Cholesterol–Heart Disease Link Illuminated

New findings explain how blood cholesterol levels are controlled and how to lower them substantially in persons at high risk of heart disease

The relationship between cholesterol and heart disease has, until recently, eluded scientific explanation. It was known that extremely high concentrations of cholesterol in the blood can—by themselves—cause heart disease. It was known that cell surface receptors for a protein that carries cholesterol in the blood somehow are necessary for the control of blood cholesterol levels. But no one knew how blood cholesterol concentrations are controlled nor any logical way to substantially lower blood cholesterol in people at very high risk of heart disease.

This state of ignorance is now changing, thanks in large part to the discovery of an animal model for a human disease of cholesterol metabolism. Not only do researchers understand how cholesterol levels are controlled but they are, at last, well on their way to answering the fundamental questions of the cholesterol–heart disease hypothesis: Does a lowering of blood cholesterol actually prevent heart disease? And how does cholesterol cause heart disease in the first place?

Until about 3 years ago, scientists were stymied. They knew that cholesterol alone can cause heart disease because patients with homozygous familial hypercholesterolemia, a genetic defect, have six to eight times the normal level of cholesterol in their blood and invariably develop heart disease. These people usually have their first heart attacks in childhood and die of heart disease by the time they reach their early 20's.

Even people who are heterozygotes for familial hypercholesterolemia and so inherit one normal gene as well as the hypercholesterolemia one have heart disease problems. Their cholesterol concentrations are two to three times higher than normal and, although they number only 1 in 500 in the population, they account for at least 5 percent of all heart attacks in persons under age 60.

Not only were these people with familial hypercholesterolemia identified, but the exact nature of their genetic defect was known. They lacked functional cell surface receptors for low density lipoproteins (LDL), which are the fundamental carriers of blood cholesterol to body cells.

Yet, says Michael Brown of the University of Texas Health Sciences Center in Dallas, "We knew that humans with familial hypercholesterolemia have this receptor defect and we knew it blocked the removal of lipoproteins from the blood. But we couldn't analyze human tissues to see which tissues are most affected and which normally use LDL receptors to obtain cholesterol. The only cells that we could remove and study were blood cells."

At about the same time as Brown together with Joseph Goldstein and their associates in Texas were trying to understand how a lack of LDL receptors and a consequent surfeit of LDL in the blood can cause heart disease, a veterinarian in Japan made a serendipitous finding that totally changed the prospect for LDL receptor research. In 1973, Yoshio Watanabe of Kobe University noticed that a male rabbit in his colony had ten times the normal concentration of cholesterol in its blood. By appropriate breeding, Watanabe obtained a strain of rabbits, all of which had this very high cholesterol level. These rabbits spontaneously developed coronary artery disease, which is extraordinary because the only way normal rabbits can be made to develop atherosclerosis is to stuff them with dietary cholesterol. The cholesterol-fed rabbits, however, develop cholesterol deposits in all of their blood vessels, not just the coronary arteries. In humans, cholesterol accumulates preferentially in the coronary arteries. Thus the Watanabe rabbits develop a heart disease that closely resembles what occurs in humans.

> The two-drug method of lowering cholesterol concentrations may be worth trying.

For years, no one made a connection between the Watanabe rabbits and persons with familial hypercholesterolemia. This was because the rabbits have high triglyceride levels as well as high cholesterol, whereas in the human disease only cholesterol is elevated.

Then, in 1980, Watanabe collaborated with biochemists at his university to look for LDL receptors on cultured skin cells from the rabbits. They found that the rabbits, like the humans, lack functional LDL receptors.

Once they realized just what the genetic defect was in Watanabe rabbits, Brown and Goldstein set out to determine which tissues take up LDL. They found that the primary sites are the liver, which excretes cholesterol from the body and also uses cholesterol to make bile acids, and the adrenal glands, which use cholesterol to make steroid hormones. Daniel Steinberg and Thomas Carew of the University of California in San Diego made similar observations.

