Hepatic HMG-CoA Reductase Expression and Resistance to Dietary Cholesterol

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The premise that the intrinsic level of expression of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase determines the relative sensitivity to the serum cholesterol raising action of dietary cholesterol was examined in 9 strains of rat. For further comparison purposes, hamsters were also examined. The basal expression of hepatic HMG-CoA reductase, extent of feedback regulation by cholesterol, and changes in serum cholesterol levels and the hepatic low-density lipoprotein (LDL) receptor in response to cholesterol challenge were determined in these animals. The Sprague-Dawley, Wistar-Furth, Spontaneously Hypertensive, Lewis, and Wistar-Kyoto rats were all very resistant to dietary cholesterol and exhibited hepatic HMG-CoA reductase activities above 150 pmol / min ^{1} / mg ^{1}. The Buffalo, Brown Norway, and Copenhagen 2331 rats had hepatic HMG-CoA reductase activities below 90 pmol / min ^{1} / mg ^{1} and had increases in serum cholesterol levels ranging from 12 to 33 mg/dl when given a 4-day, 1% cholesterol challenge. The extent of feedback regulation was reduced to only 3-fold in the Fisher 344 and Brown Norway rats that exhibited significant increases in serum cholesterol levels when given a cholesterol challenge. The Golden Syrian hamsters exhibited the largest increase (197 mg/dl) in serum cholesterol levels in response to dietary cholesterol and the lowest basal expression of hepatic HMG-CoA reductase (3.3 pmol / min^{-1} / mg^{-1}). Hepatic LDL receptor levels were not significantly decreased by dietary cholesterol in any of the animals. The data from these inbred rats and the hamsters strongly support the conclusion that the animals expressing the highest levels of hepatic HMG-CoA reductase are the most resistant to the serum cholesterol raising action of dietary cholesterol. Exp Biol Med 229:412–416, 2004

Key words: hepatic HMG-CoA reductase; LDL receptor; inbred strains of rat; feedback regulation; serum cholesterol

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Genetic variability in susceptibility to the serum cholesterol raising action of dietary cholesterol has widely been suspected. The molecular mechanism(s) underlying these genetically determined differences are not established. Polymorphisms in a multitude of gene products could be responsible. Such polymorphisms could lead to altered levels of basal expression and/or to attenuated response to environmental factors. Decreased expression of hepatic low-density lipoprotein (LDL) receptor or mutations in apo B or apo E are well-known to cause increases in serum cholesterol levels (1–5). Increased expression of cholesterol 7α-hydroxylase even in animals lacking LDL receptors lowers plasma cholesterol (6, 7). Other genes that contribute to cholesterol homeostasis include apo A-I (8), acyl CoA: cholesterol acyl transferase 2 (ACAT2; Ref. 9), ATP binding cassette A1 (ABCA1; Ref. 10), ATP binding cassette G5 and G8 (ABCG5 and 8; Refs. 11, 12), cholesterol ester transfer protein, LCAT, hepatic lipase, lipoprotein lipase, apo C-II, and the high-density lipoprotein (HDL) receptor (13). In addition to these genes, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the rate-limiting step in cholesterol biosynthesis, also plays a significant role in cholesterol homeostasis (14). This enzyme is the target for the statin class of cholesterol-lowering drugs that are currently being used so effectively. An example of these drugs is atorvastatin (15).

Although it may seem paradoxical, individuals with higher levels of expression of HMG-CoA reductase appear to be more resistant to dietary cholesterol and respond better to statins than those individuals expressing low levels of the reductase (16–18). These observations have led to the proposal that this enzyme serves as a cholesterol buffer (14). Thus, people or animals that normally express high levels of hepatic HMG-CoA reductase can extensively downregulate this enzyme to synthesize much less cholesterol in response to a dietary challenge than those that express low basal levels of reductase. This allows the high expressers the ability to compensate for the increased dietary cholesterol without a significant increase in their serum and tissue cholesterol levels.

To examine more rigorously this cholesterol-buffering aspect of hepatic HMG-CoA reductase, we have inves-

tigated this concept in 9 rat strains and in Golden Syrian hamsters. Measurements of hepatic basal HMG-CoA reductase, responses to dietary cholesterol in terms of reductase activity and immunoreactive protein, serum cholesterol levels, and hepatic LDL receptor expression were performed. A striking correlation between basal expression of hepatic HMG-CoA reductase and relative sensitivity to a cholesterol challenge was observed.

