. Each hybridization was performed in a volume of 40 μl containing 0.6 M NaCl, 30 mM Tris-HCl, pH 7.5, 4 mM EDTA, 10 mM dithiothreitol, and 25% formamide overlayered with 50 μl of mineral oil (Sigma). After overnight hybridization, samples were treated with 1 ml of a solution containing 40 μg RNase A (Boehringer Mannheim, GmbH, Germany, Cat. no. 109169), 2 μg RNase T1 (Sigma), and 100 μg salmon sperm DNA (Sigma). After 45 min of digestion at 37°C, 100 μl of 100% TCA was quickly added and samples were incubated on ice ≥ 15 min. The RNA-RNA hybrids were finally collected and washed with 70% ethanol on Whatman GF/C filters and the radioactivity on filters was determined by liquid scintillation spectrometry. The slopes of the linear hybridization signals were calculated by the method of least squares and compared to the slope generated when known amounts of a synthetic nucleotide mRNA standard were added in a range approximately 40–500 $\times 10^{-18}$ mol mRNA/tube. The mRNA copy number (molecules of mRNA/cell) was calculated assuming 5.5 pg DNA/cell and an RNA to DNA ratio of 2.7:1.

### Construction of plasmids for in vitro generation of cRNA and mRNA

Oligonucleotides were synthesized using an Applied Biosystems Inc. model 380A DNA synthesizer. The sequence used for the mouse LDL receptor was: 5′ CAGCGAGTACACCAGTCTGCTCCCCAACCTGAA-GAATGTGGTGGCTCTCGACACGGAGGTG 3′, and for the mouse HMG-CoA reductase: 5′ ACAATGTTGT CAAGACTTTTCCGGATGCATGGCCTCTTCGTGG-CCTCCCACCCCTGGGAAG 3′. These sequences correspond to nucleotides 1247–1308 in the human LDL receptor cDNA (13), and nucleotides –3 to 57 in the human HMG-CoA reductase cDNA (14). The synthetic DNA fragments were subcloned into the *Xba*I/*Eco*RI site of the pGEM-3Zf(+) vector. The sequences of the inserts were verified by automated DNA sequencing using an Applied Biosystems model 320A DNA sequencer (15). The mouse sequences were obtained by amplification of mouse genomic spleen DNA corresponding to cDNA nucleotides 1205–1344 (13) for the human LDL receptor, and cDNA nucleotides –6 to 143 (14) for human HMG-CoA reductase. The amplified genes were cloned into the *Eco*RI/*Eco*RI site of M13mp19 vector and the inserts were thereafter sequenced as described above. Amplification of genomic mouse DNA was made by the polymerase chain reaction using native Taq polymerase (Perkin-Elmer) essentially as described by the manufacturer.

A 184 base-pair mouse Cho-7-hx gene fragment was obtained by amplification of genomic mouse spleen DNA from nucleotide 646 to 813 (16). The gene fragment was cloned into the *Xba*I/*Eco*RI site in the pGEM-3Zf(+) vector. DNA sequencing (15) of the insert revealed a 90% homology between this mouse sequence and the corresponding rat Cho-7-hx sequence (16). A 60 base-pair synthetic DNA fragment corresponding to nucleotides 520 to 579 in the mouse apoE gene (17) was cloned into the *Xba*I/*Eco*RI site into the above vector. A synthetic DNA fragment, corresponding to 62 nucleotides within exon26 in the mouse apoB gene, was cloned into the same vector as described above. The partial mouse apoB sequence was kindly provided by Brian McCarthy, Gladstone Foundation, San Francisco, CA.

[$^{35}$S]α-CTP-labeled probes (cRNA) and mRNA were generated by transcription of linearized plasmids by the use of T7 (Pharmacia) or SP6 (Promega) RNA polymerase. mRNA synthesis was carried out according to the manufacturer's protocols in the presence of a trace amount of [$^{32}$P]α-CTP allowing for the quantification of the mRNA product.

