AMERICAN Scientist

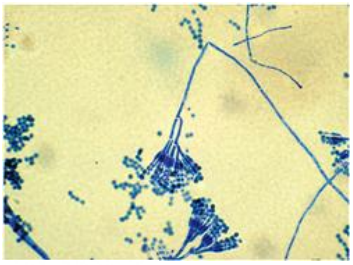
FEATURE ARTICLE

Statins: From Fungus to Pharma

The curiosity of biochemists, mixed with some obvious economic incentives, created a family of powerful cardiovascular drugs

Philip A. Rea

In 1966, Akira Endo, a young Japanese biochemist, started an adventure that would ultimately save thousands, if not millions, of lives. Only 33 years old at the time, Endo was a research scientist at Sankyo—a pharmaceutical company, later known as Daiichi Sankyo, in Tokyo—where he was looking for enzymes in fungal extracts for improving the quality of certain foodstuffs. But his research was soon to enter a new realm. As he would write years later: "In the mid-1960s, fascinated by several excellent reviews on cholesterol biosynthesis by Konrad Bloch of Harvard University, who received the Nobel Prize in 1964, I became interested in the biochemistry of cholesterol and other lipids." Endo's curiosity triggered research that eventually spawned one of today's most widely used families of drugs.



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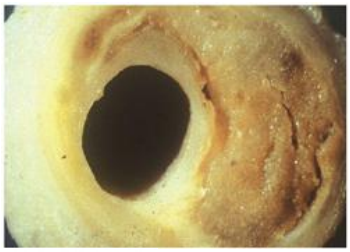
Born in 1933 on a farm in northern Japan, Endo became intrigued by mushrooms as a child. He most admired Alexander Fleming's famous work on fungi, which ultimately led to the development of penicillin. Even into adulthood, Endo remained interested in fungi and how they could be used. It was at Sankyo, by screening more than 200 fungal species, that he was able to identify enzymes capable of decreasing the pulp in fruit juices.

Endo's research, however, turned to bigger things when he took a two-year leave of absence from Sankyo to work at Albert Einstein College of Medicine in New York City. There, he entered the world-renowned biochemistry laboratory of Bernard L. Horecker and met Lawrence I. Rothfield, who is now a professor of microbiology at the University of Connecticut Health Center. Endo learned from Rothfield—who had been a physician at New York University Hospital for more than 10 years before joining Albert Einstein College of Medicine—that high blood cholesterol poses a major risk for cardiovascular disease.

As we shall see, Endo's two interests—fungi and cholesterol—merged and spurred the discovery and development of a group of cholesterol-lowering drugs called statins. The number of deaths from cardiovascular diseases has decreased by about 25 percent in the United States since 1994, not because of a radical change in lifestyle—though this is happening—but because of the ready availability of cardioprotective drugs. Of the handful of drugs out there that have fought cardiovascular diseases, statins are right at the top of the list.

Clearing the Way

According to the World Health Organization, cardiovascular diseases are the leading cause of death. In 2005, for example, about 17.5 million people died from these diseases, accounting for about 30 percent of global mortality.



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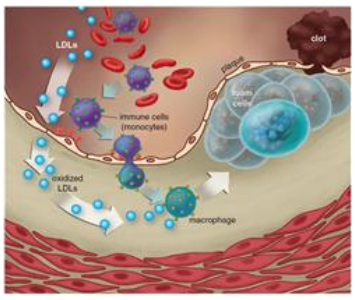
Just a few years ago, general practitioners, and even many cardiologists, would have labeled cardiovascular disease as a straightforward plumbing problem: Fat-laden gunk on the surface of artery walls blocks the flow of blood. If the tissue downstream of the blockage is cardiac muscle, a heart attack results; if it's brain tissue, a stroke ensues. We now know that there is a lot more to it than this.

