

## MINIREVIEW

# Feedback and Hormonal Regulation of Hepatic 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase: The Concept of Cholesterol Buffering Capacity (44508)

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**Abstract.** Regulation of the expression of hepatic 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase by the major end product of the biosynthetic pathway, cholesterol, and by various hormones is critical to maintaining constant serum and tissue cholesterol levels in the face of an ever-changing external environment. The ability to downregulate this enzyme provides a means to buffer the body against the serum cholesterol-raising action of dietary cholesterol. The higher the basal expression of hepatic HMG-CoA reductase, the greater the "cholesterol buffering capacity" and the greater the resistance to dietary cholesterol. This review focuses on the mechanisms of feedback and hormonal regulation of HMG-CoA reductase in intact animals rather than in cultured cells and presents the evidence that leads to the proposal that regulation of hepatic HMG-CoA reductase acts as a cholesterol buffer. Recent studies with animals have shown that feedback regulation of hepatic HMG-CoA reductase occurs at the level of translation in addition to transcription. The translational efficiency of HMG-CoA reductase mRNA is diminished through the action of dietary cholesterol. Oxysterols appear to be involved in this translational regulation. Feedback regulation by dietary cholesterol does not appear to involve changes in the state of phosphorylation of hepatic HMG-CoA reductase or in the rate of degradation of this enzyme. Several hormones act to alter the expression of hepatic HMG-CoA reductase in animals. These include insulin, glucagon, glucocorticoids, thyroid hormone and estrogen. Insulin stimulates HMG-CoA reductase activity likely by increasing the rate of transcription, whereas glucagon acts by opposing this effect. Hepatic HMG-CoA reductase activity undergoes a significant diurnal variation due to changes in the level of immunoreactive protein primarily mediated by changes in insulin and glucagon levels. Thyroid hormone increases hepatic HMG-CoA reductase levels by acting to increase both transcription and stability of the mRNA. Glucocorticoids act to decrease hepatic HMG-CoA reductase expression by destabilizing reductase mRNA. Estrogen acts to increase hepatic HMG-CoA reductase activity primarily by stabilizing the mRNA. Deficiencies in those hormones that act to increase hepatic HMG-CoA reductase gene expression lead to elevations in serum cholesterol levels. High basal expression of hepatic HMG-CoA reductase, whether due to genetic or hormonal factors, appears to result in greater cholesterol buffering capacity and thus increased resistance to dietary cholesterol. [P.S.E.B.M. 2000, Vol 224:8-19]

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The endoplasmic reticulum-bound enzyme (1), 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, is generally regarded as catalyzing the rate-limiting step (2) in the synthesis of cholesterol, a critical membrane lipid, precursor of bile acids and steroid hormones, and component of hedgehog protein, a signaling molecule involved in embryogenesis (3–6). Impairment in the synthesis of cholesterol, resulting in very low serum and

tissue cholesterol levels, occurs in patients with the Smith-Lemli-Opitz syndrome (7). Craniofacial anomalies, syndactyly and polydactyly, delayed myelination, holoprosencephaly, cleft palate, genital malformations, jaundice, and congenital heart disease characterize these patients (8). On the other hand, elevated serum cholesterol levels predispose people to atherosclerotic vascular disease (9). In healthy individuals, serum cholesterol levels are maintained within fairly narrow limits. Thus, it is not surprising that the mechanisms for maintaining cholesterol homeostasis have been the subject of intense investigation (10). Since endogenous cholesterol synthesis is a major contributor to the cholesterol that is required on a daily basis, knowledge of the mechanisms regulating this process constitutes an important key to understanding cholesterol homeostasis.

The significance of cholesterol biosynthesis to overall cholesterol homeostasis is underscored by the effectiveness of inhibitors of this process in lowering serum cholesterol levels (11–14). The statin family of drugs, which inhibit HMG-CoA reductase activity, particularly in liver (1), can lower serum cholesterol levels by as much as 45% (15). These drugs lower cholesterol levels even in mice lacking low-density lipoprotein receptor (16), consistent with a primary action on HMG-CoA reductase (17,18). Immunoblotting studies showed that hepatic LDL receptor protein levels are not increased in rats given lovastatin, pravastatin, fluvastatin, cerivastatin, or atorvastatin (19). In the case of atorvastatin, which can lower serum LDL cholesterol levels as much as 60%, neither hepatic LDL receptor mRNA nor protein is increased (18). The cholesterol-lowering action of atorvastatin is attributed to a primary and more prolonged inhibition of hepatic HMG-CoA reductase (17, 18).

Extensive studies of the regulation of HMG-CoA reductase have been carried out with cultured cells serving as experimental models, particularly UT-1 and C-100 cells. The UT-1 cells are Chinese hamster ovary tumor cells selected for growth in the presence of compactin, a potent inhibitor of HMG-CoA reductase. They express very high levels of HMG-CoA reductase due to a 15-fold amplification of the reductase gene, together with an increased rate of translation and greater stability of the reductase protein (20). The high level of HMG-CoA reductase gene expression in these cells enabled the initial isolation of a cDNA for HMG-CoA reductase (21). Studies of the regulation of HMG-CoA reductase gene expression in these cells using lipoprotein depleted media and sterols, usually cholesterol plus 25-hydroxycholesterol, led to the proposal of sterol and nonsterol regulation of the reductase (10). Sterols were shown to regulate at the level of transcription whereas nonsterols exerted regulation on translation of the mRNA (10). Both sterols and nonsterols are needed for regulation of the rate of degradation of the reductase (10). Regulation of the rate of degradation of HMG-CoA reductase has been studied extensively in the C-100 cells. These are compactin-resistant SV40-transformed baby hamster kidney cells that also overexpress HMG-CoA reductase (22). Studies with these

