

Brown and Goldstein: The Cholesterol Chronicles

Prashant Nair
Science Writer

When his parents took three-year-old John Despota to his primary care physician in Chicago in 1964, orange-tinted fat-laden bumps lined the skin on the back of his lower legs. Eruptions caused by excess cholesterol, the bumps spread across Despota's body, mirroring a total blood cholesterol level that hovered above six-times the normal range. By age 12, Despota experienced shortness of breath upon slight exertions, frequent fatigue, and chest pain: cardinal signs of a cardiac illness called angina, caused by a short supply of oxygen-rich blood to the heart muscles. "Compared with normal kids, I would get tired too soon playing baseball and hockey," recalls Despota.

Under cardiologist Neil Stone's care at a lipid clinic at the now-renamed Lurie Children's Hospital of Chicago, Despota received a diagnosis of familial hypercholesterolemia (FH), a metabolic disorder marked by high blood levels of cholesterol, artery-clogging plaques, and premature heart attacks. "When John first came to the clinic, his local cardiologist had given him a year to live," says

Stone. At 51, Despota leads a quiet life of early retirement in Tarpon Springs, a coastal town near Florida's Tampa Bay, his ailment held in abeyance by an array of drugs and medical procedures.

Described in the early 1960s as a disease that manifests in one of two genetic forms, FH is a disorder of cholesterol metabolism. Its less severe, heterozygous form—so-named because patients have one abnormal copy of a culprit gene—afflicts 1 in 500 people. Heterozygous patients have twice the normal blood levels of a particle called low-density lipoprotein, or LDL, which carries cholesterol in blood. The patients' prognosis is sobering: heart attacks strike as early as age 35. In contrast, the homozygous form, caused when both copies of the gene are defective, ails one in a million people and can send LDL levels soaring six-times above normal, potentially leading to heart attacks by the age of five years in patients without other risk factors commonly tied to heart disease.

To all appearances, Despota's condition resembled the rare but more serious homozygous

affliction. At a time when the now-ubiquitous cholesterol-lowering drugs called statins were yet to be discovered, his treatment over the next several years included a low-fat diet, cholestyramine (a drug that shunts excess cholesterol toward bile acid production), and nicotinic acid, which helps lower triglyceride fats. Because Despota was largely unresponsive to the drugs, Stone prescribed hour-long dialysis-like procedures that helped mechanically exchange his blood plasma or remove LDL from his blood every two weeks. "I never felt a 100% fine like normal adults, but after the procedures I didn't have chest pains and could perform normal activities," says Despota.

Coronary heart disease was no stranger to Despota's family; his maternal grandmother had endured two open-heart surgeries, his maternal uncle had lived for two decades on mammary artery implants, and both his parents had suffered cardiovascular symptoms. Given this grim history, Stone reasoned that a straightforward approach to unraveling Despota's biological destiny might be to perform a genetic analysis of his cells. At the very least, the analysis might uncover mechanisms of cholesterol regulation in human cells. So Stone ordered skin biopsies from Despota and his parents and sent the cells to a pair of young researchers who had carved a niche in cholesterol biology at the University of Texas Southwestern Medical School in Dallas, TX.

The Brown-Goldstein Complex

When Despota's cells arrived in Texas, Michael Brown and Joseph Goldstein, now feted in biochemists' circles, had made substantial inroads into the biology of cholesterol metabolism in human cells. Beginning in the early 1970s, the pair had uncovered findings that helped pinpoint the precise biochemical defect in FH patients. Drawn to each other by their mutual interest in metabolic disease during postdoctoral apprenticeships at the National Heart Lung and Blood Institute in Bethesda, MD, where they first encountered patients with FH, Brown and Goldstein published a series of

