

New mechanisms by which statins lower plasma cholesterol¹

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The use of an enzyme inhibitor *in vivo* to lower a metabolic rate often results in the activation of the targeted enzymatic reaction. The dose of inhibitor is adjusted so that the desired effect is achieved and maintained in spite of the activation of the enzyme. After statins were developed in the 1970s to inhibit HMG-CoA reductase and lower plasma cholesterol, it was soon reported that these drugs induce a strong activation of the reductase (1). This was not a particular concern to clinicians because, in many patients, the dose of statin could be adjusted to keep the plasma concentration of cholesterol in a range that decreased the risk of myocardial infarction. There were occasional reports suggesting that treatment with statins not only increased the activity of HMG-CoA reductase, but also increased the rate of cholesterol synthesis. In this issue of the *Journal of Lipid Research*, Schonewille et al. (2) present a comprehensive study of the influence of three different statins on a number of parameters that regulate cholesterol metabolism in normal mice: expression of genes regulating cholesterol synthesis, mRNA levels, activity of HMG-CoA reductase, absolute and fractional rates of cholesterol synthesis, concentrations of cholesterol precursors in plasma, fecal sterol and bile acids, biliary cholesterol, and fractional absorption of cholesterol by the gut.

The authors used two stable isotopic techniques to trace cholesterol synthesis in mice treated with different statins and in control mice. The first technique involves enriching body water with deuterium by an injection of saline made in ²H₂O, and giving ²H-enriched drinking water to the mice to maintain ²H-enrichment. In the reactions of *de novo* lipogenesis, protons from body water are incorporated into C-H bonds of fatty acids and cholesterol. Because the enrichment of body water is the same in all cells, absolute rates of cholesterol synthesis can be reliably calculated using the labeling of cholesterol and of body water (3). Actually, tracing fatty acid and cholesterol syntheses with ²H-enriched water has been used since the 1930s (4). The authors' data clearly show that chronic treatment of mice with statins markedly stimulates cholesterol synthesis but to a different degree for each compound (4 to 10 times).

The authors used a second technique to label cholesterol synthesized in the liver by adding 2% [¹³C]acetate to the mice's drinking water. A similar protocol had been used by Hellerstein's group (5) in humans who ingested 9 hourly doses of [¹³C]acetate. The tracer is absorbed into the portal vein blood which brings to the liver a constant supply of unlabeled acetate derived from intestinal fermentations. Such protocols induce intermittent waves of ¹³C-labeling of *i*) acetate in the portal vein, and *ii*) lipogenic acetyl-CoA in liver hepatocytes. Because most of the portal vein acetate is taken up in a single pass through the liver (6), very little [¹³C]acetate reaches peripheral tissues. Thus, this technique targets the liver and introduces ¹³C label in the fatty acid and cholesterol synthesis processes of this organ. This *in vivo* mouse protocol is followed by collection of the liver and assay of the mass isotopomer distribution of tissue cholesterol. From the M1 and M3 enrichments of cholesterol (i.e., either one or three ¹³C carbons per molecule), one calculates, not an absolute rate of cholesterol synthesis, but a fractional synthesis (7, 8). There are reservations on the use of [¹³C]acetate to trace fatty acid and cholesterol syntheses (9, 10). However, the protocol, as used by the authors, yields a useful yardstick to compare fractional rates of cholesterol synthesis during parallel pharmacological interventions with different statins. The profiles of *i*) absolute rates of whole body cholesterol synthesis traced with ²H-enriched body water, and *ii*) fractional rates of liver cholesterol synthesis traced with [¹³C]acetate were similarly affected by the different statins tested.

A remarkable finding of this study is that the increase in cholesterol synthesis induced by statins is compensated by a major increase in fecal excretion of cholesterol (up to 5-fold). All statins tested increased biliary cholesterol secretion; however, only atorvastatin also increased the transintestinal cholesterol excretion pathway (TICE) demonstrating that statins differ in their abilities to target fecal sterol excretion pathways to maintain cholesterol homeostasis. ■■

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