### Separation of plasma lipoproteins by fast protein liquid chromatography (FPLC)

Fresh mouse plasma was pooled (2.0 ml) and subjected to ultracentrifugation two times at d 1.215 g/ml after the addition of KBr. The concentrated lipoproteins were subjected to gel filtration on a Superose™ 6B (Pharmacia) column (18) after adjusting the sample volume to 3 ml with 0.15 M NaCl, 0.01% EDTA, and 0.02% sodium azide, pH 7.2. Fractions (2 ml) were collected and the concentration of total cholesterol was determined using an enzymatic method as described above.

## RESULTS

Representative raw data obtained after overnight hybridization with respective [$^{35}$S]CTP-labeled cRNA probe to hepatic RNA of four mice from experiment 2 is shown in **Fig. 1**, left column (two control mice and two mice fed 1.7% high fat diet). Graphs on the right show the signals generated by the appropriate synthetic mRNA standards. The calculated copy numbers are given in the legend. Repeated titration assays (4

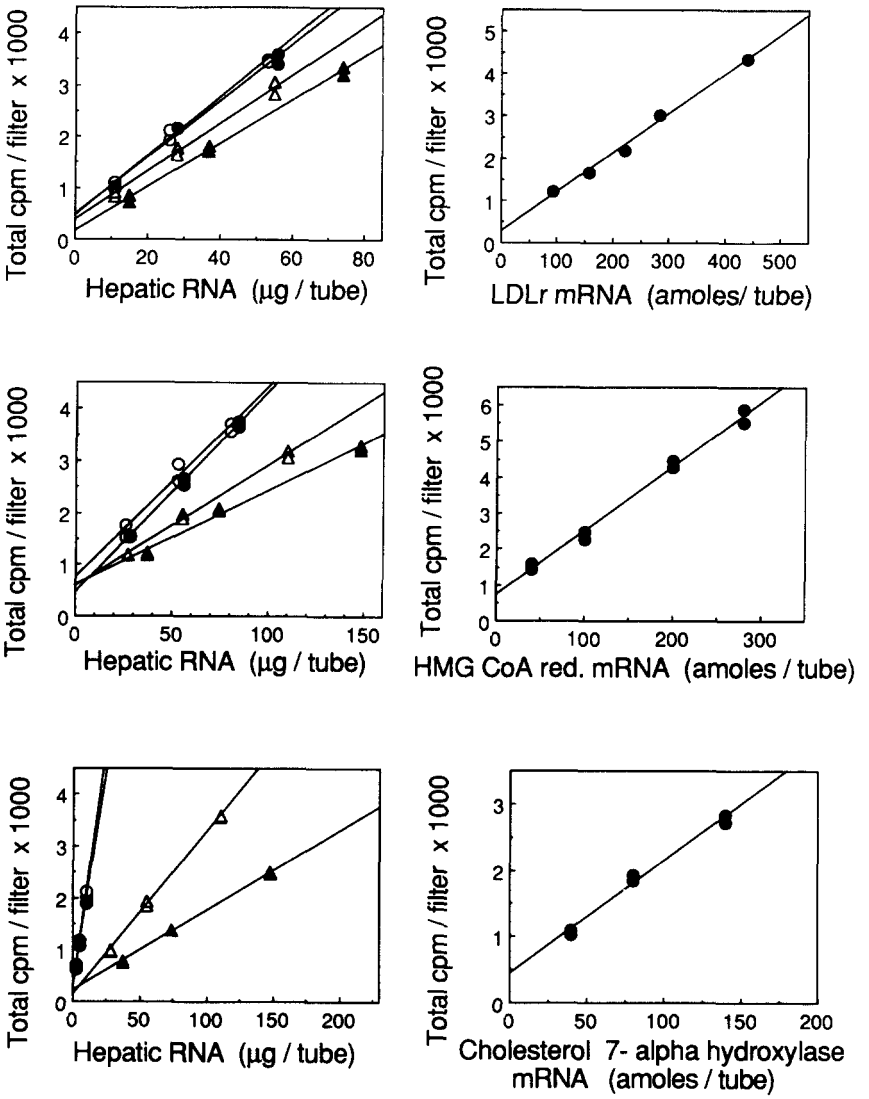


**Fig. 1.** Typical data generated by the solution hybridization assay. Raw data from three separate solution hybridization assays on hepatic RNA from four animals of experiment 2 (left column) and the corresponding standard curves (right column) generated with the indicated synthetic mRNA. (○, ●) Data from two control animals; (△, ▲) data from two animals on 1.7% high-fat diet. The calculated copy numbers (molecules of mRNA/cell) for ○, ● and △, ▲ for the LDLr were 53, 50, 43, and 39, respectively; 18, 19, 11, and 8.8 for HMG-CoA reductase, respectively; and 78, 69, 13, and 6.4 for Cho-7-hx, respectively.

times) for the LDLr mRNA abundance on one hepatic RNA extract from a normal mouse liver revealed a coefficient of variation of 5.1%.

With the aim of maximally suppressing hepatic transcript levels, mice were first given the Thomas-Hartroft diet mixed with standard mouse chow since it has been shown that this diet, a mixture of cholesterol, saturated fat, and cholic acid, induces profound hypercholesterolemia and atherosclerosis in this strain of mice (10, 11). The results from four separate experiments are shown in **Table 1.