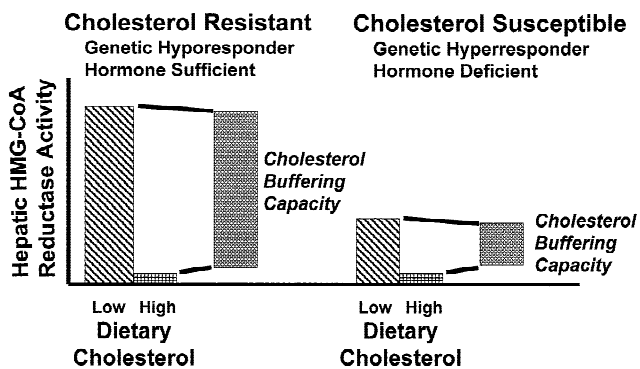
cells have concluded that the 8-membrane spanning domain of the reductase is required for sterol-mediated regulation of reductase degradation (23, 24) and that farnesol is the nonsterol regulator of degradation (25, 26). Similar findings have been reported with UT-1 cells (27) and with permeabilized Chinese hamster ovary cells (28).

Because regulation of HMG-CoA reductase gene expression in transformed cells and tumor cells may differ from that in normal cells and tissues, regulation in nontransformed cells and tissues has also been studied. It is known that the normal feedback regulation of the reductase is lost in tumors (29, 30). Regulation in animal liver has been the best-studied example. This system has allowed studies of the regulation of HMG-CoA reductase gene expression by a wide variety of physiological agents including thyroid hormone, glucocorticoids, insulin, glucagon, estrogen, diurnal rhythm, bile acids and cholesterol. Feedback regulation by cholesterol has probably received the most attention. This review will focus on regulation of HMG-CoA reductase gene expression in the liver by cholesterol, hormones, and other physiological factors.

### **Hepatic HMG-CoA Reductase in Cholesterol Homeostasis**

The liver appears to play a major role in regulating cholesterol levels (31). This organ could well be termed the “cholesterolstat.” It expresses the majority of the body’s low-density lipoprotein receptors and is the major site for degradation of cholesterol by conversion to bile acids (32, 33). Although HMG-CoA reductase is found in virtually all tissues, the liver expresses one of the highest levels of this enzyme. In some animals, it expresses the highest level of HMG-CoA reductase activity/cholesterol biosynthesis (34). In other animals, adrenals, intestine, skin, or carcass exhibit higher levels of cholesterol biosynthesis (34). However, and most importantly, feedback regulation of HMG-CoA reductase by cholesterol occurs mainly in this organ (35). Modest feedback regulation of HMG-CoA reductase also occurs in brain and testes of young male rats with other tissues essentially unresponsive to dietary cholesterol (Ness GC, unpublished data).

Decreased hepatic HMG-CoA reductase activity in response to an increase in cholesterol reaching the liver *via* chylomicron remnants (36) provides an effective means to maintain desirable cholesterol levels. Thus a high level of expression of hepatic HMG-CoA reductase can serve to buffer excess dietary cholesterol (Fig. 1). Interestingly, the Sprague-Dawley rat has a high level of hepatic cholesterol biosynthesis and is known to be rather resistant to dietary cholesterol (34). In contrast, rabbits and hamsters exhibit low levels and are very sensitive to dietary cholesterol (34). In a recent study of *Cynomolgus* monkeys (37), it was shown that animals able to resist dietary cholesterol (hypo-responders) exhibited much higher basal levels of hepatic sterol synthesis and greater downregulation when challenged with a high-cholesterol diet than monkeys that were



**Figure 1.** Dependence of cholesterol buffering capacity on basal expression of hepatic HMG-CoA reductase.

sensitive to dietary cholesterol (hyperresponders). Presumably, the level of hepatic sterol synthesis reflects the relative rate of HMG-CoA reductase activity. These findings suggest that feedback regulation of hepatic HMG-CoA reductase plays a key role in resistance/susceptibility to dietary cholesterol.

Differences among people with regard to the level of HMG-CoA reductase expression and susceptibility to dietary cholesterol-induced hypercholesterolemia also appear to exist. Plasma mevalonic acid levels have been found to be a good index of *in vivo* cholesterol biosynthesis (38). Patients with higher basal levels of plasma mevalonic acid exhibited greater cholesterol-lowering in response to treatment with the statin family of HMG-CoA reductase inhibitors (38).

### Mechanisms of Feedback Regulation

Feedback regulation of hepatic cholesterol biosynthesis was recognized nearly 50 years ago (39–41). Over the intervening years, several mechanisms for this feedback regulation of hepatic HMG-CoA reductase in response to dietary cholesterol have been advanced. Some of these mechanisms, such as allosteric regulation or regulation by changes in membrane fluidity, were discarded long ago (42, 43). The mechanisms that are currently being examined include: transcription, translation, protein turnover, and regulation of catalytic efficiency by phosphorylation/dephosphorylation (10). It has been suggested that HMG-CoA reductase may

be regulated at multiple levels (44). In studying the mechanism of feedback regulation, different species and experimental approaches have been employed. This may explain certain apparently conflicting conclusions. A summary of the presently available information concerning the regulation of hepatic HMG-CoA reductase gene expression in response to feeding cholesterol to rats, hamsters, and mice is presented in Table I. The different conditions associated with administering dietary cholesterol—dose, time of treatment, dissolving in ethanol or mixing directly in the diet, and presence or absence of HMG-CoA reductase inhibitors—are also summarized. As is readily apparent, regulation at the levels of both transcription (mRNA) and translation occur. Hamsters and mice exhibit mainly transcriptional control whereas rats display primarily translational regulation. In this review, the relative changes in HMG-CoA reductase gene expression attributable to each of the different levels of regulation will be compared with the total change in enzyme activity caused by dietary cholesterol.

