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**Statins redux: a re-assessment of how statins lower plasma cholesterol**

Raghow R. Statins and cholesterol metabolism

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**Abstract**

Obesity associated dyslipidemia and its negative effects on the heart and blood vessels have emerged as a major healthcare challenge around the globe. The use of statins, potent inhibitors of HMG Co-A reductase, a rate-limiting enzyme in cholesterol biosynthesis, has significantly reduced the rates of cardiovascular and general mortality in patients with coronary artery disease. How statins lower plasma cholesterol levels presents a mechanistic conundrum since persistent exposure to these drugs *in vitro* or *in vivo* is known to induce overexpression of the HMG Co-A reductase gene and protein. In an attempt to solve this mechanistic puzzle*,* Schonewille *et al,* studied detailed metabolic parameters of cholesterol synthesis, inter-organ flux and excretion in mice treated with 3 common statins, rosuvastatin, atorvastatin or lovastatin, each with its unique pharmacokinetics. From the measurements of the rates of heavy water (D2O) and [13C]-acetate incorporation into lipids, the authors calculated the rates of whole body and organ-specific cholesterol synthesis in control and statin-treated mice. These analyses revealed dramatic enhancement in the rates of hepatic cholesterol biosynthesis in statin-treated mice that concomitantly elicited lower levels of cholesterol in their plasma. The authors have provided strong evidence to indicate that statin treatment in mice led to induction of compensatory metabolic pathways that apparently mitigated an excessive accumulation of cholesterol in the body. It was noted however that changes in cholesterol metabolism induced by 3 statins were not identical. While sustained delivery of all 3 statins led to enhanced rates of biliary excretion of cholesterol and its fecal elimination, only atorvastatin treated mice elicited enhanced trans-intestinal cholesterol excretion. Thus, blockade of HMGCR by statins in mice was associated with profound metabolic adaptations that reset their cholesterol homeostasis. The findings of Schonewille *et al,* deserve to be corroborated and extended in patients in order to more effectively utilize these important cholesterol-lowering drugs in the clinic.

**Key words:** Statins; Atorvastatin; Lovastatin; Rosuvastatin; Cholesterol synthesis; HMG-CoA reductase

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**Core tip:** Schonewille *et al,* comprehensively studied cholesterol metabolism (*de-novo* synthesis of cholesterol and its inter-organ flux, and fecal elimination) in mice treated with rosuvastatin, atorvastatin or lovastatin. These analyses revealed that the rates of whole body and organ-specific cholesterol synthesis were boosted by all three statins. Mice treated with statins also elicited enhanced rates of biliary excretion of cholesterol and its fecal elimination. Remarkably, the process of trans-intestinal cholesterol excretion was augmented by only atorvastatin. These data shed mechanistic light on how statin treatment led to organ-specific metabolic adaptations in mice to reset their cholesterol homeostasis.

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**INTRODUCTION and COMMENTARY ON HOT TOPICS**

Statins are among the most commonly prescribed drugs across the globe[[1](#_ENREF_1),[2](#_ENREF_2)]. Statins were discovered in the 1970s, as competitive inhibitors of hydroxyl-methyl glutaryl coenzyme-A reductase (HMGCR), the rate limiting enzyme in cholesterol biosynthesis[[3](#_ENREF_3)]. Soon after their discovery, statins were shown to be relatively safe drugs that caused lowering of serum cholesterol and reduction of fatty streaks in the blood vessels of laboratory animals. The positive outcomes of statin therapy in experimental animals were corroborated and extended to patients in large-scale clinical studies that established that statin treatment was correlated with significantly reduced rates of mortality in patients with atherosclerosis[[1](#_ENREF_1),[2](#_ENREF_2)].

In addition to eliciting salutary effects in the cardiovascular system that includes improved endothelial function and neo-angiogenesis, statins exert pleiotropic actions in various tissues including the central nervous system. For instance, statins appear to exert anti-inflammatory actions in diseases such as rheumatoid arthritis, Alzheimer and Parkinson’s disease, and multiple sclerosis[[4](#_ENREF_4)]. It is worth mentioning however, that statins are far from innocuous since a sub-set of patients taking these drugs develop a range of adverse reactions that include muscle pain or myalgia, and in rare cases, rhabdomyolysis. Additionally, statin therapy has been shown to predispose some patients to develop type 2 diabetes[[5](#_ENREF_5)].