** When animals were given the high-fat diet at two different concentrations (experiments 1, 2, and 3), a dose-dependent increase in both plasma and hepatic cholesterol was found. Concomitantly, there was a dose-dependent decrease in the hepatic mRNA level for Cho-7-hx. In contrast, the mRNA levels for the LDLr and HMG-CoA reductase did not show such a dose-dependent inhibition. Regardless of the concentration of the high fat diets, the LDL receptor and the HMG-CoA reductase mRNA could only be reduced to approximately 60–70% and 25–50% of control values, respectively. It should be emphasized that this was in spite of a clear increase in both hepatic and plasma cholesterol levels that could be obtained when the fat content of the high-fat diet was increased threefold (compare data from 0.6% high-fat diet to those of 1.7% high-fat diet). The suppression of hepatic LDLr mRNA levels could not be

TABLE 1. Data of mice fed a high-fat diet

| Experiment | Total Plasma Cholesterol | Hepatic Cholesterol | mRNA Abundancy (copies/cell) LDL-Receptor | HMG-CoA Reductase | Cholesterol 7α-Hydroxylase | ApoE | ApoB |
|---|---|---|---|---|---|---|---|
| | *mg/dl* | *mg/g* | | | | | |
| Exp. 1 High fat diet (10 days) | | | | | | | |
| Controls (n = 6) | 97.5 ± 3.8 | 3.0 ± 0.1 | 69.9 ± 2.5 | 42.8 ± 5.4 | 117 ± 14.3 | 3840 ± 401 | 595 ± 161 |
| 0.6% High fat (n = 6) | 155 ± 6.9 (159) | 11.5 ± 1.6 (383) | 51.2 ± 4.1 (73) | 11.1 ± 0.9 (26) | 47.3 ± 6.3 (40) | 5560 ± 488 (145) | 509 ± 36.8 (86) |
| 1.7% High fat (n = 6) | 219 ± 10.3 (225) | 25.8 ± 1.3 (860) | 42.8 ± 3.3 (61) | 11.9 ± 0.6 (28) | 29.5 ± 5.4 (25) | 4470 ± 448 (116) | 442 ± 45.4 (74) |
| Exp. 2 High fat diet (10 days) | | | | | | | |
| Controls (n = 9) | 110 ± 3.3 | 2.6 ± 0.1 | 51.7 ± 4.3 | 15.4 ± 1.1 | 54.1 ± 6.3 | 3040 ± 344 | 619 ± 51.0 |
| 0.6% High fat (n = 10) | 140 ± 6.0 (127) | 9.9 ± 0.9 (381) | 35.4 ± 1.6 (68) | 6.8 ± 0.2 (44) | 38.8 ± 8.5 (72) | 4100 ± 238 (135) | 583 ± 19.8 (94) |
| 1.7% High fat (n = 10) | 198 ± 14.5 (180) | 18.8 ± 1.1 (723) | 38.4 ± 3.3 (74) | 8.1 ± 0.6 (53) | 11.1 ± 2.4 (21) | 3150 ± 222 (104) | 550 ± 30.9 (89) |
| Exp. 3 High fat diet (23 days) | | | | | | | |
| Controls (n = 6) | 95.7 ± 2.8 | 3.3 ± 0.1 | 57.7 ± 10.5 | 32.3 ± 5.0 | 95.3 ± 14 | 5800 ± 1080 | 320 ± 54.7 |
| 0.6% High fat (n = 6) | 130 ± 12.4 (136) | 9.0 ± 1.4 (272) | 33.5 ± 5.5 (58) | 8.6 ± 1.1 (27) | 46.7 ± 17.6 (49) | 6270 ± 742 (108) | 287 ± 50.6 (90) |
| 1.7% High fat (n = 6) | 153 ± 11.3 (160) | 21.5 ± 3.8 (651) | 64.3 ± 4.8 (111) | 25.3 ± 2.3 (78) | 40.7 ± 11.5 (43) | 10080 ± 857 (174) | 575 ± 79.8(180) |
| Exp. 4 High fat diet (43 days) | | | | | | | |
| Controls (n = 6) | 106 ± 1.3 | ND | 57.8 ± 2.7 | 13.1 ± 1.1 | 54.5 ± 15.9 | 3940 ± 258 | 525 ± 20.5 |
| 1.7% High fat (n = 5) | 225 ± 16.8 (212) | ND | 37.8 ± 4.2 (65) | 9.4 ± 2.1 (72) | 2.1 ± 0.5 (4) | 3620 ± 359 (92) | 408 ± 15.7 (78) |