Materials and Methods

Experimental Animals. All animals purchased from Harlan (Madison, WI) were males and weighed between 100 and 125 g or, in the case of the Golden Syrian hamsters (HAM), were 7 to 8 weeks old. The inbred rat strains included Wistar-Furth (WF), Spontaneously Hypertensive (SHR), Lewis (LEW), Wistar-Kyoto (WKY), Fischer 344 (F344), Brown Norway (BN), Buffalo (BUF), and Copenhagen 2331 (COP). In addition, Sprague-Dawley (SD) rats from Harlan were also studied. All animals were housed in reverse light-dark cycle rooms with the lights on from 1800 to 0600 daily. They were maintained at 21° ± 2°C and a humidity of 55% ± 5%. The animals were allowed free access to Harlan Teklad 2019 Extruded Rodent Diet (19% protein, 9% fat) and water. Some animals received this ground diet supplemented with 1% cholesterol for 4 days. Continuing the animals on the cholesterol-supplemented diets for as long as 21 days did not further affect the serum cholesterol levels. The animals were cared for according to the NIH guidelines set forth in the "Guide for the Care and Use of Laboratory Animals" (1996 edition) and specifically in accord with protocols 1749 and 2317 approved by the University of South Florida Institutional Animal Care and Use Committee. The animals were sacrificed between the third and fourth hour of the dark cycle when hepatic HMG-CoA reductase expression is at its daily peak. Groups of 4 animals were used.

Materials. Polyclonal antisera to the catalytic domain of rat liver HMG-CoA reductase (19) was generated in rabbits as previously described (20). Polyclonal antiserum to a C-terminal peptide of the LDL receptor was also generated in rabbits as previously described (21). The ECL Western blotting kit was purchased from Amersham (Chicago, IL). The goat anti rabbit secondary antibody was obtained from Jackson ImmunoLaboratories (West Grove, PA). [^{14}C] HMG-CoA (55.7 mCi/mmol) was purchased from Perkin Elmer/NEN (Boston, MA). Infinity Cholesterol Reagent, glucose-6-phosphate dehydrogenase, and NADP were purchased from Sigma Chemical Co. (St. Louis, MO).

Western Blotting Analysis. Levels of HMG-CoA reductase and LDL receptor were determined in microsomal preparations as previously described (22). Relative levels were estimated using a phosphoimager.

HMG-CoA Reductase and Cholesterol Determinations. These were determined as recently described (22). HMG-CoA reductase activity is expressed as pmol / min^{-1} / mg^{-1} of microsomal protein. Serum cholesterol levels are given in terms of mg/dl of serum.

Results

Hypo- and hyperresponding rats, in terms of responses of serum cholesterol concentration to a dietary cholesterol challenge, have previously been described (23). In this study, we sought to determine whether the sensitivity of various rat strains to dietary cholesterol is related to their basal expression of hepatic HMG-CoA reductase, extent of feedback regulation, or to modulation of hepatic LDL receptor expression. Nine rat strains were studied. In addition, the response of Golden Syrian hamsters was also compared.

Basal Expression of Hepatic HMG-CoA Reductase. The relationship between basal HMG-CoA reductase activity and change in serum cholesterol levels upon administration of a cholesterol challenge is depicted in Figure 1. Data are presented for 6 rat strains and also for Golden Syrian hamsters. As can be seen, rats with the highest basal expression show no increase in serum cholesterol levels, whereas those with intermediate levels exhibit modest elevations in serum cholesterol. Hamsters have extremely low levels of hepatic HMG-CoA reductase activity and exhibited an increase of more than 150 mg/dl in serum cholesterol levels. For the 9 rat strains, basal HMG-CoA reductase activity negatively correlated with the change in serum cholesterol levels at an r value of –0.77. This is quite significant. Levels of immunoreactive hepatic HMG-CoA reductase were also determined in all rat strains. Figure 2 presents immunoblots of samples from 8 rat strains and the hamsters. Basal expression is high in SD, F344, SHR, WKY, LEW, and WF but lower in BN, hamsters, and BUF. In all cases, a cholesterol challenge lowered the level of immunoreactive hepatic HMG-CoA reductase protein. The extent of downregulation of HMG-CoA reductase activity by dietary cholesterol in F344 and BUF rats, 3.9- and 3.7-fold, respectively, was considerably less than that in most other rat strains.

