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EDITORIAL

Statins Do More Than Lower Cholesterol—Depending on What You Eat?

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Statins (β-hydroxy β-methylglutaryl-coenzyme A [HMG-CoA] reductase inhibitors) belong to the most frequently prescribed classes of drugs worldwide, with rates amounting to 33% of the entire population >60 years of age in Denmark[1] and up to 80% of patients aged 45 to 75 years with established cardiovascular disease in the United States.[2] Statins are competitive inhibitors of HMG-CoA reductase, the rate-limiting enzyme in cholesterol biosynthesis and other isoprenoids (Figure). By reducing the intracellular concentrations of cholesterol in the liver, hepatocytes upregulate low-density lipoprotein (LDL) receptors at their surface with the consequence of lowered plasma LDL cholesterol concentrations. Consistent evidence indicates that these LDL cholesterol–lowering effects of statins account for their proven efficacy in reducing the risk of adverse cardiovascular outcomes and death.

Additional cholesterol-independent (pleiotropic) effects of statins have been reported for some years. In 1996, Vaughan and colleagues proposed that "statins do more than just lower cholesterol,"[3] based on the observation in early intervention trials such as 4S (Scandinavian Simvastatin Survival Study) and WOSCOPS (West of Scotland Coronary Prevention Study) that the clinical benefit of statin therapy emerged earlier than any expected effect on plaque morphology. A possible explanation was provided by cell culture experiments showing inhibitory effects of statins on macrophage inflammatory activity, endothelial cell function, and vascular smooth muscle cell proliferation. The cell culture effects were reversible by supplementation of mevalonate, but not squalene, a precursor of cholesterol, demonstrating HMG-CoA reductase–specific, but cholesterol-independent, effects of statins.[4] Furthermore, other studies noted improvement of nitric oxide–dependent endothelial function in patients treated with statins.[5] Since then, numerous experimental and animal studies have firmly established non–LDL-dependent anti-inflammatory, anti-atherosclerotic, anti-thrombotic, and nitric oxide–promoting effects of statins (reviewed by Oesterle et al[6]).

The cholesterol-independent effects of statins can be explained by the fact that HMG-CoA reductase not only regulates cholesterol synthesis in the liver but also provides all cells of the body with isoprenoids involved in (iso)prenylation of important signaling proteins. Isoprenoids, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate (GGPP), are short chain fatty acids that are post-translationally linked to ubiquitous signaling molecules such as Ras (farnesyl pyrophosphate) and RhoA, Rac, and Cdc42 (cell division control protein 42 homolog) proteins (GGPP). This is believed to be important for membrane anchoring and full function. The stimulatory role of these signaling molecules on cell proliferation, migration, oxidative stress, and actin polymerization and their inhibitory

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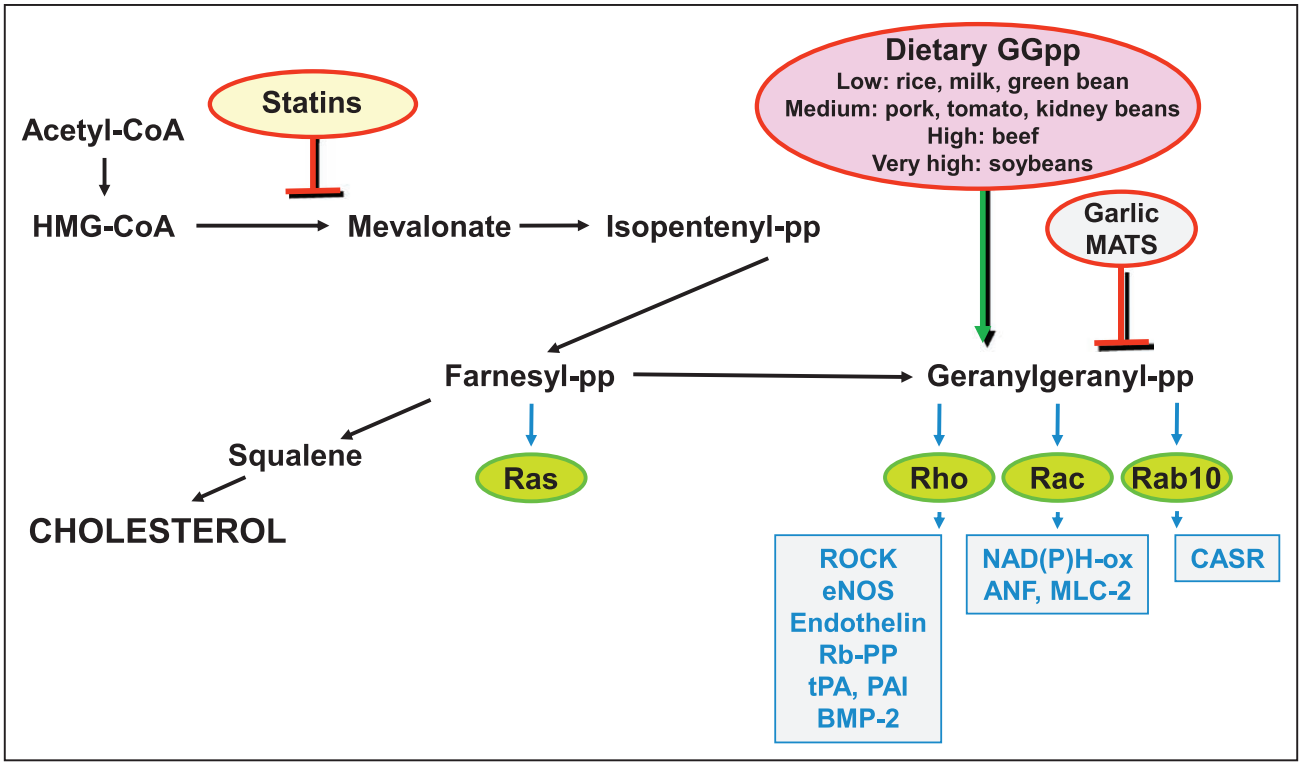