All data from four separate experiments of mice fed a high-fat diet (Materials and Methods) at two different concentrations for the indicated number of days. Values given as means ± SEM. Numbers within parentheses express the change as percent of the respective experimental control values. ND, not determined; n, number of animals.

enhanced even after mice were fed high-fat diets for as long as 23 or 43 days (experiments 3 and 4). The clear but surprisingly small suppression of hepatic LDLr and HMG-CoA reductase transcripts prompted the assay of hepatic apoE and apoB mRNAs, representing two apolipoproteins in cholesterol-carrying lipoproteins in plasma. Although the mRNA levels of apoE tended to be higher among animals receiving the high-fat diet, there was no clear-cut change for apoE or apoB mRNAs which were both found in high abundance (Table 1). To investigate the plasma lipoprotein pattern of mice fed the high-fat diets, pooled mouse plasma from each of the three groups of animals of experiment 2 (Table 1) was separated by FPLC and subsequently assayed for cholesterol (**Fig. 2**). It was found that the high-fat diet resulted in a dose-dependent increase of cholesterol predominantly within large lipoprotein particles (VLDL and IDL) while the LDL fraction only showed a slight increase. In contrast, there was a dose-dependent decrease of cholesterol within fractions corresponding to HDL particles.

To investigate the effects of feeding cholesterol only, a commonly used protocol that leads to an elevated activity of Cho-7-hx (19, 20) and a decreased activity of HMG-CoA reductase (20) and the LDL receptor, groups of animals were fed diets containing increasing cholesterol concentrations (experiment 5). As seen in **Table 2**, cholesterol feeding resulted in decreased levels of LDLr and HMG-CoA reductase mRNA, with maximal suppression already at 0.4% of dietary cholesterol. The 0.4% cholesterol diet gave only a small increase in plasma and hepatic cholesterol levels. The Cho-7-hx mRNA level was not elevated; if anything, a slight decrease was obtained. Higher concentrations of dietary cholesterol doubled the hepatic cholesterol concentration and elevated Cho-7-hx mRNA levels 1.7-fold, but the mRNA for the LDLr and HMG-CoA reductase did not decrease further and the total plasma cholesterol level was unchanged.