Extent of Feedback Regulation. The extent of feedback regulation of hepatic HMG-CoA reductase can play a major role in determining the degree of sensitivity to dietary cholesterol. Increased resistance to dietary cholesterol would be provided by greater downregulation of hepatic reductase. Table 1 presents hepatic HMG-CoA reductase activities in rats and hamsters on a chow diet and on a diet supplemented with 1% cholesterol. Data for 9 different rat strains and Golden Syrian hamsters are presented. The SD, WF, SHR, LEW, and WKY rats all exhibited at least 8-fold decreases in hepatic HMG-CoA reductase and, as shown in Table 2, were completely resistant to the serum cholesterol raising action of dietary cholesterol challenge. F344 and BUF rats displayed only 3- to 4-fold decreases in reductase activity in response to a cholesterol challenge and elevations of about 10 mg/dl in

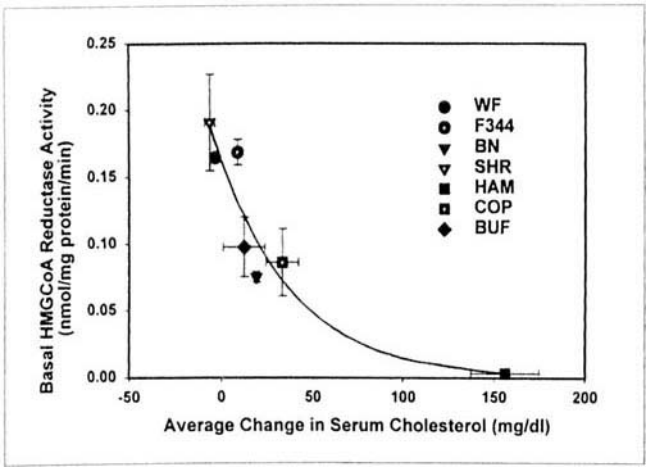

Figure 1. Relationship between basal hepatic HMG-CoA reductase activity and change in serum cholesterol levels due to dietary cholesterol. The basal expression of hepatic HMG-CoA reductase (animals on normal chow) and change in serum cholesterol level when given a diet containing 1% cholesterol for 4 days is presented for several strains of inbred rat and Golden Syrian hamsters. The means for data from 4 or more animals is given in the symbol for each animal as identified in the key. The vertical bars are the standard deviations for HMG-CoA reductase activity. The horizontal bars are the standard deviations in serum cholesterol levels.

serum cholesterol levels (Tables 1 and 2). Despite large extents of feedback regulation, the BN and COP rats had serum cholesterol increases of 18 and 33 mg/dl (Tables 1 and 2). These rats had the lowest basal levels of hepatic HMG-CoA reductase activity (Table 1). The Golden Syrian hamsters had very low hepatic reductase activity—only 2% of that of WF rats (Table 1). Despite exhibiting a 12-fold extent of feedback regulation, serum cholesterol levels increased more than 150 mg/dl in these hamsters in response to the cholesterol challenge. A comparison of immunoreactive hepatic HMG-CoA reductase protein levels in WF rats and Golden Syrian hamsters is presented in Figure 3. Although hepatic HMG-CoA reductase activity is 50-fold higher, immunoreactive protein levels are only 2.5-fold higher in the WF rats, suggesting an effect on catalytic efficiency.

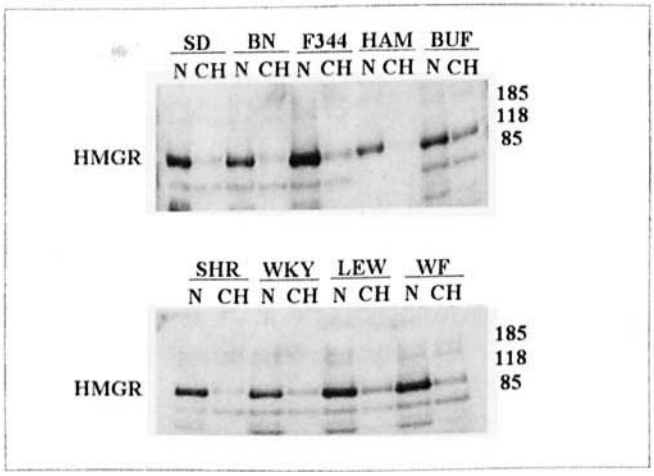

Figure 2. Representative immunoblot of hepatic HMG-CoA reductase from animals fed normal or cholesterol-supplemented chow diets. Results for 8 inbred rat strains and the hamsters are shown. N refers to normal chow diet, and CH refers to 1%-cholesterol–supplemented diet. Twenty-five micrograms of microsomal protein was applied to each lane.

