MINIREVIEW

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

GENE C. NESS[1] AND CHRISTOPHER M. CHAMBERS

Department of Biochemistry and Molecular Biology, College of Medicine, University of South Florida, Tampa, Florida 33612

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. Oxylanosterols 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]

Work from the authors' laboratory described in this review was supported in part by grant-in-aid 9950855 V from the American Heart Association—Florida/Puerto Rico Affiliate.

[1] To whom requests for reprints should be addressed at the Department of Biochemistry and Molecular Biology, College of Medicine, University of South Florida, 12901 Bruce B. Downs Blvd., Tampa, FL 33612. E-mail: gness@hsc.usf.edu

0037-9727/00/2241-0008$15.00/0

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 myelinization, 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 rhthym, 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 (hyporesponders) exhibited much higher basal levels of hepatic sterol synthesis and greater downregulation when challenged with a high-cholesterol diet than monkeys that were

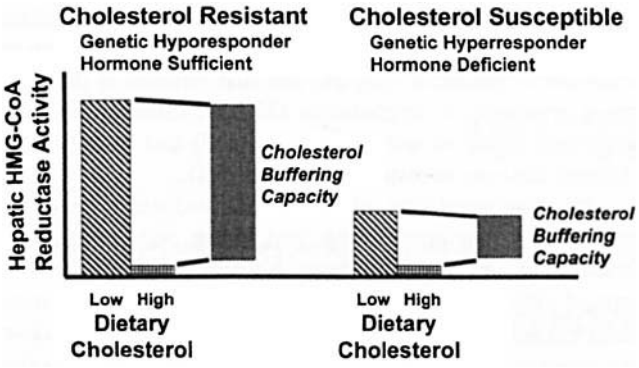

Figure 1. Dependence of cholesterol buffering capacity on basal expression of hepatic HMG-CoA reductase.

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% cholestryamine; 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 γ-glutamyl cycle (57), which removes excess amino acids from the circulation using glutathione. The first step of this cycle is catalyzed by γ-glutamyl transpeptidase, which forms γ-glutamyl-amino acid plus cysteinylglycine. The amino acid is released inside the cell. The cysteinylglycine is hydrolyzed. This process uses glutathione, which must be resynthezied. 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 β-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 elucidated.

Oxylanosterols. Kandutch 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 oxycholesterols, oxylanosterols were also found to be potent suppressors (78, 79). Whereas oxycholesterols exert their affects 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, 3β-hydroxylanost-8-en-32-al, an intermediate in the 14α 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-3β, 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, 3β-hydroxylanost-8-en-32-aldehyde accumulates when lanosterol 14α-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 14α demethylase were isolated (87, 88). Interestingly, the 5′ flanking region of this gene contained sterol responsive element-like motifs (89). Perhaps, an oxycholesterol 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_3 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 17β-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 17β 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 7α-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 7α-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 7α-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 7α-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 oxylanosterols 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.