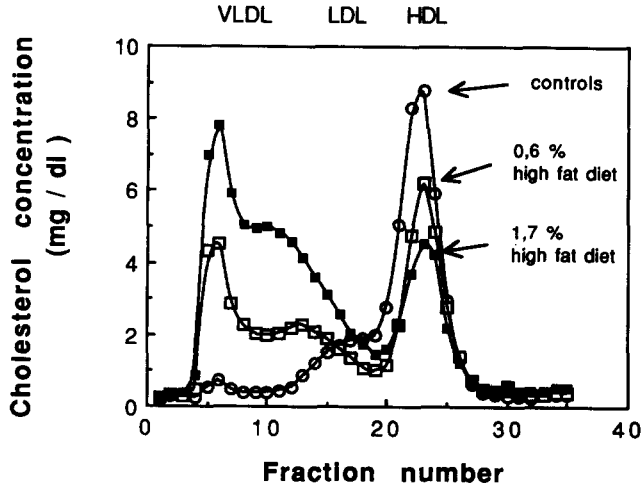


**Fig. 2.** Size fractionation of mouse plasma lipoproteins. Cholesterol concentrations of size-fractionated mouse plasma lipoproteins. Pooled plasma from all animals in experiment 2 was separated by FPLC on a Superose 6B column after lipoprotein separation by ultracentrifugation (Materials and Methods). Total cholesterol was determined by an enzymatic assay; (○) controls; (□) 0.6% high-fat diet; (■) 1.7% high-fat diet. The appropriate sites of elution of the major lipoprotein classes are indicated.

TABLE 2. Data of mice fed pure cholesterol and mevinolin + cholesterol

| Experiment | Total Plasma Cholesterol | Hepatic Cholesterol | mRNA Abundancy (copies/cell) LDL-Receptor | HMG-CoA Reductase | Cholesterol 7α-Hydroxylase |
|---|---|---|---|---|---|
| | *mg/dl* | *mg/g* | | | |
| Exp. 5 "Pure cholesterol" | | | | | |
| Controls (n = 6) | 97.3 ± 3.2 | 2.8 ± 0.11 | 52.7 ± 2.3 | 24.8 ± 3.8 | 112 ± 14.2 |
| 0.4% Pure chol. (n = 6) | 114 ± 12.8 (117) | 3.3 ± 0.1 (118) | 30.8 ± 4.5 (58) | 8.2 ± 1.1 (33) | 89.9 ± 18.4 (80) |
| 1.7% Pure chol. (n = 6) | 94.7 ± 4.5 (97) | 6.0 ± 0.3 (214) | 33.5 ± 1.3 (64) | 7.7 ± 0.5 (31) | 175 ± 20.5 (156) |
| 5% Pure chol. (n = 6) | 102 ± 1.7 (105) | 6.4 ± 0.3 (229) | 29.5 ± 2.1 (56) | 6.7 ± 0.2 (27) | 190 ± 8.1 (170) |
| Exp. 6 "Mevinolin + colestipol" | | | | | |
| Controls (n = 9) | 92.6 ± 0.3 | 2.9 ± 0.3 | 41.8 ± 6.2 | 16.1 ± 4.0 | 35.3 ± 4.2 |
| Mevinolin + colestipol (n = 10) | 77.5 ± 2.5 (84) | 2.6 ± 0.1 (90) | 70.6 ± 4.6 (169) | 87.8 ± 6.4 (545) | 111 ± 18.5 (314) |

Mice were fed normal chow supplemented with the indicated concentrations of dietary cholesterol only for 10 days (experiment 5). In experiment 6, animals received 0.25% mevinolin and 2% colestipol in the food for 10 days. Hepatic apoB or E was not determined in these experiments. Values are given as mean ± SEM; numbers within parentheses show the change as percent of respective experimental control values; n, number of animals.

Finally, with the purpose of maximally inducing hepatic transcript levels, mice were given normal chow supplemented with mevinolin and colestipol (Table 2, Exp. 6), a treatment previously shown to increase the mRNA levels for the LDLr and HMG-CoA reductase in rabbit and hamster liver (21). Although plasma and hepatic cholesterol levels showed only a slight decrease (84% and 90% of controls, respectively), there was a profound increase in the mRNA levels for HMG-CoA reductase (545%) and LDLr (169%). In addition, there was a clear (314%) increase of the Cho-7-hx mRNA level.