**Catalytic Efficiency.** The possibility that the catalytic efficiency of HMG-CoA reductase might be regulated by phosphorylation/dephosphorylation was first advanced by Beg *et al.* (45), who reported that incubation with Mg, ATP, and a cytosolic fraction inhibited enzyme activity. It was then reported that the initial decrease in hepatic HMG-CoA reductase activity that occurred after placing animals on a cholesterol-supplemented diet was due to phosphorylation of the enzyme with the decrease that occurs upon prolonged feeding resulting from a decrease in the amount of enzyme protein (46). Subsequent studies revealed that HMG-CoA could be phosphorylated and inactivated by an AMP-activated protein kinase (47–51). The actual site of phosphorylation was identified as serine 871 (49). However, replacement of this serine with an alanine failed to prevent the normal feedback regulation of reductase activity by mevalonate, 25-hydroxycholesterol, or low-density lipoprotein (52). Immunoblotting studies showed that long-term (4–5-day) feeding of a cholesterol-supplemented diet decreased both reductase protein and enzyme activity to barely detectable levels (53). Further studies showed that reductase immunoreactive protein and enzyme activity fell together during the first 72 hr after rats were placed on a diet containing 2% cholesterol (54). Thus, feedback regulation of

**Table I.** Effects of Dietary Cholesterol on Hepatic HMG-CoA Reductase Gene Expression

Diet/Animal	mRNA	Transcription	Protein	Translation	Activity	Reference
Mev, Ctx, 10 days, 2% Chol/et, Rat	↓↓↓	ND	↓↓↓	ND	ND	63
Lov. 2% Chol, 5 days, Rat	↓↓↓	↓ 50%	ND	ND	↓↓↓	66
2% Chol, 2 days, Rat	ND	ND	↓ 80%	↓ 80%	↓ 80%	56
2% Chol, 5 days, Rat	±	±	↓↓↓	ND	↓↓↓	55, 64, 66
2% Chol, 6 wks, Rat	±	ND	ND	ND	↓↓↓	65
2% Chol, 14 days, Hamster	↓ 85%	ND	ND	ND	ND	68
0.5% Chol, 12 days, Hamster	↓ 70%	ND	ND	ND	ND	69
5% Chol, 10 days, Mice	↓ 70%	ND	ND	ND	ND	67

Note. ND, not determined; ±, not significantly decreased; ↓, decreased; ↓↓↓, decreased to less than 5% of control; Mev, 0.1% mevinolin; Ctx, 4% cholestyramine; Chol, cholesterol; et, ethanol; Lov, 0.04% lovastatin.

hepatic HMG-CoA reductase by dietary cholesterol involves a decrease in the amount of enzyme protein rather than inactivation of the enzyme due to phosphorylation (54).

Another possible mechanism for regulating the activity of HMG-CoA reductase by altering the catalytic efficiency of the enzyme involves the thiol redox status of the enzyme (55). It has been shown that the thiol redox potential of HMG-CoA reductase is close to the normal ratio of cellular reduced glutathione to oxidized glutathione (56). Under most conditions, this ratio does not change. However, when a large amount of a single amino acid is given as a dietary supplement, a marked decrease in hepatic reduced glutathione levels is observed (Ness GC, unpublished data). This likely reflects increased activity of the hepatic  $\gamma$ -glutamyl cycle (57), which removes excess amino acids from the circulation using glutathione. The first step of this cycle is catalyzed by  $\gamma$ -glutamyl transpeptidase, which forms  $\gamma$ -glutamyl-amino acid plus cysteinylglycine. The amino acid is released inside the cell. The cysteinylglycine is hydrolyzed. This process uses glutathione, which must be resynthesized. It has been reported that addition of high levels of monosodium glutamate to the diets of humans or gerbils significantly lowers serum cholesterol levels and that this is associated with a decrease in the rate of cholesterol synthesis (58–60). Efficient removal of excess glutamate from the serum could lower hepatic glutathione levels. Whether this actually results in decreased levels of reduced glutathione, which in turn leads to inhibition of hepatic HMG-CoA reductase activity, remains to be determined.

**Transcription.** From even a casual inspection of the recent literature, transcriptional regulation of HMG-CoA reductase is clearly the most actively investigated mechanism. The availability of cDNA probes for HMG-CoA reductase made it possible to measure reductase mRNA levels and the rate of transcription. However, in most studies, only mRNA levels have been measured. In the initial study that used an HMG-CoA reductase cDNA probe to measure reductase mRNA levels, rats were fed a diet supplemented with 2% cholestyramine, a bile acid binding resin, and 0.1% mevinolin, a potent inhibitor of HMG-CoA reductase, and then 2% cholesterol dissolved in ethanol was added (61). These drugs are powerful inducers of HMG-CoA reductase. Under these conditions, addition of cholesterol to the diet markedly lowered hepatic HMG-CoA reductase mRNA levels. In this particular study (61), reductase mRNA could not be detected in liver from normal chow-fed rats. With the availability of improved sensitivity, HMG-CoA reductase mRNA levels were determined in liver from normal chow-fed rats and from animals given a chow diet supplemented with 2% cholesterol (62). In studies using Northern blotting analysis and ribonuclease protection assays, hepatic reductase mRNA levels were only reduced about 30%, which was not statistically significant (62, 63). In comparison, HMG-CoA reductase activity was reduced to less than 3% of

control levels. Clearly, the major site of feedback regulation in these animals is post-transcription.