The modern understanding of cardiovascular disease started to emerge in 1961, when the first reports from the Framingham Heart Study were published. This project examined 5,209 men and women, ages 30-62, who lived in Framingham, Massachusetts, a small, predominantly middle-class town just outside of Boston. The results revealed that high blood pressure, smoking and high levels of blood cholesterol are all bad for your heart. In particular, this study showed that there is a tight correlation between blood-cholesterol levels and the likelihood of later developing cardiovascular disease.

Pivotal as these findings were, they were only the prologue to a story that was to prove more complex.

First, cholesterol is not all bad news. This lipid makes up a crucial component of biological membranes and serves as a precursor for other necessary substances, including the sex hormones estrogen and testosterone. Indeed, because of its necessity cholesterol does not come exclusively from dietary sources but is also manufactured by the liver and to a lesser extent by a few other tissues, including the intestine.



Second, it is not cholesterol in general that is the problem, but rather the form it is in that matters. Atherosclerosis ("hardening of the arteries") arises from the low-density lipoprotein (LDL) form of cholesterol. These LDLs—globules of about 20 nanometers or so across—encapsulate cholesterol derivatives called cholesteryl esters.

When the bloodstream contains a surplus of LDLs, they enter the innermost layer of cells of the arterial wall and accumulate. Eventually, these lipids oxidize, which triggers metabolic and structural changes in the arterial wall, not unlike those elicited by infection from a pathogen. The immune system identifies these changes as damage, driving the formation of capped plaques replete with fat-engorged white blood cells. It is when these plaques are disrupted that trouble arises: Blood leaks through the fissure into the lipid-rich core of the structure to make contact with proteins that promote coagulation, resulting in clots. That is the downside.

The upside of cholesterol comes from the high-density lipoprotein (HDL) form, which, unlike its LDL counterpart, is cardioprotective. HDLs—globules only 8-11 nanometers across—pick up cholesterol from the blood and prevent or impede plaque progression by retrieving arterial cholesterol deposits and limiting the rate and extent of LDL oxidation. Higher levels of HDLs thereby reduce the risk of cardiovascular disease. Of

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course, that is not to say that there can never be too much of a good thing: Some studies indicate that very high levels of HDLs also increase the risk of cardiovascular diseases.

Third, cholesterol tightly regulates its own production. A seminal finding in the science of cholesterol came in 1966 when Marvin D. Siperstein and Violet M. Fagan—both then at the University of Texas Southwestern Medical School—showed how the body controls cholesterol levels. These investigators discovered that the enzyme that converts a substance named HMG-CoA to mevalonic acid, the immediate precursor of cholesterol, is inhibited by cholesterol. By feedback inhibiting the pacemaker enzyme that catalyzes the first committed and rate-limiting step in the pathway, cholesterol downregulates its own synthesis.

A major culprit in heart disease—cholesterol—and a potential therapeutic target—the enzyme HMG-CoA reductase—had been discovered.

Blue Cheese's Cousin

When Endo returned to Sankyo in 1968, he was to bring together his lifelong passion for mycology and his newfound interests in lipid metabolism. He set out to explore whether inhibiting HMG-CoA reductase could decrease blood cholesterol levels. Although other researchers had the same thing in mind, Endo took a fungal angle. He speculated that there must be at least a few fungal species capable of elaborating compounds—niche-carving antimetabolites—that target HMG-CoA reductase to do battle with fungal competitors that require cholesterol-like compounds for survival.

By 1971, Endo and his Sankyo colleague Masao Kuroda had started their search for fungal compounds that interfered with cholesterol production—via HMG-CoA reductase—in rat-liver extracts. After two years spent painstakingly screening 6,000 microbial strains, Endo and Kuroda at last found two promising cultures. The first came from *Pythium ultimum*. It inhibited HMG-CoA reductase and decreased cholesterol levels in rats, but it was eventually shown to be extremely toxic to the liver.