Figures 3–6 illustrate data from Tables 1 and 2. As seen in **Fig. 3**, a striking positive correlation between LDLr and HMG-CoA reductase mRNA levels was obtained ($r = 0.79$, n = 119, $P < 0.001$). This relationship is further illustrated in **Fig. 4**, where all 119 animals are presented in a semilog plot. When the 42 control animals were analyzed separately, a significant relation ($r = 0.59$, $P < 0.001$) remained between the mRNA levels for the LDL receptor and HMG-CoA reductase (not shown).

**Fig. 5** and **Fig. 6** show the results upon correlating Cho-7-hx mRNA with LDL receptor mRNA or HMG-CoA reductase mRNA, respectively. As seen in both figures, the regulatory responses of Cho-7-hx differed from that of the LDL receptor or HMG-CoA reductase mRNA. A striking difference was obtained after animals were fed high concentrations of pure cholesterol only; under these conditions the Cho-7-hx mRNA level was increased only in animals fed 1.7% or 5% cholesterol.

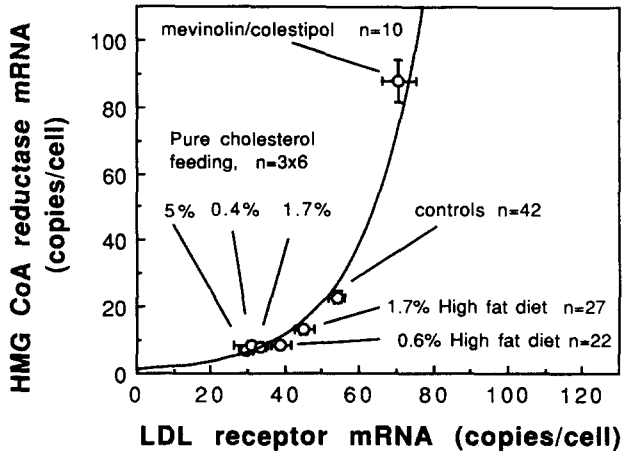


**Fig. 3.** Relation between hepatic mRNAs for LDLr and HMG-CoA reductase. Illustration of the data presented in Tables 1 and 2; circles, mean; bars, SEM. The respective groups are indicated.

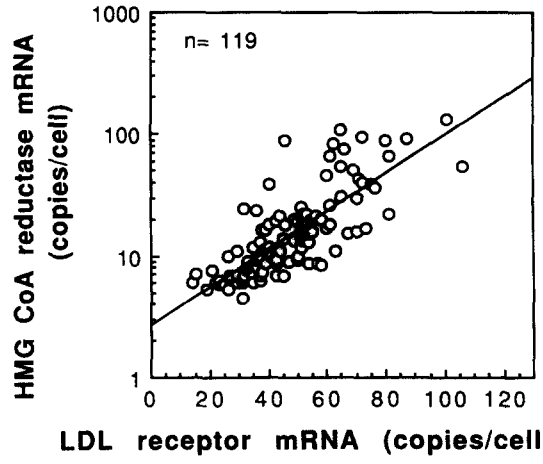


**Fig. 4.** Relationship between hepatic mRNAs for LDLr and HMG-CoA reductase of all individual 119 animals investigated.

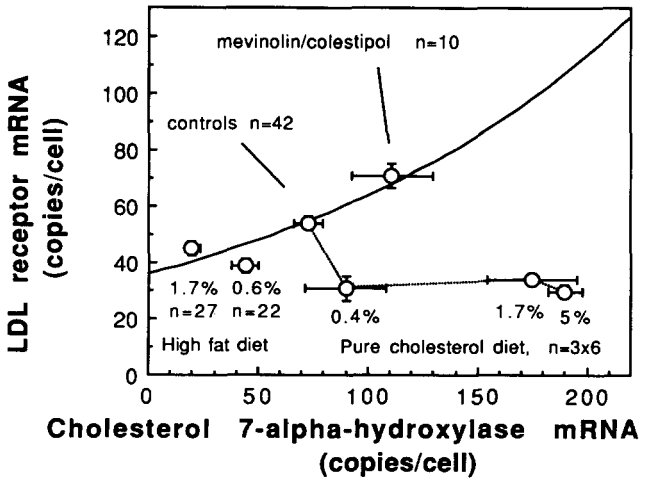


**Fig. 5.** Illustration of relationship between hepatic Cho-7-hx and LDLr mRNA levels obtained from the data in Tables 1 and 2; circles, mean; bars, SEM.