The responses of rats fed chow or an HMG-CoA reductase inhibitor, lovastatin (mevinolin), to dietary cholesterol were compared directly. It was found that marked decreases in hepatic reductase mRNA levels and rates of transcription only occurred in the drug-treated animals (64). In the chow-fed rats, no decrease in transcription was observed in response to dietary cholesterol. It was concluded that HMG-CoA reductase inhibitors could unmask cryptic transcriptional regulation.

In contrast with the studies of rats, investigations of cholesterol feedback regulation of hepatic HMG-CoA reductase in both hamsters and mice have revealed apparent transcriptional regulation. Feeding mice diets containing up to 5% cholesterol for 10 days caused 3–4-fold decreases in hepatic reductase mRNA levels when compared with mice that received diets supplemented with only corn oil (65). How this compared with the decrease in enzyme activity was not addressed. Also the effect of dietary cholesterol on the rate of transcription was not determined in these animals. In experiments with Syrian hamsters, feeding diets containing 2% cholesterol for 14 days reduced hepatic HMG-CoA reductase mRNA levels about 6-fold (66). In a more recent study of Golden Syrian hamsters, feeding diets containing 0.5% cholesterol for 12 days lowered hepatic reductase mRNA levels 3-fold (67). Transcription rates were not determined in either of these studies (66, 67). It was concluded that the reduction in mRNA together with post-transcriptional regulation was responsible for the profound fall in hepatic cholesterol synthesis that occurred when hamsters consumed cholesterol (67). Comparisons of the reduction in mRNA with the corresponding decreases in reductase immunoreactive protein or enzyme activity levels were not carried out in these studies of mice or hamsters.

The data obtained with these different animals indicate that transcription may be the major mechanism for feedback regulation in cholesterol-sensitive animals such as mice and hamsters whereas it is only a minor mechanism in rats, which are known to be rather resistant to dietary cholesterol. Feeding hamsters diets enriched in cholesterol increases serum cholesterol levels 2-fold and liver cholesterol 7-fold (67). In contrast only a 10%–15% increase is seen in serum cholesterol and a 2-fold increase in liver cholesterol in Sprague-Dawley rats (68). Whether all resistant and sensitive animals exhibit different mechanisms of feedback regulation requires further investigation. It is known that people differ in their sensitivities to dietary cholesterol (69). There exist several cholesterol-sensitive and resistant strains of rats, mice, pigeons, rabbits, and monkeys (69). Studies of these animals should help to explain variable responses in humans.

The detailed mechanism of transcriptional regulation of HMG-CoA reductase has been studied extensively in recent years (70). The central role of sterol response element binding proteins has emerged from these studies. Overexpres-

sion of the active form of sterol response element binding protein markedly increases hepatic mRNA levels for HMG-CoA reductase and several other enzymes involved in cholesterol and fatty acid biosynthesis (71). These transgenic mice exhibit an enlarged liver filled with cholesterol and triglycerides (71). The SREBP pathway has been reviewed elsewhere in depth (70) and will not be reviewed further here.

**Translation.** It has become apparent from the studies reviewed above that feedback regulation of hepatic HMG-CoA reductase involves considerable post-transcriptional regulation. One of the obvious post-transcriptional regulatory sites is translation. Early studies, using UT-1 cells, a line of Chinese Hamster Ovary cells selected for growth in the presence of the reductase inhibitor, compactin, concluded that nonsterols regulate HMG-CoA reductase gene expression at the level of translation (10). It was suggested that the production of mRNAs with different length 5' untranslated regions ranging from 68 to 670 nucleotides with or without AUG codons upstream of the initiator methionine might be responsible (72). In studies of cultured C100 cells grown in the presence of lovastatin, a potent inhibitor of HMG-CoA reductase, reductase mRNAs with the shorter 5' untranslated regions were found associated with the actively translating polysomal fraction (73).

Interest in translation as a site for feedback regulation by dietary cholesterol emerged from studies showing a much greater decline in hepatic reductase immunoreactive protein than in mRNA levels (53). Pulse labeling studies of HMG-CoA reductase were carried out using liver slices to measure the rate of synthesis of reductase protein (54). It was found that feeding diets containing 2% cholesterol to rats for 48 hr caused a 6-fold decrease in the rate of synthesis of reductase protein without affecting the synthesis of total hepatic protein. This decrease accounted for the decreases seen in this time period for both reductase protein and enzyme activity (54). Liver slices prepared from rats given mevalonolactone as a source of endogenous cholesterol exhibited a similar decrease in the rate of synthesis of reductase protein. Thus, translation appears to be the major mechanism of feedback regulation of hepatic HMG-CoA reductase in rats in response to both exogenous and endogenous cholesterol. The extent to which this mechanism also contributes to feedback regulation exhibited by cholesterol-sensitive animals remains to be determined.

The question of whether the decreased translational efficiency of HMG-CoA reductase mRNA in livers from rats fed cholesterol-supplemented diets was due to a shift from association with actively translating polysomes to inactive monosomes was investigated by performing polysome profile studies (74). It was found that the monosomal fraction from livers of rats given dietary cholesterol contained over 10 times more reductase mRNA. This was associated with decreases in reductase mRNA found in the polysomal fractions. In contrast, the distribution of  $\beta$ -actin mRNA was unaffected. Thus, dietary cholesterol clearly decreases the

translational efficiency of hepatic HMG-CoA reductase mRNA. Whether the underlying mechanism involves the synthesis of a protein that binds to reductase mRNA or different forms of reductase mRNA remains to be elucidated.