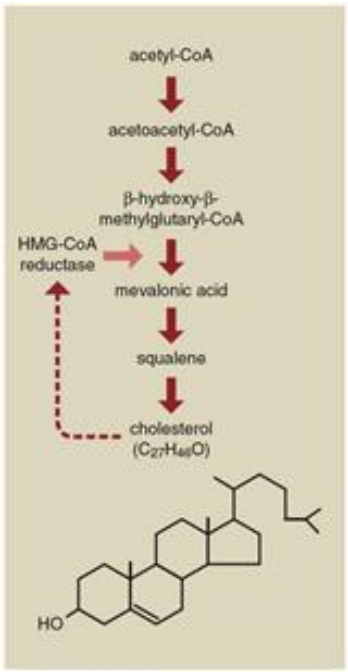
The second, a true hit this time, came from *Penicillium citrinum*, a relative of the organism responsible for the blue in blue cheese and the fungal mats that grow on old oranges—surely a thrilling result if only because of Endo's admiration for Fleming's exploits with this genus more than 40 years previously. By purifying active compounds from 2,900 liters of filtered liquid drawn from *P. citrinum* cultures, Endo and Kuroda isolated compound ML-236B. This is the compound that became known as mevastatin, signifying a substance that stops (where "stat" suggests static, or not changing) mevalonic acid synthesis. Mevastatin is a structural analogue of HMG-CoA: It is able to dock onto the enzyme HMG-CoA reductase and obstruct HMG-CoA binding, thus preventing its conversion into mevalonic acid for the synthesis of cholesterol.

With such a potentially promising inhibitor in hand, Sankyo faced two make-or-break questions: Does mevastatin do what it should *in vivo*, and if so is it free of deleterious side effects? Endo started exploring these questions in depth with rats. Much to his dismay, he found that mevastatin was effective only in the short term. Over longer trials, even at relatively high doses, it produced no consistent effect. That was very bad news—news that could have easily brought work on this compound as a cholesterol-lowering drug to an abrupt end.

By chance, though, one of Endo's colleagues offered some hens for testing. Given the high levels of cholesterol in chicken eggs, these birds seemed perfect for studying this strategy for cholesterol reduction. So Endo and his colleagues fed egg-laying hens with commercial chicken feed supplemented with mevastatin and then measured their blood cholesterol levels. It worked—decreasing cholesterol by as much as 50 percent, while leaving body weight, food intake and egg production unaffected.

Cholesterol's Complexities

Before further examining the history of the statins, it is instructive to consider a perplexing question, or at least a question that is perplexing with the benefit of hindsight. That is, if statins diminish all types of cholesterol, why do they reduce the risk of cardiovascular diseases? Surely, a block on cholesterol production should decrease both the good and the bad, the HDLs as well as the LDLs. Well, the short answer to this question is: Luckily, these drugs are more selective than could have been anticipated when they were first discovered.



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The empirical results speak for themselves. Treatment with statins does appreciably decrease LDLs, as expected. But, in addition, statins increase HDLs, and by more than 7.5 percent, according to some studies.

The liver is the hub when it comes to LDLs. When the production of cholesterol in liver cells is diminished by the inhibition of HMG-CoA reductase, fewer LDLs enter the circulation. And because the liver cells have fewer LDLs entering to contribute to the cholesterol pool, they generate more LDL receptors on their surfaces to grab more of this substance from the blood. The combination of producing less cholesterol in general—including the LDL fraction—and pulling more LDLs from the blood into liver cells serves to deplete circulating levels of LDL cholesterol. All other things being equal, high numbers of LDL receptors in liver cells equate with low levels of LDLs in the blood.

All well and good, but if a statin decreases the overall production of cholesterol, shouldn't circulatory HDL levels also decrease? Logically they should, but fortunately they don't. Instead, circulatory HDLs increase, making statins even more cardioprotective than they might otherwise be.