Table 1. Response of Rat and Hamster Hepatic HMG-CoA Reductase to Dietary Cholesterol[a]

	Hepatic HMG-CoA reductase (pmol / min^{-1} / mg^{-1})	
Animal	Chow diet	1% cholesterol diet
Rat strain		
SD	190 ± 3	13 ± 3
WF	165 ± 2	17 ± 6
SHR	191 ± 60	11 ± 2
LEW	215 ± 77	24 ± 12
WKY	125 ± 6	15 ± 1
F344	169 ± 9	44 ± 13
BUF	97 ± 22	26 ± 9
BN	75 ± 4	2 ± 1
COP	86 ± 25	5 ± 1
Hamsters		
Golden Syrian	3.3 ± 0.3	0.2 ± 0.1

[a] HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; SD, Sprague-Dawley; WF, Wistar-Furth; SHR, Spontaneously Hypertensive; LEW, Lewis; WKY, Wistar-Kyoto; F344, Fisher 344; BUF, Buffalo; BN, Brown Norway; COP, Copenhagen 2331.

Hepatic LDL Receptor Expression. Animals may also respond to a dietary cholesterol challenge by increasing the expression of the hepatic LDL receptor in order to help maintain cholesterol homeostasis. This receptor is primarily expressed in liver (24). As shown in Figure 4, immunoblots show that in most animals (SD, WF, WKY, SHR, BUF, BN, and HAM) hepatic LDL receptor protein levels are unaffected by adding 1% cholesterol to the diet. However, in the LEW and F344 rats, increases of up to 2-fold were

Table 2. Responses of Rat and Hamster Serum Cholesterol to Dietary Cholesterol[a]

	Serum cholesterol (mg/dl)	
Animal	Chow diet	1% cholesterol diet
Rat strain		
SD	107 ± 9	104 ± 4
WF	110 ± 1	107 ± 3
SHR	82 ± 2	77 ± 4
LEW	98 ± 1	99 ± 6
WKY	134 ± 2	132 ± 3
F344	65 ± 1	71 ± 4
BUF	90 ± 18	102 ± 4
BN	85 ± 1	103 ± 3
COP	132 ± 6	166 ± 12
Hamsters		
Golden Syrian	38 ± 3	235 ± 32

[a] SD, Sprague-Dawley; WF, Wistar-Furth; SHR, Spontaneously Hypertensive; LEW, Lewis; WKY, Wistar-Kyoto; F344, Fisher 344; BUF, Buffalo; BN, Brown Norway; COP, Copenhagen 2331.

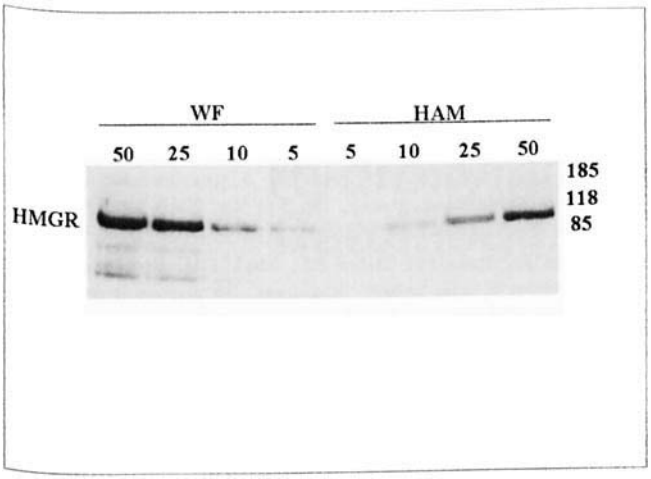

Figure 3. A comparison of immunoreactive hepatic HMG-CoA reductase protein levels in Wistar-Furth rats and Golden Syrian hamsters. Increasing amounts of microsomal protein ranging from 5 to 50 μg were loaded as indicated.

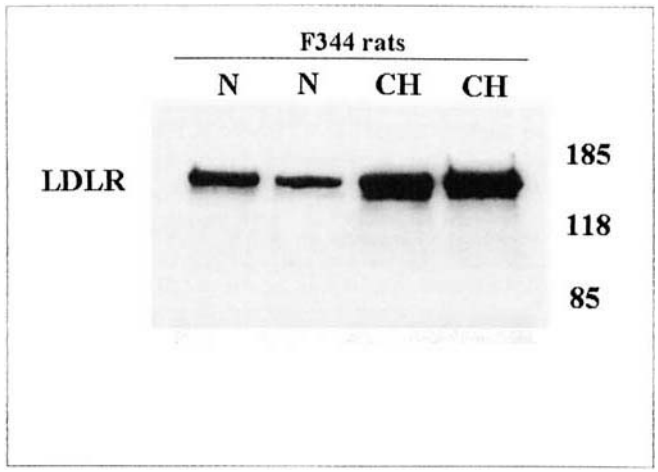

Figure 5. Effect of feeding a diet supplemented with 1% cholesterol on hepatic LDL receptor protein levels in F344 rats. Twenty-five micrograms of microsomal protein was applied to each lane.