Figure. Simplified scheme of the synthesis pathway of cholesterol and other isoprenoids.
The 3-hydroxy 3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) is the rate-limiting enzyme in the synthesis of mevalonate, which is the precursor of farnesyl pyrophosphate, the branch point of the synthesis of cholesterol (via squalene) on the one hand and geranylgeranyl pyrophosphate (GGPP) on the other. Farnesyl pyrophosphate is used for the prenylation of Ras; GGPP for the prenylation of RhoA, Rac, Rab10, and other signaling nodes. Prenylation is thought to be necessary for membrane anchoring and thereby proper function of the respective signaling pathways. Inhibition of HMG-CoA reductase by statins inhibits both cholesterol and farnesyl pyrophosphate and GGPP synthesis. The latter is believed to be the major mechanism of pleiotropic, cholesterol-independent effects of statins. The study by Zhu et al[13] shows that common foods such as beef and soybeans contain pharmacologically relevant concentrations of GGPP, which is absorbed and can abolish the GGPP-lowering and thus pleiotropic effects of statins. On the other hand, methyl-allylthiosulfinate (MATS), which exists at high concentrations in garlic, competes with GGPP and can thereby restore the GGPP-dependent effects of statins. ANF indicates atrial natriuretic factor; BMP-2, bone morphogenetic protein-2; CASR, Ca^{2}+-sensing receptor; eNOS, endothelial nitric oxide synthase; MLC-2, myosin light chain 2; NAD(P)H-ox, nicotinamide adenine dinucleotide phosphate oxidase; PAI, plasminogen activator inhibitor; ROCK, Rho associated coiled-coil containing protein kinase; and tPA, tissue plasminogen activator.

effect on the endothelial nitric oxide synthase explain the salutary effects of statin-mediated inhibition of their isoprenylation.[6,7]

This ubiquitous mechanism was the reason to test statins in a wide range of clinical indications, and, indeed, evidence accumulated for clinical benefits of statin therapy in patients with vascular disease (eg, kidney disease, venous thrombosis, erectile dysfunction), autoimmune disease (eg, multiple sclerosis, rheumatoid arthritis), and connective tissue diseases (eg, osteoporosis, periodontal disease; reviewed by Oesterle et al[6]). However, the evidence for relevant cholesterol-independent effects on human diseases is overall inconsistent and has not led to guideline recommendations for the use of statins in noncardiovascular indications. Moreover, larger trials did not confirm cholesterol-independent benefits of statin therapy (for example, in postoperative atrial fibrillation[8] or heart failure[9]), and the quantitative comparison of lipid-lowering and anti-inflammatory effects (surrogate C-reactive protein) in large trials such as JUPITER (Justification for the Use of Statin in Prevention: An Intervention Trial Evaluating Rosuvastatin), HOPE-3 (Heart Outcomes Prevention Evaluation), and A to Z (Early Intensive vs a Delayed Conservative Simvastatin Strategy in Patients With Acute Coronary Syndromes Phase Z of the A to Z Trial) did not reveal consistent evidence for cardiovascular benefit of statins exceeding that of cholesterol lowering.[6]

The clinical relevance of cholesterol-independent effects of statins remains difficult to define for 2 main reasons. By virtue of their mechanism of action, statins always inhibit both cholesterol and isoprenoid synthesis. Moreover, clinical studies in the cardiovascular space always test novel lipid-lowering therapies, such as ezetimibe, PCSK9 inhibitors, and bempedoic acid, on the background of statin therapy. Thus, the best evidence for an LDL-independent benefit of statins would be expected from head-to-head comparisons of a drug expected to only lower LDL cholesterol with a statin. Although large trials are lacking, a small clinical study in patients with heart failure found that simvastatin improved radial flow–dependent blood flow whereas ezetimibe did not.[10] Others reported larger effects of high-dose statins versus a combination of

a statin with ezetimibe,[11] but again this could not be confirmed in other studies with similar design.[12] Thus, the quantitative role of pleiotropic effects in humans remains unresolved.