1. Singer II, Kawaka DW, Kazazis DM, Alberts AW, Chen JS, Huff JW, Ness GC. Hydroxymethylglutaryl coenzyme A reductase containing hepatocytes are distributed periportally in normal and mevinolin-treated rat livers. Proc Natl Acad Sci U S A **81**:5556–5560, 1984.
2. Bucher NLR, Overath P, Lynen F. 3-Hydroxy-3-methylglutaryl coenzyme A reductase, cleavage, and condensing enzymes in relation to cholesterol formation in rat liver. Biochim Biophys Acta **40**:491–501, 1960.
3. Bloch K. Summing up. Ann Rev Biochem **56**:1–19, 1987.
4. Brown MS, Goldstein JL. A receptor-mediated pathway for cholesterol homeostasis. Science **232**:34–47, 1986.
5. Bjorkhem I. Mechanism of bile acid biosynthesis in mammalian liver. In: Danielsson H, Sjovall J, Eds. Sterols and Bile Acids. Amsterdam: Elsevier, pp231–278, 1985.
6. Porter JA, Young KE, Beachy PA. Cholesterol modification of hedgehog signaling proteins in animal development. Science **274**:255–259, 1995.
7. Tint GS, Irons M, Elias MR, Batta AK, Frieden R, Chen TS, Salen G. Defective cholesterol biosynthesis associated with the Smith-Lemli–Opitz syndrome. N Engl J Med **330**:107–113, 1994.
8. Irons M, Elias MR, Tint GS, Salen G, Frieden R, Buie TM, Ampola M. Abnormal cholesterol metabolism in the Smith-Lemli–Opitz syndrome-TypeII: Report of clinical and biochemical findings in four patients and treatment in one patient. Am J Med Genet **50**:347–352, 1994.
9. Grundy SM. Cholesterol and coronary heart disease: A new era. JAMA **256**:2849–2858, 1986.
10. Goldstein JL, Brown MS. Regulation of the mevalonate pathway. Nature **343**:425–430, 1990.
11. Bradford RH, Shear CL, Chremos AN, Dujovne C, Downton M, Franklin FA, Gould AL, Hesney M, Higgins J, Hurley DP, Langendorfer A, Nash DT, Pool JL, Schnaper HW. Expanded clinical evaluation of Lovastatin (EXCEL) study results. I. Efficacy in modifying plasma lipoproteins and adverse event profile in 8245 patients with moderate hypercholesterolemia. Arch Intern Med **151**:43–49, 1991.
12. Waters D, Higginson L, Gladstone P, Kimball B, Le May M, Boccuzzi SJ, Lesperance J. Effects of monotherapy with an HMG-CoA reductase inhibitor on the progression of coronary atherosclerosis as assessed by serial quantitative arteriography: The Canadian coronary atherosclerosis intervention trial. Circulation **89**:959–968, 1994.
13. Scandinavian Simvastatin Survival Study Group. Randomized trial of cholesterol-lowering in 4444 patients with coronary heart disease: The Scandinavian simvastatin survival study. Lancet **344**:1383–1389, 1994.
14. Shepherd J, Cobbe SM, Ford I, Isles CG, Lorimer AR, MacFarlane PW, McKillop JH, Packard CJ. Prevention of coronary heart disease with pravastatin in men with hypercholesterolemia. N Engl J Med **333**:1301–1307, 1995.
15. Nawrocki JW, Weiss SR, Davidson MH, Sprecher DL, Schwartz SL, Lupien PJ, Jones PH, Haber HE, Black DM. Reduction of LDL cholesterol by 25% to 60% in patients with primary hypercholesterolemia by atorvastatin, a new HMG-CoA reductase inhibitor. Arterioscler Thromb Vasc Biol **15**:678–682, 1995.
16. Bisgaier CL, Essenburg AD, Auerbach BJ, Pape ME, Sekerke CS, Gee A, Newton RS. Attenuation of plasma low-density lipoprotein cholesterol by select 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors in mice devoid of low-density lipoprotein receptors. J Lipid Res **38**:2502–2515, 1997.
17. Naoumova RP, Dunn S, Rallidis L, Abu-Muhana O, Neuwirth C, Rendell NB, Taylor GW, Thompson GR. Prolonged inhibition of cholesterol synthesis explains the efficacy of atorvastatin. J Lipid Res **38**:1496–1500, 1997.
18. Ness GC, Chambers CM, Lopez D. Atorvastatin action involves diminished recovery of hepatic HMG-CoA reductase activity. J Lipid Res **39**:75–84, 1998.