In spite of decades of research in cultured cells and experimental animals[[6](#_ENREF_6)], the mechanistic details underlying diverse actions of statins *in vivo* are poorly understood. According to the prevailing mechanism of action, statins competitively block HMGCR enzyme that leads to reduced biosynthesis of cholesterol. Statin-induced depletion of cholesterol in the endoplasmic reticulum (ER) activates the sterol response element binding proteins (SREBP-1 and SREBP-2) cleavage and activation protein, SCAP, that chaperones the full-length transcription factor SREBP2 from ER to the Golgi. The nascent SREBP-2 is sequentially cleaved by regulated intra-membrane proteolysis catalyzed by two proteases, named S1P and S2P. The truncated nSREBP-2 then moves into the nucleus to activate transcription of its downstream targets that include the gene encoding the low-density lipoprotein receptor (LDLR). The LDLRs are the primary regulators of cholesterol homeostasis and their increased abundance on plasma membranes is inversely coupled to levels of LDL-cholesterol in circulation[[7](#_ENREF_7),[8](#_ENREF_8)].

This relatively straightforward relationship between statin-induced blockade of HMGCR and reduced concentration of LDL-cholesterol in plasma fails to reconcile a key observation that persistent exposure to statins leads to enhanced expression of HMG Co-A reductase gene and its enzyme, both in cells in culture and in intact animals[[6](#_ENREF_6)]. This behavior of statins is not unusual since targeted inhibition of enzymatic pathways often leads the emergence of adaptive mechanisms to cope with such metabolic blockades. The compensatory up regulation of HMGCR expression in patients in response to statins does not hinder their clinical utility since doses of statins are invariably adjusted to achieve the desired range of plasma cholesterol.

Although the pharmacology of statins has been extensively studied, attempts to decipher whether and to what extent these drugs modulate rates ofcholesterol biosynthesis and its tissue-specific sequestration and excretion *in vivo* have yielded conflicting results. For example, based on measurements of serum concentration of surrogates of cholesterol (*e.g.*, mevalonic acid, squalene, cholestenol, lathosterol and desmosterol), some investigators have claimed that statin therapy was associated with reduced rates of cholesterol biosynthesis[[9](#_ENREF_9),[10](#_ENREF_10)]. Similarly, the results of direct quantification of cholesterol have also yielded apparently conflicting conclusions. Thus, while one study[[11](#_ENREF_11)] reported that rates of cholesterol synthesis remained unaltered in patients taking pravastatin or lovastatin, two other reports claimed that statin treatment led to reduced rates of cholesterol biosynthesis[[12](#_ENREF_12),[13](#_ENREF_13)] and, yet another study reached an exactly opposite conclusion[[14](#_ENREF_14)]. We believe that a clear-cut interpretation of earlier studies has been partially confounded by relative paucity of experiments that were designed to directly measure the rates cholesterol biosynthesis *in vivo*. Additionally, most earlier studies did not rigorously address the relationship between mechanisms involved in the biosynthesis of cholesterol and its inter-organ flux in statin-treated animal or patients. A number of studies have shown the inadequacy of molecular surrogates of cholesterol to accurately reflect the rates of cholesterol synthesis *in vivo* (discussed in detail in the publication of Schonewille *et al*., which is the subject of the current Commentary). Finally, studies to date have not undertaken a sufficiently comprehensive analysis of cholesterol metabolism to specifically reconcile the paradoxical observation that statin therapy was associated with increased expression of HMGCR messenger RNA and protein in the livers of laboratory rodents and patients while concomitantly leading to lower levels of serum cholesterol[[15-21](#_ENREF_15)].