**Oxysterols.** Kandutich and Chen (75, 76) first advanced the idea that oxygenated sterols rather than cholesterol per se might mediate the feedback regulation of HMG-CoA reductase. It was found that oxysterols, such as 25-hydroxycholesterol, bind to a cytosolic protein and act as potent repressors of HMG-CoA reductase in cultured cells (77). In addition to oxysterols, oxylanosterols were also found to be potent suppressors (78, 79). Whereas oxysterols exert their effects at the level of transcription, oxylanosterols appear to regulate HMG-CoA reductase gene expression at the level of translation in cultured cells (80–82). An oxylanosterol, 3 $\beta$ -hydroxy- $\Delta^8$ -lanost-8-en-32- $\alpha$ l, an intermediate in the 14 $\alpha$  demethylation of lanosterol, accumulates when cholesterol biosynthesis is inhibited (83). This compound acts post-transcriptionally, possibly at the level of translation. To circumvent the rapid metabolism of this compound, nonmetabolizable analogs have been used to study the regulatory effects of oxylanosterols. One such compound is 15-oxa-32-vinyl-lanost-8-ene-3 $\beta$ , 32 diol (DMP 565). When rats were treated with this drug, hepatic reductase protein levels were markedly reduced without affecting mRNA levels (84). In liver slices, the rate of HMG-CoA reductase protein synthesis was reduced by over 85% (84). Thus, oxylanosterols appear to mimic the effect of dietary cholesterol.

The regulatory oxylanosterol, 3 $\beta$ -hydroxy- $\Delta^8$ -lanost-8-en-32-aldehyde accumulates when lanosterol 14 $\alpha$ -demethylase activity is low (85). The activity of this enzyme is reduced by a factor of 10 when rats are fed a diet containing cholesterol (86). Recently, cDNAs for lanosterol 14 $\alpha$  demethylase were isolated (87, 88). Interestingly, the 5' flanking region of this gene contained sterol responsive element-like motifs (89). Perhaps, an oxysterol acts to decrease transcription of this gene which in turn leads to accumulation of an oxylanosterol that in turn acts to decrease translation of hepatic HMG-CoA reductase mRNA. The oxylanosterol could act either by binding to a preexisting mRNA binding protein or by inducing the synthesis of an mRNA binding protein, for example. These are testable proposals.

**Protein Degradation.** The level of HMG-CoA reductase protein could also be determined in part by changes in its rate of degradation. The half-life of hepatic HMG-CoA reductase in chow-fed rats is about 2.5 hr (90). This is a fairly short half-life. Modulation of this half-life could markedly and rapidly change the level of reductase protein. In early studies (91), it was shown that giving rats diets supplemented with the potent HMG-CoA reductase inhibitor, mevinolin (lovastatin), and the bile acid binding resin, cholestyramine, increased the half-life of reductase protein to about 12 hr. It was felt that this was due to depletion of either cholesterol or a mevalonate-derived product (10). Based on this observation, it was reasoned that giving cho-

lesterol and/or mevalonate should exert the opposite effect and decrease the half-life of HMG-CoA reductase protein. However, it was found that the half-life of hepatic HMG-CoA reductase protein was unaffected by adding cholesterol to a normal chow diet (54).

Most of the studies of the degradation of HMG-CoA reductase have been carried out using Chinese Hamster Ovary cells grown in lipoprotein-depleted media in the presence of a potent inhibitor of the reductase such as compactin (10, 24, 25, 28). Addition of cholesterol, 25-hydroxycholesterol, and mevalonate to these cells typically reduces the half-life of HMG-CoA reductase from 8 to 5 hr (25). This sterol- plus nonsterol-mediated effect appears to depend upon the eight-membrane spanning region of the reductase (28). Considerable effort has been directed at determining the identity of the nonsterol mevalonate-derived regulator of reductase degradation. Reports that it is farnesol, derived from dephosphorylation of farnesyl pyrophosphate, have been published (26, 27, 92). These reports were based on studies of cells grown in the presence of compactin, a potent inhibitor of HMG-CoA reductase.

Recently, it was shown that lovastatin (formerly called mevinolin) inhibits the activity of the proteasome and that mevalonate increases this activity (93). Several endoplasmic reticulum proteins are degraded by the proteasome (24). It has also been reported that HMG-CoA reductase is degraded by the proteasome in yeast (94, 95). Some involvement of the proteasome in the degradation of HMG-CoA reductase in C-100 cells has been suggested by lactacystin inhibition studies (96). However, the reductase does not become ubiquitinated (24, 55). Further protease inhibitor studies suggested that a membrane-bound cysteine protease is responsible for the *in vitro* degradation of the reductase with a more indirect involvement of the proteasome system (24). An ATP requirement for the degradation of HMG-CoA reductase has been demonstrated using digitonin-permeabilized cells (97). The oligomerization state of the reductase may also influence its rate of degradation (98). Radiation inactivation and immunoblotting studies have shown that HMG-CoA reductase exists as a dimer in liver from normal, chow-fed rats and predominately as a monomer in liver from rats fed diets supplemented with mevinolin (56, 99). The mevinolin-induced monomer form has a much longer half-life (65). In contrast with these findings, studies of monomer and oligomer constructs of the HMG-CoA reductase membrane domain fused to FK 506-binding protein 12 expressed in CHO cells showed that the monomeric state was unstable whereas the oligomeric forms were relatively stable (98).