In this issue of *Circulation*, Zhu et al[13] present a novel perspective on the story of pleiotropic effects of statins. They start with the notion that GGPP is derived from mevalonate in almost all living organisms but that an alternative pathway exists in plants. This raised the possibility of relevant differences in GGPP concentration in diets. Indeed, chromatographic assessment of GGPP concentrations showed that GGPP was undetectable in flour, lettuce, broccoli, and potatoes, and its concentration was low in rice, milk, and green beans (6 to 16 ng/g); medium in pork, tomato, and kidney beans; high in beef (≈70 ng/g); and very high in soybeans (≈130 ng/g). These differences translated well in terms of differential effects on GGPP plasma concentrations (from baseline of 50 ng/mL to ≈200 ng/mL) in rats that were fed with the respective food supplements at 2 g/100 g body weight.

Given their interest in potential therapeutic efficacy of statins in pulmonary arterial hypertension (PAH), the authors then focused on the effects of diet on pulmonary circulation. Whereas the different GGPP diets did not affect baseline hemodynamics or the pulmonary vascular response to chronic hypoxia (4 weeks at 10% oxygen), a high GGPP-containing diet (as well as direct supplementation with GGPP) abolished the beneficial effects of simvastatin treatment (10 mg/kg body weight) both in the hypoxia and a monocrotaline model of PAH. Further elegant work both in rats in vivo and in cell culture showed that simvastatin inhibited RhoA and its downstream target ROCK (Rho associated coiled-coil containing protein kinase), the Ras-related protein Rab10 involved in membrane trafficking, and Ca^{2+}-sensing receptor, previously shown to be involved in PAH (Figure).[14] All effects of simvastatin were prevented by dietary supplementation with GGPP (2 μg/kg body weight). Conversely, the effects of GGPP (diet) were reversed by concomitant oral administration of garlic extracts containing high concentrations of methyl-allylthiosulfinate or methyl-allylthiosulfinate directly (0.5 mg/kg body weight), a competitive inhibitor of GGPP, for its use by geranylgeranyl transferases. Furthermore, the authors generated genetically modified rats in which 2 Rab10 interaction sites on Ca^{2+}-sensing receptor were mutated and showed that the rats were partially protected from PAH under hypoxia, substantiating an important role of Ca^{2+}-sensing receptor. Possible translation to humans was investigated in a small prospective, blinded clinical trial in volunteers. It showed that a high-GGPP diet (beef, soybeans; ≈1.2 μg per meal) increased GGPP plasma concentrations compared with a standard Chinese diet (0.3 μg/meal) by a factor of 1.5 to 2 and that simvastatin lowered GGPP plasma concentrations and decreased RhoA activity in blood monocytes (under hypoxia). As shown before in rats, all statin effects were abolished by beef or soybean diet and were restored by concomitant intake of high–methyl-allylthiosulfinate garlic extract.

This elegant, data-rich study provides a number of important novel insights. In retrospect, it is almost surprising that no one has ever looked at the dietary content of GGPP with regard to statin therapy. An earlier study reported similar or higher GGPP levels in polished rice (0 to ≈1000 ng/g)[15] but linked this observation to possible anticancer actions of GGPP. The proposed mechanism of action of statins in the animal model of PAH and their antagonism by GGPP are well in line with former work and significantly extend it, for example, in terms of the role of Rab10 and Ca^{2+}-sensing receptor. Strengths of the present study include the careful GGPP analytic procedures, the strength of mechanistic insights including several loss-of-function approaches in vitro and in vivo, and the pharmacologic consistency with similar plasma concentrations in rats and humans and realistic effects of statins and diet/GGPP, respectively.

Of course, open questions remain. The present therapeutic effects in rats with PAH cannot be directly translated to patients. As clearly stated by the authors, statins failed to show consistent therapeutic efficacy in patients with PAH. In fact, the inconsistencies of the clinical data were one reason for the study. The dose of simvastatin in rats was much higher than maximal therapeutic doses in patients (10 mg/kg versus maximally 1 mg/kg in patients). However, even the low 20-mg dose of simvastatin affected GGPP and RhoA activity in the human study, indicating that statins in standard dose are able to change GGPP plasma concentration. The very low systemic bioavailability of statins (≈5% for simvastatin) suggests that inhibition of hepatic HMG-CoA reductase reduces serum concentrations of isoprenoids, but this requires further analysis. The study does not answer whether dietary GGPP affects statin efficacy in the prevention of cardiovascular events and other diseases.

The study by Zhu et al[13] convincingly demonstrates that different diets contain widely varying levels of GGPP and that dietary GGPP affects plasma GGPP concentrations and modifies pleiotropic effects of statins in animal models of PAH and human volunteers (RhoA activity). The data raise the provocative question of whether populations in which the typical diet contains high amounts of soybeans or beef benefit less from statins. The study should be considered hypothesis-generating and stimulate retrospective analyses of clinical registries and the existing large intervention trials to validate or refute this hypothesis. Whatever the outcome, the study is a fitting example of thorough

scientific underpinning of the widely held maxim that we are what we eat.

ARTICLE INFORMATION

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Disclosures

None.

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