In a recent paper (Schonewille M, Freark de Boer J, Mele L, Wolters H, Bloks VW, Wolters JC, Kuivenhoven JA, Tietge UJ, Brufau G, Groen AK. Statins increase hepatic cholesterol synthesis and stimulate fecal cholesterol elimination in mice. *J Lipid Res* 2016; **57**: 1455-1464), Schonewille *et al*, have attempted to re-examine the mechanistic paradox of how statins affect cholesterol metabolism *in vivo*. To achieve this goal, the authors undertook a comprehensive analysis of putative pathways involved in *de novo* biosynthesis of cholesterol and its elimination in mice that were fed rosuvastatin, atorvastatin or lovastatin, mixed with regular rodent chow. The authors chose these 3 common statins because they have unique pharmacokinetics properties. Thus, lovastatin, a pro-drug that needs to be metabolically activated in the enterohepatic circulation, was given to a group of mice; a parallel cohort of mice received rosuvastatin which is a more hydrophilic and liver-selective statin. The third group of mice were fed chow mixed with atorvastatin. Both atorvastatin and lovastatin are more lipophilic and have longer half-lives than rosuvastatin. After 5 d of statin treatment, rates of cholesterol synthesis, sequestration and elimination were assessed in various tissues of control and statin-treated mice.

The authors used two complementary, stable isotope-labeling techniques to compare the rates of whole body and organ-specific cholesterol synthesis. Using the classic method[[22](#_ENREF_22)], based on the incorporation of deuterium-labeled water (D2O) into newly synthesized lipids, Schonewille *et al,* assessed *de novo* cholesterol synthesis. Since deuterium-enriched body water becomes uniformly distributed in all organs, this technique allows determination of absolute rates of cholesterol synthesis from quantification of deuterium incorporated into lipids. Schonewille *et al,* also studied cholesterol metabolism in mice that drank water spiked with 2% [13C]-acetate. Under these conditions, acetate was transported into the liver via the portal vein which continuously fluxes acetate from the gastrointestinal (GI) tract to the liver[[23](#_ENREF_23)]. As documented earlier, the majority of the exogenously supplied [13C]-acetate enters the liver in an intermittent, yet highly efficient manner and gets assimilated into acetyl-CoA and newly synthesized lipids[[24](#_ENREF_24),[25](#_ENREF_25)]. This technique enabled the authors to calculate fractional rates of cholesterol synthesis in control and statin-treated mice. It is noteworthy that although the two techniques measured different facets of *de novo* lipid synthesis, the profiles of absolute rates of cholesterol synthesis derived from incorporation of H3-enriched drinking water into newly synthesized lipids or fractional rates of hepatic synthesis of cholesterol, as assessed by rates of incorporation of a bolus of exogenous [13C]-acetate, showed similar qualitative and quantitative trends in statin-treated mice that were significantly different from untreated animals.

Based on the kinetics of incorporation of either 13C-acetate or D2O water into lipids, the authors surmised that atorvastatin and lovastatin treatments led to a robust increase in *de novo* cholesterol synthesis in the liver. The authors simultaneously measured changes in organ-specific expression of genes relegated to the biosynthesis of cholesterol and molecular pathways that were putatively involved in the accumulation of cholesterol in various tissues. Concomitant quantification of rates of lipid synthesis and gene expression analyses led to three important insights. Thus, *Schonewille et al.,* observed that (i) treatment with rosuvastatin, atorvastatin and lovastatin was associated with 6-, 15-, and 11-fold greater expression of hepatic HMGCR protein, respectively, (ii) treatment of all 3 statins also led to greater expression of SREBP-2 gene and some of its downstream targets (e.g., mevalonate kinase, phosphomevalonate kinase, farnesyl-diphosphate farnesyltransferase 1 and squalene epoxidase), and (iii) plasma concentrations of 3 cholesterol surrogates (e.g., lathosterol, lanosterol and desmosterol) failed to accurately reflect the rates of cholesterol synthesis in statin-treated mice.