## DISCUSSION

To study discrete regulatory changes of mRNA levels, it is advantageous to perform hybridization reactions in solution. The rationale for adopting the current solution hybridization titration assay was its previously documented high sensitivity (9). Furthermore, the RNA–RNA duplexes formed show almost absolute resistance to RNase digestion rendering these hybrids less sensitive to particular assay conditions as compared to DNA–RNA hybrids (22). Previously used assays (21, 23) have been used in order to monitor pronounced increases of hepatic mRNA levels for the LDL receptor and HMG-CoA reductase. The assay used in the present study was shown to faithfully measure endogenous mRNA levels for the LDL receptor, HMG-CoA reductase, and Cho-7-hx, three transcripts found to be present in low abundance. Also, discrete decreases from normal endogenous levels could be determined with accuracy. The expression of mRNA abundancy as copies of mRNA molecules/cell was chosen since it is a common way to express this kind of data and is not a quantification in absolute terms.

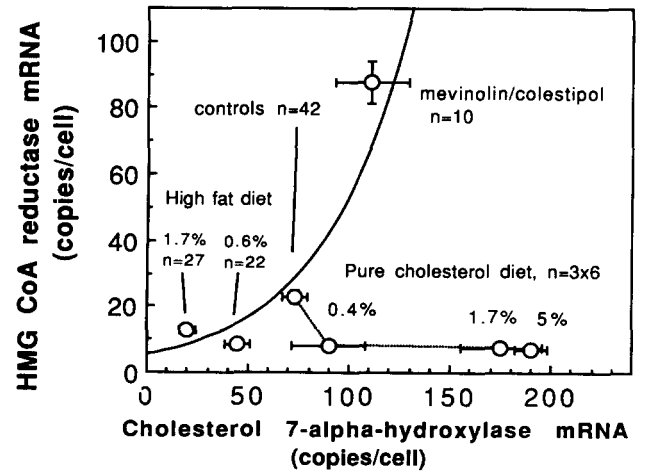


**Fig. 6.** Illustrated relationship between hepatic Cho-7-hx and HMG-CoA reductase mRNA levels presented in Tables 1 and 2; circles, mean; bars, SEM.

A striking feature of the current study was the fact that the LDLr and HMG-CoA reductase mRNA both showed a pronounced resistance to dietary suppression upon feeding diets previously developed to induce hypercholesterolemia and atherosclerosis in mice (high-fat diets, experiments 1–4). Ligand blots of hepatic membranes immobilized on nitrocellulose filters after separation on SDS-PAGE could detect only slightly decreased levels of hepatic LDL receptors when animals fed 1.7% cholesterol + 2% cholic acid were compared to controls (not shown). This indicates that the hepatic mRNA level for the LDL receptor mirrors LDL receptor protein in mouse liver, as has previously been shown for rabbits and hamsters (21, 23).

The strong positive correlation between the hepatic LDLr and HMG-CoA reductase mRNA levels (Figs. 3 and 4) indicates that these genes, under the conditions tested, are regulated in parallel at the mRNA level in vivo. The relationship was still significant upon analyzing control animals only, indicating that parallel regulation is present also under normal physiologic circumstances. The relation obtained between the LDL receptor and HMG-CoA reductase mRNA levels showed a curvilinear pattern (Fig. 3). It is of interest to note that Spady, Turley, and Dietschy (7) found a strikingly similar pattern upon relating hepatic cholesterol synthesis (measured in vitro) with hepatic in vivo LDL uptake in male hamsters. Also data from a study in humans (24), where the enzyme activities for HMG-CoA reductase and Cho-7-hx as well as the LDL receptor binding activity were determined in hepatic biopsies from gallstone patients, indicated that the regulatory pattern for HMG-CoA reductase and the LDLr in humans appears similar to that presently found at the mRNA level in the mouse.