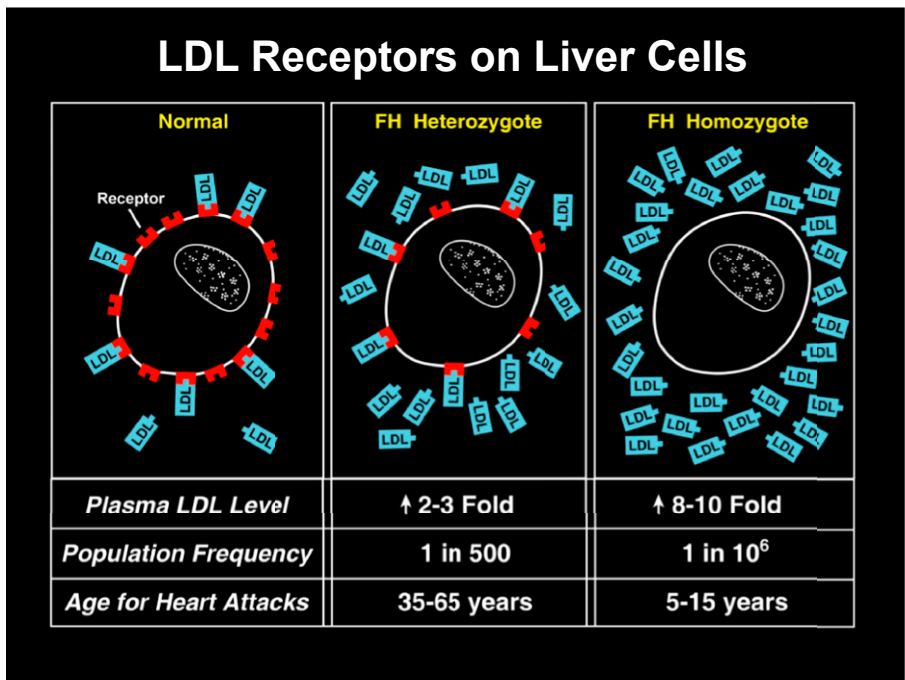


Fig. 1. LDL in FH: A comparative look. Courtesy of Brown and Goldstein.

See Classic Article "Familial hypercholesterolemia: Defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity" on page 788 in issue 3 of volume 71.

See Classic Perspective, 10.1073/pnas.1312967110.



Fig. 2. Partners for life: Brown and Goldstein circa 1975. Courtesy of Brown and Goldstein.

reports on cellular LDL uptake that came to be regarded as finely wrought dispatches from a fast-growing scientific field.

Among those reports was a 1974 PNAS paper entitled “Familial hypercholesterolemia: Defective binding of lipoproteins to cultured fibroblasts associated with impaired regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase activity,” which laid the ground for discoveries that earned the pair the 1985 Nobel Prize in Physiology or Medicine for their decadal effort (1). “The disease attracted our attention because no one knew the basis for the elevated cholesterol, and it was the most dramatic example of an inherited form of heart attacks,” says Goldstein. In a report redolent of pregenomics era biochemistry and peppered with prescient insights, the duo described differences in the binding of radioactively labeled LDL by cultured skin cells taken from healthy people and FH patients, uncovering hints to the cause of excess cholesterol in patients.

“The 1974 paper nailed the fact that there was something on the surface of the cells that likely mediated the delivery of LDL into the cells. It was a completely novel approach and a key technical advance,” says University of California, San Francisco biochemist Richard Havel, a long-time maven of lipoprotein research.

Part of Brown and Goldstein’s recipe for success is their reductionist knack for breaking down complex scientific problems into their components. Thus, they sought to apply the techniques of cell culture and enzymology to first develop an assay for a key enzyme involved in making cholesterol in human cells. In skin cells from healthy people, the enzyme’s activity was suppressed by

the presence of LDL in the culture medium. In striking contrast, no such suppression occurred in skin cells from the homozygous FH patients. Next, Brown and Goldstein set out to find whether skin cells of homozygous FH patients could bind LDL, as did the cells of healthy people. (Although the liver is the primary source of cholesterol made by the body, skin cells lent themselves to the experimental task at hand).

Because the patients’ cells failed to adequately bind LDL, the reasoning went, they were unable to keep cholesterol synthesis in check through feedback control, thus leading to runaway cholesterol levels. More intriguingly, the genetic defect in the FH cells suggested that LDL likely bound with high affinity to a receptor on the surface of cells that ferried the lipoprotein into the cells. In the 1970s, the concept of transport receptors on cell surfaces had not yet entered the scientific canon. Yet, only a decade later, Brown and Goldstein purified the LDL receptor from the adrenal glands of cows, identified the genetic sequence of its human counterpart, and isolated the gene encoding the human LDL receptor, setting the stage for the genetic analysis of mutations underlying FH. Today, more than 1,200 distinct mutations have been identified in the LDL receptor gene in FH patients.