To seek an explanation for the discrepancy between enhanced rates of synthesis of hepatic cholesterol and its lower concentration in the plasma of statin-treated mice, Schonewille *et al,* undertook additional experiments aimed at discovering putative pathways involved in inter-organ flux of cholesterol and its elimination. The authors found that hepatic expression of genes encoding low-density lipoprotein receptor (LDLR) and two ATP-binding cassettes-containing cholesterol transporters, ABCG5 and ABCG8, was greatly enhanced in mice receiving statin treatment. The authors also experimentally probed inter-organ fluxes of cholesterol to determine if excessive cholesterol was removed from the liver and plasma via biliary secretion or via fecal elimination. Since accumulation of cholesterol in the feces represents 3 potential sources i.e., unabsorbed dietary cholesterol, cholesterol secreted from the gall bladder, and trans-intestinal cholesterol elimination (TICE), Schonewille *et al,* sought evidence for all three sources of cholesterol in the feces of statin-treated mice. Based on these analyses, the authors concluded that while statins did not alter intake of dietary cholesterol or its absorption, statin-mediated inhibition of HMGCR was associated with increased cholesterol in the bile acids; with regard to biliary secretion, lovastatin was found to be particularly potent. It was noted however that the metabolic adaptations induced by 3 statins were not identical. For instance, the atorvastatin-treated mice uniquely elicited increased rates of TICE. These data were consistent with the conclusion that treatment with either rosuvastatin and lovastatin was associated with robustly increased rates of secretion of hepatobiliary cholesterol and its fecal elimination, atorvastatin treatment also impinged on the mechanism of TICE. Thus, although enhanced cholesterol catabolism might have partially contributed to increased cholesterol synthesis in atorvastatin-treated mice, apparently, this was not the case in mice treated with rosuvastatin or lovastatin. Whether or not statin treatment is associated with enhanced rates of TICE in humans remains to be experimentally established.

Although the question of how statins lower plasma cholesterol has been studied in many laboratories, the report of Schonewille *et al*, is remarkable with regard to the comprehensive nature of their experiments aimed at answering this question. Thus, the authors not only explored the molecular mechanisms of biosynthesis of cholesterol (stable isotope-labeling of newly synthesized lipids, gene and protein expression) and its inter-organ flux but also the processes of biliary and fecal elimination of cholesterol in statin-treated mice. A key insight of experiments reported by Schonewille *et al*, is that sustained presence of statins *in vivo* leads to significant inter-organ metabolic interactions that profoundly altered cholesterol homeostasis in statin-treated mice. These data also revealed that all statins were not alike with respect to their ability to alter the mechanisms of cholesterol homeostasis.

While the report of Schonewille *et al*, contains novel data that bear directly on the actions of statins on whole body and organ-specific cholesterol homeostasis, it raises a number of key mechanistic questions that need to be addressed in follow-up studies. For example, it is not clear from the data of Schonewille *et al,* if age of the mice or their gender played a significant role in altered cholesterol metabolism in response to statin treatment. In a similar vein, additional investigations are needed to reveal if effects of statins on the biosynthesis and inter-organ flux of cholesterol are dose-dependent. Finally, future studies need to address if the effects of statins on lipoprotein profiles (HDL *vs* LDL) are species-specific, and if so, what are the underlying molecular mechanisms that explain variable responses of rodents and humans to statin treatment. These caveats notwithstanding, the findings of Schonewille *et al*, in mice deserve to be corroborated and extended in other animals, and in patients. Such investigations ought to be launched with an aim to decipher the cellular and molecular mechanisms involved in species-specific differences in statin-induced alterations in the pathways of *de novo* biosynthesis of cholesterol and its inter-organ flux[[21](#_ENREF_21),[26](#_ENREF_26),[27](#_ENREF_27)].

**CONCLUSION**

To sum up, the tantalizing discovery of a novel mechanism of action of statins in mice, reported by Schonewille *et al*, has important implication for patients receiving statin therapy. In my opinion, analogous investigations in patients have generally lacked the experimental rigor and scope of the present study, that have perhaps contributed to apparently conflicting data[[6](#_ENREF_6),[28](#_ENREF_28)]. This situation can only be mitigated by more comprehensive studies aimed at directly measuring the rates of *de novo* cholesterol synthesis, inter-organ cholesterol flux and the processes of sequestration and elimination of neutral sterols in humans taking statins. Accomplishing this mission will be predicated on the development of innovative, noninvasive methods to study the mechanisms of cholesterol homeostasis in humans in greater detail. Such analyses are likely to yield new insights that will enable a more judicious use of statins in millions of patients worldwide.

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