The mRNA level for Cho-7-hx showed a different regulatory pattern from that of LDLr and HMG-CoA reductase mRNA in line with previous results. First, a dose-dependent suppression of Cho-7-hx mRNA was seen upon feeding the high fat diet (Table 1). This is likely an effect of the cholic acid present in the diet since this agent is known to reduce the activity of Cho-7-hx as well as its mRNA level (4). Second, Cho-7-hx mRNA levels were increased 1.6- to 1.7-fold upon feeding pure cholesterol only (Table 2, Exp. 6) in agreement with the findings of Li, Wang, and Chiang (19)

who found a 2-fold increased mRNA level upon feeding rats 2% cholesterol. It has been suggested that the availability of substrate (cholesterol) should be important for the increased activity of Cho-7-hx upon feeding cholesterol (25), and that steroid responsive elements in the promotor region for Cho-7-hx may be of importance for the increased mRNA levels seen upon feeding animals 2% cholesterol (26). The present findings of elevated mRNA levels for Cho-7-hx upon feeding diets with high cholesterol concentrations together with the finding of a slight decrease of the mRNA level for Cho-7-hx upon feeding 0.4% cholesterol, a dose that still exerted maximal suppression of both the LDLr and the HMG-CoA reductase mRNA levels (Table 2, Exp. 6) may suggest that the other mechanisms may also operate. One possibility could be that artificially high cholesterol concentrations in the gut lead to an increased loss of bile acids via the gut resulting in a compensatory increase in enzyme synthesis.

When the measured transcript levels were related to total plasma cholesterol levels in all animals (not shown), Cho-7-hx showed the best correlation ($r = -0.52$, $P < 0.001$). When the relation between HMG-CoA reductase or LDLr mRNA and plasma cholesterol were tested the relationships were not as strong ($r = -0.40$ and $-0.29$, respectively). However, size-fractionations of mouse plasma (Fig. 2) revealed that animals fed the high-fat diet showed increased cholesterol levels predominantly within large lipoprotein particles (VLDL-IDL) while the LDL fraction only showed a slight increase. On the other hand, the cholesterol content in the HDL fractions showed a clear reciprocal decrease. Thus, changes of the total plasma cholesterol level in the mouse are complex. Quantitation of mouse hepatic mRNA levels for apoE and apoB in animals fed the high-fat diet (experiments 1 to 4, Table 1) did not reveal clear-cut changes in the mRNA levels for apoE or B. However, apoE mRNA levels tended to be higher upon feeding the high-fat diet, in agreement of what has been shown by Lusis et al. (27). Thus, at least at the mRNA level, the largest hepatic regulatory changes seem to take place at the catabolic side upon feeding mice this high-fat diet.

The small suppression of the LDLr mRNA level in combination with the weak relationship between plasma cholesterol levels and LDL receptor mRNA in the liver could suggest that hepatic LDL receptors may be of little importance for the development of hypercholesterolemia in the mouse upon challenge with high-fat diets. An alternative and more likely interpretation is that the strong resistance to down-regulation of the LDL receptors in mouse liver is important for the pronounced resistance to dietary fat in this species. Only when animals were given highly artificial diets consisting of a mixture of cholesterol, saturated fat, and bile acids, all at high concentrations, could significant hypercholesterolemia be obtained. Support for the latter interpretation has indeed been provided by Yokode et al. (28); they demonstrated that a transgenic strain of C57BL/6J mice was significantly even more resistant to this high-fat diet after the introduction of a human LDL receptor minigene leading to a fivefold increase of the hepatic expression of LDL receptors. The high resistance to dietary fat observed in mice and rats may thus be due to some endogenous factor that makes the hepatic LDL receptor in these species extraordinarily resistant to feedback inhibition by cholesterol. A better understanding of the mechanism of resistance to dietary fat in mice and rats is therefore important, since this may provide information of value in order to counteract and reduce hypercholesterolemia.

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