observed in response to the cholesterol-supplemented diet. This is more clearly shown in Figure 5 for the F344 rats. The COP rats also showed at least 2-fold increases in LDL receptor protein levels (data not shown). Thus, these rats upregulate hepatic LDL receptor expression in an effort to compensate for the dietary cholesterol insult. The F344 and COP rats exhibit low basal expression of hepatic HMG-CoA reductase.

Discussion

A striking correlation between basal expression of hepatic HMG-CoA reductase and resistance to dietary cholesterol was observed in the 9 inbred rat strains and the Golden Syrian hamsters investigated in this study. Animals with the highest hepatic HMG-CoA reductase activity were completely resistant to dietary cholesterol (Fig.1 and Tables 1 and 2). The Golden Syrian hamsters expressed the lowest level of hepatic HMG-CoA reductase activity and exhibited by far the greatest increase in serum cholesterol levels when given a cholesterol challenge. These results are in agreement with the observations made in the Scandinavian Simvastatin Survival Study ("4S study") of patients on simvastatin (16). Expression of relatively high basal levels of hepatic HMG-CoA reductase allows a greater degree of downregulation to compensate for increased absorption of dietary cholesterol (14).

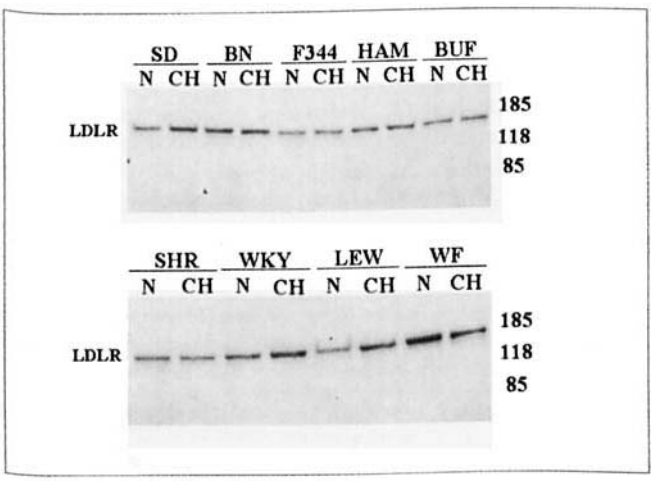

Figure 4. Representative immunoblot of hepatic LDL receptor from animals fed normal or cholesterol-supplemented chow diets. Results for 8 inbred rat strains and the hamsters are shown. N refers to normal chow diet, and CH refers to 1%-cholesterol–supplemented diet. Twenty-five micrograms of microsomal protein was applied to each lane.

A couple of inbred rat strains (F344 and BUF) displayed low degrees of feedback regulation of hepatic HMG-CoA reductase (Table 1). These rats exhibited moderate increases in serum cholesterol levels in response to the challenge (Table 1). In most animals, adding cholesterol to the diet did not affect hepatic LDL receptor expression. The LEW, F344, and COP rats exhibited increases of up to 2-fold. Increased expression of the hepatic LDL receptor can help to establish cholesterol homeostasis by removing LDL cholesterol from the blood. The increased dietary cholesterol reaches the liver by way of chylomicron remnants. The excess hepatic cholesterol can be incorporated into VLDL and processed to intermediate-density lipoprotien (IDL) and LDL by the action of lipoprotein lipase (13). However, for the most part, decreased expression of hepatic HMG-CoA reductase is the primary response employed by the animals studied to achieve cholesterol homeostasis in the face of a dietary cholesterol challenge. This decrease in hepatic HMG-CoA reductase expression is accomplished by a decrease in translational efficiency of the HMG-CoA reductase mRNA (25–27).

Taken together, the data in the current study provide strong support for the concept that hepatic HMG-CoA reductase functions as a cholesterol buffer (14). When animals face a cholesterol challenge, they respond by

markedly downregulating the expression of hepatic reductase protein (Fig. 2) with little if any affect on hepatic LDL receptor protein levels (Fig. 4). Thus, animals that express high basal levels of hepatic HMG-CoA reductase are resistant to dietary cholesterol, whereas those expressing low levels of reductase (hamsters) are very susceptible to the serum cholesterol raising action of dietary cholesterol (Fig. 1).

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