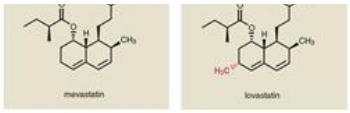
Currently, there is no consensus on just how statins increase blood HDL levels. Some scientists suspect that statins inhibit the transfer protein responsible for unloading the cholesteryl ester cargo of HDLs. A variety of experiments on animals and humans show that blocking the cholesteryl ester transfer protein triggers increases in the levels of HDLs. Another possibility is that statins stimulate the expression of HDL transport proteins, which in turn ferry this form of cholesterol from the liver to the blood.

It is intriguing to consider that the mechanisms that nearly stalled Endo's first screens of mevastatin, because they were done on rats, are the very mechanisms that make these drugs so effective therapeutically in humans. Rats are an exception because their steady-state blood levels of LDLs are low; most of their blood cholesterol is in HDLs. What this means is that even if statins decreased blood LDL levels enough to be noticeable in the short term in rats, any long-term effects at the level of total blood cholesterol would be offset by a subsequent increase in HDLs. As Endo's work with chickens and subsequently other animals (including humans and other primates) was to show, a lowering of total blood cholesterol is typically seen because LDL cholesterol ordinarily represents a sizeable fraction of the total—a much larger fraction than in rats and other rodents. And to think that egg-laying hens were to pave the way for the use of statins in humans.

Rumors of Tumors

In April 1976, Boyd Woodruff, then executive administrator of Merck, heard through the pharmaceutical network that Sankyo had a patent application covering mevastatin, and he inquired about obtaining a sample for evaluation under a confidentiality agreement. Sankyo assented and provided samples of the compound

together with a report on its properties.



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During the next couple of years, Merck studied mevastatin in cultured mammalian cells and in rats and dogs. By October 1978, they had obtained results that largely confirmed those of Endo and colleagues. Impressed by its efficacy, Alfred W. Alberts, a lipidologist at Merck, initiated fungal-culture screens to find another statin, one that Merck could call its own. In a matter of only two weeks, Alberts and colleagues found one. Sample number 18—derived from the common soil fungus *Aspergillus terreus*—contained lovastatin, which is structurally identical to mevastatin except for a single methyl group. Better still, at least for Merck, lovastatin proved every bit as effective as mevastatin.

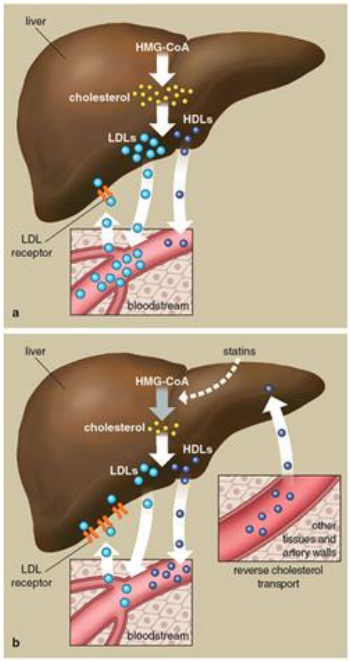
By then, Sankyo was running clinical trials on mevastatin, but it hit a deadly snag. Rumor—and that is all that we have to this day—had it that some of the dogs treated with mevastatin developed intestinal tumors. So in the fall of 1980, Sankyo halted its trials.

Knowing that only a methyl group distinguished lovastatin from mevastatin, Merck, too, immediately halted its testing on people, recalling all outstanding samples from clinical investigators and notifying the U.S. Food and Drug Administration (FDA). Although only a few patients had received lovastatin, and only at low doses, Merck still advised the physicians who were conducting the trials to check very carefully for signs of cancer in their subjects.

Merck then set out to determine the exact nature and extent of the problems that Sankyo had seemingly encountered. Failing to track down any solid evidence of harm, P. Roy Vagelos, then president of Merck Research Laboratories, and Barry Cohen, Merck's head of international business, flew to Japan. As he was to relate years later in his 2004 autobiography (coauthored with historian Louis Galambos of Johns Hopkins University), Vagelos took aim at getting to the bottom of the problem by making a business proposition to Sankyo: "If you help us solve this problem, we'll share Mevacor [trade name for lovastatin] with you in Japan, and you can share your second-generation product with us when you're ready." Sankyo executives said that they wanted to cooperate, but couldn't. Apparently, their bosses at Sankyo saw it as bad for business.