19. Ness GC, Zhao Z, Lopez D. Inhibitors of cholesterol biosynthesis increase hepatic low-density lipoprotein receptor protein degradation. Arch Biochem Biophys **325**:242–248, 1996.
20. Luskey KL, Faust JR, Chin DJ, Brown MS, Goldstein JL. Amplification of the gene for 3-hydroxy-3-methylglutaryl coenzyme A reductase, but not for the 53-kDa protein, in UT-1 cells. J Biol Chem **258**:8462–8469, 1983.
21. Chin DJ, Gil G, Russell DW, Liscum L, Luskey KL, Basu SK, Okayama H, Berg P, Goldstein JL, Brown MS. Nucleotide sequence of 3-hydroxy-3-methylglutaryl coenzyme A reductase, a glycoprotein of endoplasmic reticulum. Nature **308**:613–617, 1984.
22. Skalnik DG, Brown DA, Brown PC, Friedman RL, Hardeman EC, Schimke RT, Simoni RD. Mechanisms of 3-hydroxy-3-methylglutaryl coenzyme A reductase overaccumulation in three compactin-resistant cell lines. J Biol Chem **260**:1991–1994, 1985.
23. Kumagai H, Chun KT, Simoni RD. Molecular dissection of the role of the membrane domain in regulated degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase. J Biol Chem **270**:19107–19113, 1995.
24. Moriyama T, Sather SK, McGee TP, Simoni RD. Degradation of HMG-CoA reductase *in vitro*: Cleavage in the membrane domain by a membrane-bound cysteine protease. J Biol Chem **273**:22037–22043, 1998.
25. Chun KT, Bar-Nun S, Simoni RD. The regulated degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase requires a short-lived protein and occurs in the endoplasmic reticulum. J Biol Chem **265**:22004–22010, 1990.
26. Meigs TE, Roseman DS, Simoni RD. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase degradation by the nonsterol mevalonate metabolite farnesol *in vivo*. J Biol Chem **271**:7916–7922, 1996.
27. Meigs TE, Simoni RD. Farnesol as a regulator of HMG-CoA reductase degradation: Characterization and role of farnesyl pyrophosphatase. Arch Biochem Biophys **345**:1–9, 1997.
28. Gil G, Faust JR, Chin DJ, Goldstein JL, Brown MS. Membrane-bound domain of HMG-CoA reductase is required for sterol-enhanced degradation of the enzyme. Cell **41**:249–258, 1985.
29. Siperstein MD, Fagan V. Deletion of the cholesterol-negative feedback system in liver tumors. Cancer Res **24**:1108–1115, 1964.
30. Azrolan NI, Coleman PS. A discoordinant increase in the cellular amount of 3-hydroxy-3-methylglutaryl coenzyme A reductase results in the loss of rate-limiting control over cholesterogenesis in a tumor cell-free system. Biochem J **258**:421–425, 1989.
31. Dietschy JM, Turley SD, Spady DK. Role of liver in the maintenance of cholesterol and low-density lipoprotein homeostasis in different animal species, including humans. J Lipid Res **34**:1637–1659, 1993.
32. Bilheimer DW, Goldstein JL, Grundy SC, Starzl TE, Brown MS. Liver transplantation to provide LDL receptors and lower plasma cholesterol in a child with homozygous familial hypercholesterolemia. N Engl J Med **311**:1658–1664, 1984.
33. Turley SD, Spady DK, Dietschy JM. Role of liver in the synthesis of cholesterol and the clearance of low-density lipoproteins in the cynomolgus monkey. J Lipid Res **36**:67–79, 1995.
34. Spady DK, Dietschy JM. Sterol synthesis *in vivo* in 18 tissues of the squirrel monkey, guinea pig, rabbit, hamster, and rat. J Lipid Res **24**:303–315, 1983.
35. Andersen JM, Turley SD, Dietschy JM. Relative rates of sterol synthesis in the liver and various extrahepatic tissues of normal and cholesterol-fed rabbits: Relationship to plasma lipoprotein and tissue cholesterol levels. Biochim Biophys Acta **711**:421–430, 1982.
36. Kita T, Goldstein JL, Brown MS, Watanabe Y, Hornick CA, Havel RJ. Hepatic uptake of chylomicron remnants in WHHL rabbits: A mechanism genetically distinct from the low-density lipoprotein receptor. Proc Natl Acad Sci U S A **79**:3623–3627, 1982.
37. Turley SD, Spady DK, Dietschy JM. Identification of a metabolic difference accounting for the hyper- and hyporesponder phenotypes of cynomolgus monkey. J Lipid Res **38**:1598–1611, 1997.
38. Naoumova RP, Marais AD, Mountney J, Firth JC, Rendell NB, Taylor GW, Thompson GR. Plasma mevalonic acid, an index of cholesterol synthesis *in vivo*, and responsiveness to HMG-CoA reductase inhibitors in familial hypercholesterolaemia. Atherosclerosis **119**:203–213, 1996.