Studies of HMG-CoA reductase degradation in normal rat liver have yielded surprisingly different results (55). It was found that administration of either mevalonate or farnesol to rats on a normal chow diet had no effect on the half-life of hepatic HMG-CoA reductase (91). In contrast, rats that received a diet supplemented with the HMG-CoA reductase inhibitor, lovastatin, had a reduction in half-life of

hepatic reductase from over 12 hr to about 4 hr (65). Thus, administration of this drug appeared to uncover regulation of the rate of turnover of the reductase. Interestingly, hepatic farnesol levels were unaffected by treatment with lovastatin, which markedly stabilized hepatic HMG-CoA reductase (90). On the other hand, treatment with zaragozic acid A, an inhibitor of squalene synthase, caused a 10-fold rise in hepatic farnesol levels and decreased the half-life of the reductase to about 15 min (91). Further analysis revealed that levels of farnesol in rat liver were unaffected by the addition of 2% cholesterol to the diet. Most importantly it was found that the half-life of hepatic HMG-CoA reductase is unaffected by dietary cholesterol (55). Thus, in the absence of drugs, the rate of degradation of hepatic HMG-CoA reductase appears to be unaffected by either sterols or nonsterols.

### Regulation by Hormones and Other Physiological Factors

Although, feedback regulation of hepatic HMG-CoA reductase by cholesterol is critical to maintaining cholesterol homeostasis, several other physiological factors also exert profound regulatory effects on the expression of this enzyme. Hepatic HMG-CoA reductase activity undergoes a marked diurnal rhythm, high when animals are feeding and low when animals are fasting (100, 101). Since the major fate, quantitatively, of cholesterol is conversion to bile acids, the need for these emulsifying agents affects the expression of hepatic HMG-CoA reductase. Several hormones are involved in modulating hepatic HMG-CoA reductase gene expression. These include: insulin, glucagon, thyroid hormone, and glucocorticoids (102–108). These hormones appear to be involved in regulation of this enzyme as needed in fasting, feeding, growth, and development.

**Diurnal Rhythm.** A pronounced diurnal change in the levels of hepatic HMG-CoA reductase activity was one of the earliest regulatory features observed for this enzyme (100, 101). The enzyme activity changes as much as 10-fold during a 24-hr period in rats (102). Peak enzyme activity occurs during the dark portion of the daily cycle when the animals consume most of their food. Since the changes in reductase activity occur rapidly, it was originally thought that the diurnal variation in reductase activity was mediated by alterations in the state of phosphorylation of the enzyme (109–111). More recent studies, which used immunoblotting, showed that the rise and fall in enzyme activity was associated with corresponding changes in the level of reductase protein (112). Thus the catalytic efficiency of the enzyme remained unchanged. This ruled out regulation by a phosphorylation/dephosphorylation mechanism. Since serum insulin levels increase upon consumption of a meal, the possibility that an increase in the level of this hormone might be responsible for the increase in reductase activity and protein was considered. It was found that when rats were rendered diabetic, hepatic reductase decreased to low

levels, and the diurnal variation was markedly diminished (102).

**Insulin and Glucagon.** Insulin has been shown to act rapidly to increase hepatic HMG-CoA reductase activity. A maximal increase of 10-fold is seen within 2 hr after treating a diabetic rat with insulin (102). There have been some reports that this is due to a post-translational effect involving alterations in the degree of phosphorylation or thiol status of the enzyme (109–111). In more recent studies (107, 108), it has been demonstrated that hepatic HMG-CoA reductase mRNA levels decline to 12% of control in diabetic rats within 18 hr after giving the streptozotocin to render the animals diabetic. Administration of insulin restored these mRNA levels to near normal within 1 hr. This occurred with low physiological doses of insulin. The restoration of hepatic HMG-CoA reductase gene expression correlated closely with the fall in blood glucose levels. Experiments with the protein synthesis inhibitor, cycloheximide, revealed that protein synthesis was not required for this increase in reductase mRNA, indicating a rather direct action of the hormone, most likely at the level of transcription. Presumably, an insulin response element is involved.

Immunoblotting analysis showed that hepatic HMG-CoA reductase protein was reduced to undetectable levels in diabetic rats (107). Administration of low doses of insulin restored reductase immunoreactive protein levels in parallel with enzyme activity. This increase in reductase protein followed the very rapid increase in mRNA levels by about 1 hr.

Administration of glucagon to insulin-treated diabetic rats inhibited the expected increase in hepatic reductase activity. This correlated with decreases in both immunoreactive protein and mRNA levels (108).

The question of whether the decrease in hepatic HMG-CoA reductase activity caused by fasting was due to decreases in mRNA and immunoreactive protein levels was investigated. Fasting lowers insulin levels while raising serum glucagon levels. It was found that 24 and 48 hr of fasting progressively reduced mRNA and protein levels (108). The fall in immunoreactive protein levels correlated very well with that in reductase enzyme activity.

**Thyroid Hormone.** Of the various physiological factors that modulate hepatic HMG-CoA reductase gene expression, the thyroid hormone, L-triiodothyronine ( $T_3$ ), appears to exert the greatest effect. Increases of over 30-fold in reductase enzyme activity were observed upon treatment of hormone-deficient (hypophysectomized) rats with a near receptor-saturating dose of  $T_3$  (103–105). This large increase in enzyme activity was associated with similar increases in levels of reductase mRNA and immunoreactive protein (104). These increases followed a lag of about 36 hr. Treatment with cycloheximide attenuated these increases suggesting that the effects were indirect and required protein synthesis.