Merck Moves Ahead

Knowing Sankyo's concerns over the tumors that mevastatin allegedly induced, and given the general lack of cooperation that they encountered in Japan, Merck executives didn't know what to do with lovastatin. Their ultimate decision proved to be a wise one. Instead of pursuing a drug to treat the general population, they focused their efforts on the small fraction with a condition called heterozygous familial hypercholesterolemia, which afflicts approximately 1 in 500 people. In this genetic disease, a person carries two different copies of a gene, hence the term heterozygous. One copy works normally, and one does not. The possession of one aberrant copy of the gene in this autosomal-dominant disease is sufficient to cause problems because of a deficiency in the cellular receptors responsible for the removal of circulatory LDLs. As a result, people with this disease have a severe elevation of LDLs, and they typically develop cardiovascular disease between the ages of 30 and 40 if left untreated.



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Since Sankyo had already used mevastatin on similar patients with some favorable results, the case was compelling: After all, people with hypercholesterolemia faced a great and certain danger, whereas the cancer potential of mevastatin remained only speculation. For this reason, many of the clinicians involved in the early trials of lovastatin urged Merck to continue. Merck, therefore, did what had to be done: It presented all of its data to the FDA and sought approval for further studies on patients with this condition.

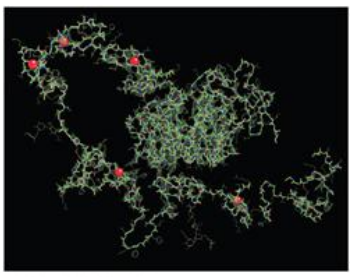
The FDA consented, and Merck's clinical trials on lovastatin resumed in 1982. The early results suggested that this compound dramatically reduces LDL levels with few side effects. The company continued its work, moving through the drug-development process. By 1987, the FDA approved Mevacor (alias lovastatin) for use in patients whose cholesterol levels could not be adequately controlled by diet or by using other drugs, such as inhibitors of the intestinal resorption of cholesterol derivatives or absorption of the parent compound from dietary sources.

Despite the FDA's approval, Merck still faced a fundamental question: Although it was clear that statins decreased the level of blood LDL-cholesterol, did these drugs necessarily protect against cardiovascular disease? To find out, Merck sponsored the Scandinavian Simvastatin Survival Study (the "4S study" for short), which was completed in 1994. In a group of patients diagnosed with "moderate" hypercholesterolemia (defined at the time as a total blood cholesterol level of 200-300 milligrams per deciliter), 2,223 patients received a placebo, and 2,221 took simvastatin, a second-generation statin from Merck produced by the synthetic modification of lovastatin. The results of this study, a milestone in cardiology and evidence-based medicine, were conclusive. The group of patients taking simvastatin not only showed statistically significant decreases in total blood cholesterol and LDL cholesterol of 25 and 35 percent, respectively, but also a 42 percent decrease in death rate.

These stunning results and the successful trials with Mevacor drew other pharmaceutical companies into the statin market. Sankyo, for instance, eventually teamed up with Bristol-Myers Squibb to distribute and sell a derivative of mevastatin with increased efficacy, pravastatin (marketed as Pravachol).

Improving on Nature

Although Sankyo and Merck both originally isolated valuable statins from natural sources, their later efforts were directed at manufacturing them synthetically. In the early 1980s, Merck filed a patent that showed that some of the complexities of the fungal metabolites were superfluous and could be eliminated without compromising biological activity.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At about this time, five years before the FDA approved Mevacor, Bruce D. Roth, then a 28-year-old postdoc in the chemistry department at the University of Rochester, started tinkering with statins, exploring their synthetic chemistry. In the spring of 1985, he succeeded in synthesizing one of the fungal statins that Endo and Kuroda had isolated a few years before.