39. Gould RG, Taylor CB. Effect of dietary cholesterol on hepatic cholesterol synthesis. Fed Proc **9**:179, 1950.
40. Gould RG. Lipid metabolism and atherosclerosis. Am J Med **11**:209–227, 1951.
41. Gould RG, Taylor CB, Hagerman JS, Warner I, Campbell DJ. Cholesterol metabolism I. Effect of dietary cholesterol on the synthesis of cholesterol in dog tissue *in vitro*. J Biol Chem **201**:519–528, 1953.
42. Mittopoulos KA. Molecular control of HMG-CoA reductase: The role of nonesterified cholesterol. In: Sabine J, Ed. 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase. Boca Raton, FL: CRC Press, pp107–127, 1983.
43. Sabine JR, James MJ. The intracellular mechanism responsible for dietary feedback control of cholesterol synthesis. Life Sci **18**:1185–1192, 1976.
44. Brown MS, Goldstein JL. Multivalent feedback regulation of HMG-CoA reductase, a control mechanism coordinating isoprenoid synthesis and cell growth. J Lipid Res **21**:505–517, 1980.
45. Beg ZH, Allmann DW, Gibson DW. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity with cAMP and protein fractions of rat liver cytosol. Biochem Biophys Res Commun **54**:1362–1369, 1973.
46. Arebalo RE, Tormanen CD, Hardgrave JE, Noland BJ, Scallen TJ. *In vivo* regulation of HMG-CoA reductase: Immunotitration of enzyme after short-term mevalonate or cholesterol feeding. Proc Natl Acad Sci U S A **79**:51–55, 1982.
47. Ingebritsen TS. Molecular control of HMG-CoA reductase: Regulation by phosphorylation/dephosphorylation. In: Sabine JR, Ed. 3-Hydroxy-3-methylglutaryl Coenzyme A Reductase. Boca Raton, FL: CRC Press, pp129–152, 1983.
48. Parker RA, Miller SJ, Gibson DM. Phosphorylation of native 97-kDa 3-hydroxy-3-methylglutaryl coenzyme A reductase from rat liver: Impact on activity and degradation of the enzyme. J Biol Chem **264**:4877–4887, 1989.
49. Clarke PR, Hardie DG. Regulation of HMG-CoA reductase: Identification of the site phosphorylated by the AMP-activated protein kinase *in vivo* and in intact rat liver. EMBO J **9**:2439–2446, 1990.
50. Hardie DG. Regulation of fatty acid and cholesterol metabolism by the AMP-activated protein kinase. Biochim Biophys Acta **1123**:231–238, 1992.
51. Omkumar RV, Darnay BG, Rodwell VW. Modulation of Syrian hamster 3-hydroxy-3-methylglutaryl coenzyme A reductase activity by phosphorylation: Role of serine 871. J Biol Chem **269**:6810–6814, 1994.
52. Sato R, Goldstein JL, Brown MS. Replacement of serine 871 of hamster 3-hydroxy-3-methylglutaryl CoA reductase prevents phosphorylation by AMP-activated protein and blocks inhibition of sterol synthesis induced by ATP depletion. Proc Natl Acad Sci U S A **90**:9261–9265, 1993.
53. Ness GC, Eales S, Lopez D, Zhao Z. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression by sterols and non-sterols in rat liver. Arch Biochem Biophys **308**:420–425, 1994.
54. Chambers CM, Ness GC. Dietary cholesterol regulates hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase gene expression in rats primarily at the level of translation. Arch Biochem Biophys **354**:317–322, 1998.
55. Ness GC, McCreery MJ, Sample CE, Smith M, Pendleton LC. Sulfhydryl-disulfide forms of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase. J Biol Chem **260**:16395–16399, 1985.
56. Cappel RE, Gilbert HF. Oxidative inactivation of HMG-CoA reductase and subunit cross-linking involve different thiol/disulfide centers. J Biol Chem **268**:342–348, 1993.
57. Meister A. On the enzymology of amino acid transport. Science **180**:33–39, 1974.
58. Bazzano G, D'Elia JA, Olson RE. Monosodium glutamate: Feeding of large amounts in man and gerbils. Science **169**:1208–1209, 1970.
59. Olson RE, Bazzano G, D'Elia JA. The effects of large amounts of glutamic acid upon serum lipids and sterol metabolism in man. Trans Assoc Am Physicians **83**:196–210, 1970.
60. Bazzano G, Olson RE. Hypocholesterolemic effect of glutamic acid in the Mongolian gerbil. Proc Soc Exp Biol Med **131**:1463–1465, 1969.
61. Liscum L, Luskey KL, Chen DJ, Ho YK, Goldstein JL, Brown MS. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase and its mRNA in rat liver as studied with a monoclonal antibody and a cDNA probe. J Biol Chem **258**:8450–8455, 1983.
62. Ness GC, Keller RK, Pendleton LC. Feedback regulation of hepatic