Further studies demonstrated that the 30-fold  $T_3$ -mediated increase in hepatic HMG-CoA reductase mRNA

was due to a 5-fold increase in the rate of transcription coupled with a marked stabilization of the reductase mRNA (105). The half-life of reductase mRNA was increased from 2.5 to about 15 hr. Both the increase in transcription and the stabilization of the mRNA required protein synthesis. This is similar to the effect of  $T_3$  on malic enzyme gene expression where both transcriptional and post-transcriptional regulation is sensitive to inhibitors of protein synthesis (113). The identity of a presumed thyroid response element in the reductase promoter and of a protein involved in mRNA stability remains to be established.

Several lines of evidence suggest that thyroid hormone may play a key role in cholesterol homeostasis. Hypothyroidism is associated with elevated serum cholesterol levels (114). This is strikingly illustrated in a study of a woman heterozygous for familial hypercholesterolemia who also had hypothyroidism (115). She had a serum cholesterol level of 700 mg/dl whereas her two siblings who also had heterozygous familial hypercholesterolemia but were not hypothyroid had serum cholesterol levels of only 300 mg/dl. Interestingly, giving animals large doses of  $T_3$ , which markedly increases hepatic HMG-CoA reductase activity and cholesterol production, does not increase serum cholesterol levels, but rather reduces them (116). In fact thyromimetics are known to be potent cholesterol-lowering agents (116). Thyroid function decreases with age whereas serum cholesterol levels increase (117). This may reflect diminished cholesterol buffering capacity (Fig. 1) in individuals expressing lower levels of hepatic HMG-CoA reductase.

**Glucocorticoids.** Interestingly, glucocorticoids were found to block effectively the large increase in hepatic HMG-CoA reductase caused by  $T_3$  (106). The  $T_3$ -induced increases in both reductase immunoreactive protein and mRNA were drastically reduced by treatment with dexamethasone. The natural glucocorticoid, hydrocortisone, also effectively antagonized these  $T_3$ -mediated increases. The biologically inactive analog, epihydrocortisone, was completely ineffective. Surprisingly, administration of dexamethasone did not decrease the  $T_3$ -mediated stimulation of transcription of hepatic HMG-CoA reductase in hypophysectomized rats (106). Rather dexamethasone treatment decreased the half-life of hepatic HMG-CoA reductase mRNA from 13.5 to 2.5 hr, thus accounting for the decreases in mRNA, protein, and enzyme activity (106). This indicates that glucocorticoids act to destabilize reductase mRNA. The finding that the half-life of hepatic reductase mRNA was increased in adrenalectomized rats provides further support for this view (106). The antagonism by glucocorticoids of the large  $T_3$ -mediated increase in reductase gene expression provides an explanation for the difference between hypophysectomized and thyroidectomized rats in terms of their relative responses to  $T_3$  with regard to stimulating HMG-CoA reductase gene expression (116). Thyroidectomized animals still have endogenous glucocorticoids that act to blunt the  $T_3$  response whereas these are absent from hypophysectomized rats. Treatment of thyroidectomized

rats with T<sub>3</sub> only increases reductase gene expression 2–3 fold (116).

**Estrogen.** It is well known that premenopausal women have a significantly lower risk of atherosclerotic vascular disease than do men of the same age. This suggests that estrogen may play a significant role in cholesterol homeostasis. Thus, studies of the effects of estrogen or gender on hepatic HMG-CoA reductase gene expression have been carried out. It has been shown that hepatic cholesterol synthesis is several-fold higher in female as compared with age-matched male hamsters (118). Direct studies of the effects of estrogen on hepatic HMG-CoA reductase enzyme activity have also been done. It was found that female rats expressed 2–3-fold higher levels of reductase activity than male rats at all times during the diurnal cycle (119). These levels declined in gonadectomized animals and were restored by treatment with implanted silastic capsules of 17 $\beta$ -estradiol (120). It was concluded that a physiological level of estrogen acts to increase hepatic HMG-CoA reductase activity (120). These results were confirmed in a study showing that administration of estradiol benzoate, ethinyl estradiol, and mestranol to ovariectomized rats all increased hepatic HMG-CoA reductase activity (121). Administration of estrogen in pharmacological doses increased hepatic HMG-CoA reductase activity in men about two-fold (122). Studies in *Xenopus laevis* revealed that treatment with estradiol 17 $\beta$  stimulated liver reductase enzyme activity more than 40-fold (123). Hepatic HMG-CoA reductase mRNA levels were increased as much as 23-fold (124). Interestingly, the rate of transcription was not increased by estrogen treatment, suggesting that the hormone may act to stabilize reductase mRNA (124). Studies of the effect of estrogen on the half-life of hepatic HMG-CoA reductase mRNA have not been performed. Estrogen stimulation of hepatic HMG-CoA reductase gene expression would provide premenopausal women with a greater cholesterol buffering capacity.

**Bile Acids.** Quantitatively, the major products derived from cholesterol are the bile acids. Thus it is not surprising that the bile acids exert feedback regulation on hepatic HMG-CoA reductase activity. Feeding diets supplemented with cholate, deoxycholate, chenodeoxycholate, or taurocholate to rats markedly decreases hepatic reductase mRNA, reductase activity and immunoreactive protein levels (125, 126). Supplementing diets with ursodeoxycholate had no effect. Interruption of the return of bile acids to the liver by feeding a bile acid binding resin such as cholestyramine caused an increase of 3-fold in reductase mRNA levels (125). It appears that bile acids act transcriptionally to regulate hepatic HMG-CoA reductase gene expression. A nuclear receptor for bile acids, farnesoid X receptor (FXR), has been identified (127). When bound to bile acids, it acts to repress transcription of cholesterol 7 $\alpha$ -hydroxylase, the enzyme that catalyzes the rate-limiting step in the synthesis of bile acids. Whether FXR might also act on a bile acid response element in the promoter of HMG-CoA reductase remains to be investigated.