“This paper was a blockbuster, and its binding studies, which suggested a role for the receptor, represent a landmark discovery,” says Washington University, St. Louis cell biologist Stuart Kornfeld, a pioneer in the field of cellular protein transport. “It’s beautifully done, impactful work that’s as exciting today as it was 40 years ago,” he adds.

But when the paper appeared in PNAS in the spring of 1974, its findings were far from unquestioningly embraced. Despite appreciative reviews, the report stirred deep misgivings among its detractors, who attempted to weaken its central claim with an array of countervailing arguments, including the significance of the small fraction of LDL that bound to cells during the experiment, the

nonspecific stickiness of LDL to a variety of surfaces, and the heretical nature of the suggestion of a receptor for LDL. “There was a tremendous amount of skepticism about the findings, especially among lipid biologists” recalls Brown.

Yet the paper has stood the test of time, and when its facts were pursued to their logical conclusion, it furnished a nuanced view of the fate of cholesterol in cells and spawned paradigms that have since become staples of cell biology texts.

“There was a lot of resistance to the idea that lipids needed a receptor to enter cells, but Brown and Goldstein’s experiments were so elegantly optimized, the problem so neatly dissected, and the observations so crisp that the evidence proved to be compelling,” says Helen Hobbs, Southwestern Medical School geneticist and Brown and Goldstein’s erstwhile postdoctoral fellow, who identified molecular abnormalities in their collection of FH cell lines.

Despota’s cells, Brown and Goldstein found, harbored two kinds of mutations in the LDL receptor gene: one kind led to a deficiency of the receptor, and the other hobbled the receptor’s entry into cells, blocking its function. Despota’s sky-high cholesterol levels, then, were a sum of those defects. “It was quite a detective story trying to find out what was wrong in his cells,” remembers Brown. “The boy had inherited the mother’s null gene—so that chromosome didn’t produce any LDL receptor—and the father’s mutant gene, so the other chromosome produced a receptor that bound LDL on the surface but couldn’t enter cells,” he explains. The pair summarized their findings in a 1976 paper in *Cell* (2).

“Despota was the first case with a defect in LDL-receptor internalization, and Brown and Goldstein’s groundbreaking work on his cells made me realize that standard medicines were not going to work in his case,” recalls Stone.

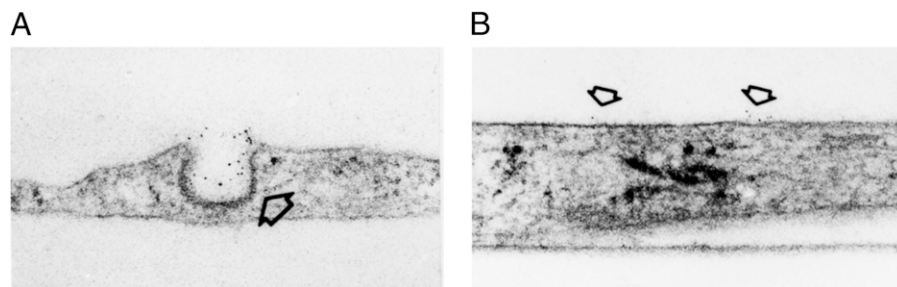


Fig. 3. Electron micrographs of normal human skin cells (A) showing labeled LDL particles and the LDL receptor clustered in cell membrane pockets called coated pits. In Despota’s skin cells (B), however, most of the LDL receptor was found to be remote from the coated pits and randomly scattered across the membrane. Reprinted by permission of Macmillan Publishers, Ltd: *Nature* (3), copyright 1977.

Early Start, Tangled Paths

The groundbreaking effort stems from the intertwined biographies of Brown and Goldstein, whose storied research partnership is now the stuff of legend. Born in Sumter, SC, Goldstein went to the University of Texas Southwestern Medical School in Dallas in 1962, a chemistry degree in hand. There, he met his mentor Donald Seldin, chairman of the university's internal medicine department, who urged him to pursue academic medicine, brokering a deal for a future faculty appointment at Southwestern on the condition that Goldstein specialize in human genetics elsewhere before returning to Dallas to set up a genetics division there. But Goldstein decided to first pursue an internship and residency in medicine at Massachusetts General Hospital in Boston.