3-hydroxy-3-methylglutaryl coenzyme A reductase activity by dietary cholesterol is not due to altered mRNA levels. J Biol Chem **266:**14854–14857, 1991.
63. Spady DK, Cuthbert JA. Regulation of hepatic sterol metabolism in the rat: Parallel regulation of activity and mRNA for 7α hydroxylase but not for 3-hydroxy-3-methylglutaryl coenzyme A reductase or low-density lipoprotein receptor. J Biol Chem **267:**5584–5591, 1992.
64. Lopez D, Chambers CM, Ness GC. 3-Hydroxy-3-methylglutaryl coenzyme A reductase inhibitors unmask cryptic regulatory mechanisms. Arch Biochem Biophys **343:**118–122, 1997.
65. Rudling M. Hepatic mRNA levels for the LDL receptor and HMG-CoA reductase show coordinate regulation *in vivo*. J Lipid Res **33:**493–501, 1992.
66. Gil G, Goldstein JL, Slaughter CA, Brown MS. Cytoplasmic HMG-CoA synthase from the hamster I: Isolation and sequencing of a full-length cDNA. J Biol Chem **261:**3710–3716, 1986.
67. Shimomura I, Bashmakov Y, Shimano H, Horton JD, Goldstein JL, Brown MS. Cholesterol feeding reduces nuclear forms of sterol regulatory element binding proteins in hamster liver. Proc Natl Acad Sci U S A **94:**12354–12359, 1997.
68. Lopez D, Ness GC. Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase unmask transcriptional regulation of hepatic low-density lipoprotein receptor gene expression by dietary cholesterol. Arch Biochem Biophys **344:**215–219, 1997.
69. Beynen AC, Katan MB, Van Zutphen LFM. Hypo- and hyperresponders: Individual differences in the response of serum cholesterol concentration to changes in diet. Adv Lipid Res **22:**115–171, 1987.
70. Brown MS, Goldstein JL. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. Cell **89:**331–340, 1997.
71. Shimano H, Horton JD, Hammer RE, Shimomura I, Brown MS, Goldstein JL. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. J Clin Invest **98:**1575–1584, 1996.
72. Reynolds GA, Goldstein JL, Brown MS. Multivalent control of 3-hydroxy-3-methylglutaryl coenzyme A reductase determined by multiple transcription initiation sites and intron splicing sites in the 5′ untranslated region. J Biol Chem **260:**10369–10377, 1985.
73. Gayen AK, Peffley DM. The length of 5′ untranslated leader sequences influences distribution of 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in polysomes: Effects of lovastatin, oxysterols, and mevalonate. Arch Biochem Biophys **322:**475–485, 1995.
74. Chambers CM, Ness GC. Translational regulation of hepatic HMG-CoA reductase by dietary cholesterol. Biochem Biophys Res Commun **232:**278–281, 1997.
75. Kandutsch AA, Chen HW, Heiniger HJ. Biological activity of some oxygenated sterols. Science **201:**498–501, 1978.
76. Kandutsch AA, Chen HW. Consequences of blocked sterol synthesis in cultured cells. J Biol Chem **252:**409–415, 1979.
77. Taylor FB, Saucier SE, Shown EP, Parish EJ, Kandutsch AA. Correlation between oxysterol binding to a cytosolic binding protein and potency in the repression of hydroxymethylglutaryl coenzyme A reductase. J Biol Chem **259:**12382–12387, 1984.
78. Gibbons GF, Pullinger CR, Chen HW, Cavenee WK, Kandutsch AA. Regulation of cholesterol biosynthesis in cultured cells by probable natural precursor sterols. J Biol Chem **255:**395–400, 1980.
79. Panini SR, Delate TA, Sinensky M. Post-transcriptional regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by 24 (S), 25-oxidolanosterol. J Biol Chem **267:**12647–12654, 1992.
80. Trzaskos JM, Magolda RL, Favata MF, Fischer RT, Johnson PR, Chen HW, Ko SS, Leonard DA, Gaylor JL. Modulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by 15 α-fluorolanost-7-en-3β-ol. J Biol Chem **268:**22591–22599, 1993.
81. Leonard DA, Kotarski MA, Tessiatore JE, Favata MF, Trzaskos JM. Post-transcriptional regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by 3β-hydroxy-lanost-8-en-32al, an intermediate in the conversion of lanosterol to cholesterol. Arch Biochem Biophys **310:**152–157, 1994.
82. Trzaskos JM. Oxylanosterols as modifiers of cholesterol biosynthesis. Prog Lipid Res **34:**99–116, 1995.
83. Tabacik C, Aliau S, Seriou B, Crastes de Paulet A. Post-HMG-CoA reductase regulation of cholesterol biosynthesis in normal human lymphocytes: Lanosten-3β-ol-32al, a natural inhibitor. Biochem Biophys Res Commun **101:**1087–1095, 1981.