Within only two years, Roth found himself heading up an 18-scientist atherosclerosis group at Parke-Davis Pharmaceutical Research. And while they made remarkable progress in fabricating their first synthetic HMG-CoA-reductase inhibitor, this was scuttled when they were notified that Sandoz AG, a Swiss drug company that is now part of Novartis, had obtained a patent for the very same compound. Parke-Davis was left with no other option but to turn its efforts toward what started out as a second-tier drug called atorvastatin—now known better by its trade name, Lipitor.

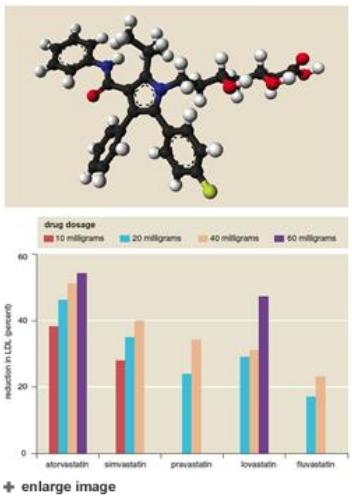
By the time Parke-Davis had Lipitor ready to present to the FDA, it was late 1989, and three statins—Mevacor, Zocor and Pravachol—were already approved

for sale. Did this mean that the market was already saturated? According to Ron Winslow, later writing in the *Wall Street Journal*, the decision Parke-Davis made arose from economic rather than scientific considerations. First, the patent for Parke-Davis's Lopic—a treatment for high serum-triglyceride levels, which was also the company's most-successful drug with annual sales of about $600 million—was about to expire. Second, the statin market looked likely to grow into one of the biggest pharmaceutical boons of all time. In short, if Parke-Davis could capture even a small piece of the action, Lipitor could become the company's best seller.

These considerations were to be the tipping point. Parke-Davis moved the compound into clinical trials to show that doses of Lipitor of 10 milligrams and 80 milligrams per day decreased LDL-cholesterol levels by 40 and 60 percent, respectively. These were decreases in LDL-cholesterol levels that beat every other available statin—at any dose—by at least 40 percent.

Needs for Proceeds

With the niches for statin drugs quickly filling up, Parke-Davis wanted to get Lipitor to customers as soon as possible. To do that, the company needed to convince the FDA that Lipitor should be put on the fast track for approval, a privilege reserved for products that address an "unmet medical need."



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To find that need, Parke-Davis used a version of Merck's earlier technique. Parke-Davis contacted two South African doctors who were treating a group of young patients with the homozygous form of familial hypercholesterolemia. This is where an individual has not just one but two copies of the disease-causing gene. Characterized by cholesterol levels of 600 milligrams per deciliter or more, children with this condition typically suffer a heart attack or undergo bypass surgery before or soon after entering their teens. Because these doctors had previously tried Merck's Zocor (trade name for simvastatin) with little effect, these children faced a grim prognosis. With very little to lose they agreed to try Lipitor.

The blood cholesterol levels of the children in the trial started to come down within a month. One child with an untreated blood cholesterol level of 1,100 milligrams per deciliter achieved a new steady state of 700. Others with somewhat lower untreated levels achieved 300-400 milligrams per deciliter. Having at least gone some way toward meeting this unmet medical need, Lipitor received a priority review from the FDA.

To get a similarly fast and favorable "review" from customers, Parke-Davis made an equally strategic marketing decision: They elected to co-market Lipitor with Pfizer. At the time, 1996, Pfizer was the fifth most-profitable pharmaceutical company, renowned for its acumen in sales and marketing, but also a company that lacked a statin of its own.