The decision to go to Massachusetts General marked a pivotal moment in Goldstein's now-historic trajectory, leading him to the man who would become his lifelong research partner. Brown, a native of Brooklyn, NY, with degrees in chemistry and medicine from the University of Pennsylvania, was also a young intern at Massachusetts General. After two fruitful years, when their wide-ranging erudition, love of scientific pursuits, and fondness for Duplicate Bridge helped forge a deep friendship, the duo left for research positions at the National Institutes of Health. Brown studied with enzymologist Earl Stadtman, while Goldstein trained with Nobel laureate Marshall Nirenberg.

Around this time, Goldstein became enchanted with lipid metabolism as a result of his experience working with FH patients under the care of National Institutes of Health physician Donald Fredrickson. Convinced that they had found their calling, the pair resolved to unravel the genetics of FH. The time had come to take up Seldin on his offer, and to that end—upon Goldstein's urging—Brown accepted a faculty position at Southwestern Medical School in Texas, where Goldstein joined him after a two-year postdoctoral stint in medical genetics at the University of Washington, Seattle. In 1974, Brown and Goldstein merged their laboratories at Southwestern; four decades later, their names continue to be mentioned in conjunction.

Thanks to their seamless collaboration and electron microscopic analysis carried out by cell biologist Richard Anderson, the pair soon found that the LDL receptors on Despotas's skin cells failed to be taken up by the cells because they were scattered randomly across the membrane surrounding the cells instead of being clustered into regions called coated pits, which pinch off into vesicles that ferry the LDL-laden receptor from the cell's

surface to its interior, where the lipoprotein is broken down (3). The receptor then shuttles back to the surface, facilitating further rounds of LDL transport. (In its 20-hour lifespan, they later showed, the LDL receptor makes several hundred round trips, each lasting 10 minutes).

"[Despotas's] mutation was terribly instructive because he had a specific amino acid substitution in the cytoplasmic domain of the LDL receptor, and that single point mutation rendered the receptor incapable of clustering into coated pits," says University of California, Berkeley cell biologist Randy Schekman, who has long studied how proteins reach their proper destinations in cells.

Published in a 1977 *Nature* report (3), the findings gave birth to the concept of receptor-mediated endocytosis, a highly selective process by which cells internalize macromolecules, until then largely believed to enter cells through nonspecific means. "[Despotas's] cells gave us the first genetic evidence for receptor-mediated endocytosis, and the subsequent review we wrote in *Nature* put us on the cell biology map," recalls Goldstein. In the wake of this demonstration, researchers elsewhere found that other biologically important molecules, such as insulin and the iron-carrying protein transferrin, were similarly internalized by specific receptors.

"The way we've always done science is to go from the particular to the general," says Brown. As a testament to that approach, they went on to demonstrate that LDL, once internalized, generates cholesterol, which not only quickens the breakdown of a key cholesterol-making enzyme in cells but suppresses the enzyme's synthesis through the action of a gene switch, called sterol regulatory-element binding protein (SREBP). Through the same switch LDL suppresses the synthesis of its own receptor, allowing cells to adjust the number of LDL receptors according to their metabolic needs.

Lending heft to what was an incipient theory of cholesterol regulation in 1974, Brown and Goldstein had uncovered by the turn of the decade how cells keep cholesterol levels in membranes strikingly constant, despite varying supply and demand (4). "A few years after we described this process, a handful of similar paradigms came to light," says Goldstein. For example, their work on SREBP revealed how membrane-spanning proteins are cleaved in the membrane to release fragments that can enter the cell's nucleus to act as gene switches. The pair also found that this process, dubbed regulated intramembrane proteolysis, or Rip, activates a gene switch involved in cellular stress response (5). Rip was rediscovered by other researchers working on the amyloid precursor protein, implicated

in Alzheimer's disease (6), and on the signaling protein Notch, which controls embryonic development (7).

"The connection between receptor internalization and regulation of metabolism was a unique contribution by Brown and Goldstein," says Schekman.