84. Ness GC, Lopez D, Chambers CM, Zhao Z, Beach DL, Ko SS, Trzaskos JM. Effects of 15 oxa-32-vinyl-lanost-8-ene-3β, 32 diol on the expression of HMG-CoA reductase and LDL receptor in rat liver. Arch Biochem Biophys **357:**259–264, 1998.
85. Trzaskos JM, Fischer RT, Favata MF. Mechanistic studies of lanosterol C-32 demethylation: Conditions which promote oxysterol intermediate accumulation during the demethylation process. J Biol Chem **261:**16937–16942, 1986.
86. Trzaskos JM, Bowen WD, Shafiee A, Fischer RT, Gaylor JL. Cytochrome P-450–dependent oxidation of lanosterol in cholesterol biosynthesis: Microsomal electron transport and C-32 demethylation. J Biol Chem **259:**13402–13412, 1984.
87. Aoyama Y, Funae Y, Noshiro M, Horiuchi T, Yoshida Y. Occurrence of a P450 showing high homology to yeast lanosterol 14 α-demethylase (P45014DM) in the rat liver. Biochem Biophys Res Commun **329:**73–1326, 1994.
88. Stromstedt M, Rozman D, Waterman MR. The ubiquitously expressed human CYP51 encodes lanosterol 14 α-demethylase, a cytochrome P450 whose expression is regulated by oxysterols. Arch Biochem Biophys **329:**73–81, 1996.
89. Rozman D, Stromstedt M, Tsui LC, Scherer SW, Waterman MR. Structure and mapping of the human lanosterol 14 α-demethylase gene (CYP51) encoding the cytochrome P450 involved in cholesterol biosynthesis: Comparison of exon/intron organization with other mammalian and fungal CYP genes. Genomics **38:**371–381, 1996.
90. Keller RK, Zhao Z, Chambers CM, Ness GC. Farnesol is not the nonsterol regulator mediating degradation of HMG-CoA reductase in rat liver. Arch Biochem Biophys **328:**324–330, 1996.
91. Sinensky M, Logel J. Inhibition of degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by mevinolin. J Biol Chem **258:**8547–8549, 1983.
92. Correll CC, Edwards PA. Mevalonic acid-dependent degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase *in vivo* and *in vitro*. J Biol Chem **269:**633–638, 1994.
93. Rao S, Porter DC, Chen X, Herliczek T, Lowe M, Keyomarsi K. Lovastatin-mediated G_1 arrest is through inhibition of the proteasome, independent of hydroxymethylglutaryl CoA reductase. Proc Natl Acad Sci U S A **96:**7797–7802, 1999.
94. Hampton RY, Gardner R, Rine J. Role of 26S proteasome and HRD genes in the degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase, an integral endoplasmic reticulum membrane protein. Mol Biol Cell **7:**2029–2044, 1996.
95. Hampton RY, Bhakta H. Ubiquitin-mediated regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase. Proc Natl Acad Sci U S A **94:**12944–12948, 1997.
96. McGee TP, Cheng HH, Kumagai H, Omura S, Simoni RD. Degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in endoplasmic reticulum membranes is accelerated as a result of increased susceptibility to proteolysis. J Biol Chem **271:**25630–25638, 1996.
97. Leonard DA, Chen HW. ATP-dependent degradation of 3-hydroxy-3-methylglutaryl coenzyme A reductase in permeabilized cells. J Biol Chem **262:**7914–7919, 1987.
98. Cheng HH, Xu L, Kumagai H, Simoni RD. Oligomerization state influences the degradation rate of 3-hydroxy-3-methylglutaryl CoA reductase. J Biol Chem **274:**17171–17178, 1999.
99. Ness GC, Pendleton LC, McCreery MJ. *In situ* determination of the functional size of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase by radiation inactivation analysis. Biochim Biophys Acta **953:**361–364, 1988.
100. Kandutsch AA, Saucier SE. Prevention of cyclic and Trition-induced increases in 3-hydroxy-3-methylglutaryl coenzyme A reductase and sterol synthesis by puromycin. J Biol Chem **244:**2299–2305, 1969.
101. Shapiro DJ, Rodwell VW. Regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol synthesis. J Biol Chem **246:**3210–3216, 1971.
102. Lakshmanan MR, Nepokroeff CM, Ness GC, Dugan RE, Porter JW. Stimulation by insulin of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol synthesizing activity. Biochem Biophys Res Commun **50:**704–710, 1973.
103. Ness GC, Dugan RE, Lakshmanan MR, Nepokroeff CM, Porter JW. Stimulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity in hypophysectomized rats by l-triiodothyronine. Proc Natl Acad Sci U S A **70:**3839–3842, 1973.