Disadvantages would seem to be inevitable when entering a market late in the game, but if there is an advantage to be had it is that the latecomer has ready access to the competitor's products to test against their own. To set off Lipitor from other statins, Parke-Davis and Pfizer therefore commissioned a head-to-head trial—called the CURVES study—of comparative-dose efficacies of Lipitor (atorvastatin) against four other statins in patients with hypercholesterolemia. Lipitor won hands down. For example, at a dose of 10 milligrams per day, it lowered LDL cholesterol by 38 percent, which was significantly better than what patients experienced with equivalent doses of fluvastatin (17 percent), lovastatin (29 percent), pravastatin (24 percent) and simvastatin (28 percent).

In January 1997, when the FDA approved Lipitor, it went so far as to allow Parke-Davis and Pfizer to include data from the CURVES study in every package of the drug sold. Lipitor captured 18 percent of the statin market over the next 18 months, in large part through Pfizer's sales prowess—making it second only to Merck's Zocor, which had 37 percent. In 2000, Pfizer purchased Warner-Lambert—the parent company of Parke-Davis—and became the sole owner of Lipitor. By 2004, annual sales of Lipitor surpassed $12 billion, making it the world's best-selling drug by a wide margin.

In one case, though, even Congress believed that Pfizer's marketing of Lipitor went too far. In January 2006, Pfizer launched a Lipitor-advertising campaign centered on Robert Jarvik—known for developing the Jarvik artificial heart. Under Congressional criticism, Pfizer pulled this $258 million advertising campaign early in 2008. As Stephanie Saul wrote in the February 25, 2008, issue of *The New York Times*: "[T]he campaign had come under scrutiny from a Congressional committee that is examining consumer drug advertising and has asked whether the ads misrepresented Dr. Jarvik and his credentials. Although he has a medical degree, Dr. Jarvik is not a cardiologist and is not licensed to practice medicine." Saul went on to note: "One television ad depicted Dr. Jarvik as an accomplished rower gliding across a mountain lake, but the ad used a body double for the doctor, who apparently does not row."

As of March 2010 Lipitor's patent is due to expire, at which point it will face competition from generic versions. In this eventuality, Pfizer will need more than sheer marketing power to replace Lipitor's revenue stream.

Adding Applications

Akira Endo's decades-old adventure continues. In the mid-1990s, the U.K. Heart Protection Study—sponsored by the U.K. Medical Research Council, the British Heart Foundation, Merck and Hoffman-La Roche—examined 20,000 volunteers who were 40 to 80 years old. These subjects were considered at high risk of cardiovascular disease because of factors such as diabetes, but their physicians did not consider them candidates for statin therapy because their blood LDL-cholesterol levels were within the normal range. Nonetheless, this study put these people on either 40 milligrams per day of simvastatin or a placebo for an average of 5.5 years. The results were impressive. In those individuals with diabetes but no obvious arterial disease who were put on simvastatin, the risk of a heart attack or stroke decreased by about 20 percent. Rory Collins, who together with Richard Peto, headed this study, estimates that up to 20 million people worldwide would be eligible for statin therapy. On TheHeart.org (www.theheart.org), Collins noted that "even if an extra 10 million people took them, we would save 50,000 lives a year" and prevent untold numbers of debilitating heart attacks and strokes.

Other work shows the potential for still broader applications of statins. In April 2008, for example, Beatrice A. Golomb, a professor of medicine at the San Diego School of Medicine and director of the University of California, San Diego's statin study, and colleagues reported that statins lower blood pressure. Pending their confirmation or otherwise, these findings point to further expansion of the types of patients who stand to gain from being prescribed statins. Stroke prevention, not just of the occlusive but also of the hemorrhagic variety, is an obvious target in that statins may alleviate not just one but both of the main predisposing factors, atherosclerosis and high blood pressure.

The applications of statins might not stop here, however. Some studies suggest that these drugs can also help prevent Alzheimer's dementia, age-related bone loss and even prostate cancer. As is only becoming obvious now, one young man's curiosity about fungi is helping to fight a growing list of debilitating and life-threatening diseases. Stated plainly, the discovery of statins and the new insights into cardiovascular and other diseases that have and continue to come from their implementation represents one of the most significant accomplishments of the biomedical sciences in the 20th century.

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