Conversion of cholesterol to bile acids represents the major pathway for elimination of cholesterol from the body. Stimulation of hepatic cholesterol 7 $\alpha$ -hydroxylase by hormones, bile acid depletion, or overexpression of this enzyme significantly lowers serum cholesterol levels (128–130). This decrease occurs even in the absence of LDL receptor function (129, 130). Thus, cholesterol 7 $\alpha$ -hydroxylase appears to play a very important role in overall cholesterol homeostasis.

**Genetic Differences.** It is generally believed that differences exist among people with respect to their sensitivities to dietary cholesterol. An extreme example of this view is the report of an 88-year-old man who consumed 25 eggs a day but yet had a normal plasma cholesterol (131). Obviously, resistance to dietary cholesterol can be due to any of several factors ranging from absorption of cholesterol to expression of lipoprotein receptors, apolipoproteins, cholesterol 7 $\alpha$ -hydroxylase, or hepatic HMG-CoA reductase. In a recent study of a subgroup (868 Finnish patients) of the Scandinavian Simvastatin Survival Study (132), a set of individuals in whom coronary events were not reduced by simvastatin was identified. These individuals had low baseline rates of cholesterol synthesis as judged by their serum cholestanol levels. These authors concluded that patients with high baseline rates of synthesis of cholesterol seemed to be responders whereas those with low rates of synthesis of cholesterol were nonresponders (132). This same conclusion was also reached in a study of 35 patients with familial hypercholesterolemia where plasma mevalonate levels were used as an index of the rate of *in vivo* cholesterol biosynthesis (39).

The availability of several inbred strains of rats, which differ in their sensitivities to dietary cholesterol (133), provides an appropriate experimental model in which to test the relationship between basal expression of hepatic HMG-CoA reductase and sensitivity to dietary cholesterol. For example, feeding diets containing 2% cholesterol to Brown Norway rats increases serum cholesterol levels two-fold but has little effect on serum cholesterol levels of Sprague-Dawley rats (132). In preliminary studies we have shown that hepatic HMG-CoA reductase activity and immunoreactive protein are more than three-fold higher in the Sprague-Dawley rats.

## Conclusions

Feedback regulation of hepatic HMG-CoA reductase gene expression plays a key role in cholesterol homeostasis. In addition to the well-characterized transcriptional regulation of this enzyme, it is evident that translational regulation is also involved and appears to be the major form of regulation in certain species. Further studies are needed to determine just how much translational regulation contributes. The relative level of hepatic HMG-CoA reductase gene expression may, in part, determine an animal's degree of susceptibility to cholesterol by serving to help offset the effect of dietary cholesterol on serum and tissue levels. We refer



to this concept as cholesterol buffering capacity. Although, on the surface, it may seem paradoxical, high levels of hepatic HMG-CoA reductase gene expression, whether caused by genetic or hormonal factors, are associated with low serum cholesterol levels.

Both insulin and triiodothyronine act to increase hepatic HMG-CoA reductase gene expression. Insulin likely acts at the level of transcription whereas  $T_3$  acts by stabilizing reductase mRNA in addition to increasing the rate of transcription. The importance of these hormones to cholesterol homeostasis is emphasized by the findings that hypothyroidism and diabetes are often associated with increased serum cholesterol levels.

Several important areas may prove fertile for future investigations. The relationship between resistance to dietary cholesterol and hepatic HMG-CoA reductase gene expression needs to be examined in several additional species and strains of animals that are known to differ in their sensitivities to dietary cholesterol. The molecular mechanism of translational regulation of hepatic HMG-CoA reductase needs to be elucidated. The questions of whether oxysterols and/or mRNA binding proteins are involved needs to be resolved. Possible insulin and thyroid hormone response elements in the reductase gene should be identified. The mechanism by which  $T_3$  and glucocorticoids alter the stability of reductase mRNA needs to be established. The regulatory mechanisms by which estrogen and bile acids affect hepatic HMG-CoA reductase gene expression need to be investigated. Answers to these questions should provide a better understanding of the physiological regulation of hepatic HMG-CoA reductase and in turn of cholesterol homeostasis. With such knowledge, better control of serum cholesterol levels, a major risk factor for atherosclerotic vascular disease, may be attainable.

Hepatic HMG-CoA reductase gene expression appears to play a critical role in governing serum cholesterol levels. Numerous correlations between enhanced levels of this enzyme (whether due to genetic or hormonal factors) and increased resistance to elevated serum cholesterol levels has been noted. These correlations have led us to postulate a cholesterol buffering capacity role for hepatic HMG-CoA reductase. One must be cautious of conclusions drawn from correlations; however, the formation of testable postulates is useful. The cholesterol buffering capacity postulate could be tested directly by creating transgenic mice that overexpress hepatic HMG-CoA reductase and determining whether these animals exhibit increased resistance to dietary cholesterol in proportion to the degree of overexpression.

## Comment

Although much has been learned from studies of HMG-CoA reductase in cultured cells, certain cautions are also warranted. In some cases, the culture conditions (addition of an HMG-CoA reductase inhibitor) appear to have unmasked regulation that is not observed in normal cells. The use of tumor cells does not seem appropriate since these cells are

known to have lost their normal feedback regulation. Further studies using intact animals are needed to understand the human situation better, particularly feedback regulation by cholesterol and hormonal control.

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