104. Sample CE, Pendleton LC, Ness GC. Regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA levels by l-triiodothyronine. Biochemistry **26**:727–731, 1987.
105. Simonet WS, Ness GC. Transcriptional and post-transcriptional regulation of rat hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase by thyroid hormones. J Biol Chem **263**:12448–12453, 1988.
106. Simonet WS, Ness GC. Post-transcription regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in rat liver: Glucocorticoids block the stabilization caused by thyroid hormones. J Biol Chem **264**:569–573, 1989.
107. Ness GC, Wiggins L, Zhao Z. Insulin increases hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA and immunoreactive protein levels in diabetic rats. Arch Biochem Biophys **309**:193–194, 1994.
108. Ness GC, Zhao Z, Wiggins L. Insulin and glucagon modulate hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase activity by affecting immunoreactive protein levels. J Biol Chem **269**:29168–29172, 1994.
109. Ingebritsen TS, Geelen MJ, Parker RA, Evenson KJ, Gibson DM. Modulation of hydroxymethylglutaryl–CoA reductase activity, reductase kinase activity, and cholesterol synthesis in rat hepatocytes in response to insulin and glucagon. J Biol Chem **254**:9986–9989, 1979.
110. Beg ZH, Stonik JA, Brewer HB. *In vitro* and *in vivo* phosphorylation of rat liver 3-hydroxy-3-methylglutaryl coenzyme A reductase and its modulation by glucagon. J Biol Chem **255**:8541–8545, 1980.
111. Henneberg R, Rodwell VW. Altered modulation state of rat hepatocyte HMG-CoA reductase in response to insulin, glucagon, cAMP, cGMP, and epinephrine. Fed Proc **40**:1604, 1981.
112. Ness GC, Chambers CM. The diurnal variation of hepatic HMG-CoA reductase activity is due to changes in the level of immunoreactive protein. Arch Biochem Biophys **327**:41–44, 1996.
113. Back DW, Wilson SB, Morris SM, Goodridge AG. Hormonal regulation of lipogenic enzymes in chick embryo hepatocytes in culture: Thyroid hormone and glucagon regulate malic enzyme mRNA level at post-transcriptional steps. J Biol Chem **261**:12555–12561, 1986.
114. Ness GC. Thyroid hormone: Basis for its hypocholesterolemic effect. J Fla Med Assoc **78**:383–385, 1991.
115. Illingworth D, McClung MR, Conner WE, Alaupovic P. Familial hypercholesterolaemia and primary hypothyroidism: Coexistence of both disorders in a young woman with severe hypercholesterolaemia. Clin Endocrinol **14**:145–152, 1981.
116. Ness GC, Lopez D, Chambers CM, Newsome WP, Cornelius P, Long CA, Harwood HJ. Effects of l-triiodothyronine and the thyromimetic L-94901 on serum lipoprotein levels and hepatic low-density lipoprotein receptor, 3-hydroxy-3-methylglutaryl coenzyme A reductase and apo A-I gene expression. Biochem Pharmacol **56**:121–129, 1998.
117. Walker RF, Ness GC, Zhao Z, Bercu BB. Effects of stimulated growth hormone secretion on age-related changes in plasma cholesterol and hepatic low-density lipoprotein mRNA concentrations. Mech Ageing Dev **75**:215–226, 1994.
118. Spady DK, Turley SD, Dietschy JM. Dissociation of hepatic cholesterol synthesis from hepatic low-density lipoprotein uptake and biliary cholesterol saturation in female and male hamsters of different ages. Biochim Biophys Acta **753**:381–392, 1983.