The Age of Statins

The contribution gained greater currency when epidemiological studies in the 1960s and 1970s established a link between LDL cholesterol levels and coronary heart disease in the general population. And when in 1973 Japanese biochemist Akira Endo identified the LDL-lowering compound compactin in fungal extracts, ushering in the era of statin treatment for heart attacks, Brown and Goldstein demonstrated how statins worked to regulate cholesterol levels in blood. Endo's drug was designed to block the action of a key cholesterol-making enzyme, but Brown and Goldstein's partnership proved that when people at risk for coronary heart disease ingest statins, the amount of cholesterol in their liver cells drops, leading to a corresponding rise in the number of LDL receptors on the cells' surfaces. The receptors soak up excess blood LDL cholesterol, which is later metabolized, thus keeping heart attacks at bay (8). "Although the discovery of compactin was independent of that of the LDL receptor pathway by Brown and Goldstein, their PNAS paper of 1974 strongly supported and encouraged our idea that inhibition of elevated [cholesterol-making enzyme] by compactin might reduce blood cholesterol levels in FH," says Endo. According to the National Heart, Lung, and Blood Institute, coronary heart disease is the number one killer in the United States, with 405,000 people succumbing to the illness in 2008. Today, more than 30 million people worldwide take statins.

Statin therapy received a boost in 2006 when Hobbs and her collaborator Jonathan Cohen found that African Americans with a mutation in a protein dubbed PCSK9, which destroys LDL receptors in the liver, had remarkably lower LDL-cholesterol levels in their blood, reflecting an 88% drop in the incidence of coronary heart disease before the age of 60 in this group of people (9). A handful of drug companies are hot on the trail of PCSK9-blocking drugs, but the finding, published in the *New England Journal of Medicine*, helped explain why statin treatment trials had achieved only modest reductions in the incidence of heart attacks, despite lowering LDL-cholesterol levels dramatically: Because people entering such trials already have plaques in their arteries and are typically treated for no more than five years, the finding suggested that early and prolonged

treatment might be a key to truly effective statin therapy against heart attacks.

"That said, you can't treat everyone with a statin starting in middle age," says Brown. After all, statins can have side effects, such as muscle pain, cramping, and fatigue. Which is why, he adds, "The cardiologist's challenge is to identify people at the earliest stages of atherosclerosis. So far, genomic studies have not yielded such a signature, so we believe that noninvasive imaging techniques to visualize coronary arteries and screen people are sorely needed."

Today, the statin drug Crestor figures prominently in Despota's drug regimen, which also includes Zetia, a cholesterol-lowering drug; metformin, a type 2 diabetes drug; coumadin, a blood thinner; isosorbide, an angina drug; and cardoval, a heart supplement. Over the years, Despota has endured a coronary bypass and a heart valve replacement, soldiering through bimonthly procedures for LDL removal from his blood, now performed at Mease Countryside Hospital near Tampa, FL.

Though he never met Brown and Goldstein during the course of his treatment, Despota says he is pleased his cells could contribute to furthering the cause of science. "I am happy that my case has helped them come up with solutions that might help other people with cholesterol problems," he adds.

Immortalized in the annals of biochemistry as the first LDL-receptor internalization mutants to be characterized, Despota's cells helped open a line of experimentation that

led to the discovery of hundreds of LDL-receptor mutations in thousands of patients. "Today, almost every amino acid in the receptor is known to be mutated in one FH patient or another," says Goldstein.

The discovery of those defects owes a clear debt to Brown and Goldstein's technical acumen. "They don't publish anything unless they're 100% confident in the results. The standards they uphold make them second to none," says Schekman.

"Their work isolating, characterizing, sequencing, and cloning the LDL receptor was unbelievably beautiful and based on well-thought out, linear, and methodical experiments. It was an amazing example of how biochemical research should be done," says University of California, San Diego biochemist Daniel Steinberg, who has helped unravel the pathogenesis of atherosclerosis.

"Their conceptual contributions to our knowledge of endocytosis are unsurpassed.

What made their work so seminal is that Brown and Goldstein called upon their backgrounds in biochemistry, genetics, and cell biology to cross disciplines, which wasn't so common those days but is typically true of breakthrough studies," says University of California, San Diego cell biologist Marilyn Farquhar, who studies cellular protein transport.

The strength of Brown and Goldstein's work lies as much in their practice of unassailable science as in the plenitude of their accomplishments. The renowned 19th century playwright Oscar Wilde once told the English designer William Morris, "Your work comes from the sheer delight of making beautiful things," so that "in its singleness of aim, as well as in its perfection of result, it is pure art" (10). By the same token, if there is a single body of scientific work worthy of art's exalted status, Brown and Goldstein's continuing oeuvre might be a serious contender.

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