119. Carlson SE, Mitchell AD, Goldfarb S. Sex-related differences in diurnal activities and development of hepatic microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase and cholesterol 7α-hydroxylase. Biochim Biophys Acta **531**:115–124, 1978.
120. Carlson SE, Mitchell AD, Carter ML, Goldfarb S. Evidence that physiologic levels of circulating estrogens and neonatal sex-imprinting modify postpubertal hepatic microsomal 3-hydroxy-3-methylglutaryl coenzyme A reductase activity. Biochim Biophys Acta **633**:154–161, 1980.
121. Abul-Hajj YJ. Effect of estrogens on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and cholesterol levels. Steroids **37**:601–607, 1981.
122. Angelin B, Olivecrona H, Reihner E, Rudling M, Stahlberg D, Eriksson M, Ewerth S, Henriksson P, Einarsson K. Hepatic cholesterol metabolism in estrogen-treated men. Gastroenterology **103**:1657–1663, 1992.
123. Philipp BW, Shapiro DJ. Estrogen regulation of hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase and acetyl CoA carboxylase in *Xenopus laevis*. J Biol Chem **256**:2922–2927, 1981.
124. Chen H, Shapiro DJ. Nucleotide sequence and estrogen induction of *Xenopus laevis* 3-hydroxy-3-methylglutaryl coenzyme A reductase. J Biol Chem **265**:4622–4629, 1990.
125. Shefer S, Nguyen LB, Salen G, Ness GC, Chowdhary IR, Lerner Batta AK, Tint GS. Differing effects of cholesterol and taurocholate on steady-state hepatic HMG-CoA reductase and cholesterol 7α-hydroxylase activities and mRNA levels in the rat. J Lipid Res **33**:1193–1200, 1992.
126. Duckworth PF, Vlahcevic ZR, Studer EJ, Gurley EC, Heuman DM, Beg ZH, Hylemon PB. Effect of hydrophobic bile acids on 3-hydroxy-3-methylglutaryl coenzyme A reductase activity and mRNA levels in the rat. J Biol Chem **266**:9413–9418, 1991.
127. Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, Shan B. Identification of a nuclear receptor for bile acids. Science **284**:1362–1365, 1999.
128. Ness GC, Lopez D. Transcriptional regulation of rat hepatic low-density lipoprotein and cholesterol 7α-hydroxylase by thyroid hormone. Arch Biochem Biophys **323**:404–408, 1995.
129. Xu G, Salen G, Shefer S, Ness GC, Nguyen LB, Tint GS, Parker TS, Roberts J, Batta AK, Chen TS, Zhao Z, Kong X. Increasing hepatic cholesterol 7α-hydroxylase reduces plasma cholesterol concentrations in normocholesterolemic and hypercholesterolemic rabbits. Hepatology **24**:882–887, 1996.
130. Spady DK, Cuthbert JA, Willard MN, Meidell RS. Overexpression of cholesterol 7α-hydroxylase in mice lacking the low-density lipoprotein receptor gene. J Biol Chem **273**:126–132, 1998.
131. Kern F Jr. Normal plasma cholesterol in an 88-year-old man who eats 25 eggs a day. N Engl J Med **324**:896–899, 1991.
132. Miettinen TA, Gylling H, Strandberg T, Sarns S. Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of Scandinavian simvastatin survival study. Finnish 4S Investigators. Br Med J **316**:1127–1130, 1998.
133. Van Zutphen LF, Den Bieman MG. Cholesterol response in inbred strains of rats, *Rattus norvegicus*. J Nutr **111**:1833–1838, 1981.