In addition, says Brown, "The second thing we learned—and perhaps the most important—is the nature of the overproduction of LDL in patients with familial hypercholesterolemia. It was observed a long time ago that if you study the turnover of LDL you can show a deficiency in the removal of LDL from the blood. That is easy to explain on the basis of the lack of receptors. But patients also seemed to produce more LDL than normal individuals. We didn't know how to account for that."

Researchers were faced with a single gene disease that caused both a slowdown in the removal of LDL and an increase in the synthesis of this cholesterol-carrying protein. How, they wondered, could one mutation cause both of these effects? "The study of the Watanabe rabbits allowed us to explain it," says Brown.

It was known that LDL are secreted from the liver in the form of a precursor, called very low density lipoproteins (VLDL), which carry triglycerides as well as relatively small amounts of cholesterol. The VLDL go to fatty tissues which remove the triglycerides from them. What remains is a remnant particle that must be removed from the blood.

"What we learned from the Watanabe rabbit is that the removal of the VLDL remnant requires the LDL receptor,"

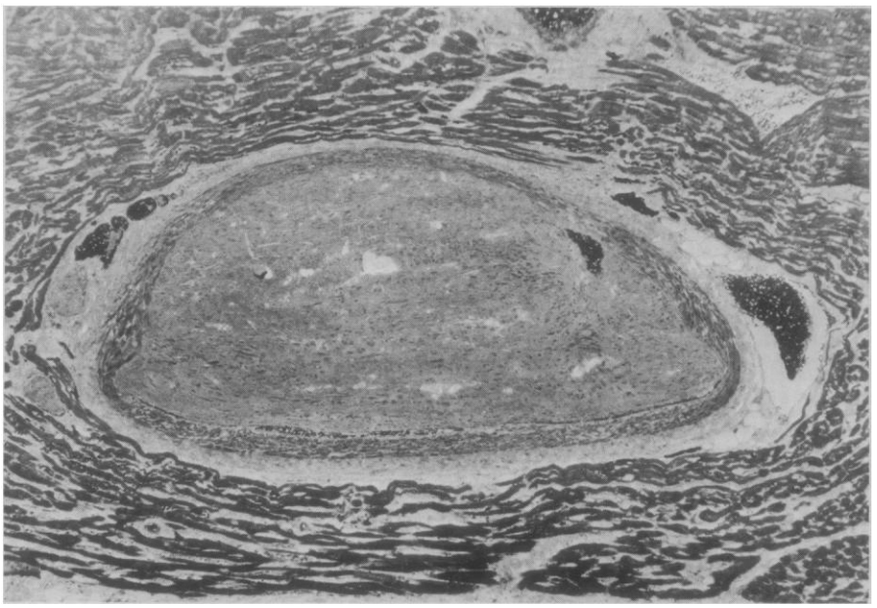
Brown remarks. The LDL receptors on the liver cells grab the VLDL remnants and degrade them. "In the Watanabe rabbit, VLDL can't get back into the liver. It remains in the plasma and is eventually converted to LDL. We call this the shunt pathway for LDL production."

In normal persons, by far the majority of the VLDL remnants go to the liver where they bind to LDL receptors and are degraded. A small portion of them escape this degradation and are converted to the cholesterol-carrying protein LDL. This LDL is bound by bodily tissues, especially the adrenal gland, and supplies them with cholesterol. According to Brown and Goldstein, the shunt pathway is thought to be the sole source of LDL in plasma. In patients with familial hypercholesterolemia, VLDL remnants remain in the plasma and are converted to LDL, thereby accounting for the very high LDL levels in these patients. The LDL receptors thus have a dual effect in controlling LDL levels. They are necessary to prevent over synthesis of LDL from VLDL remnants and they are necessary for the normal removal of LDL from the blood plasma.

Persons who are heterozygotes for familial hypercholesterolemia have one normal gene for LDL receptors, but it is not fully expressed. Based on their new understanding of the control of LDL, David Bilhiemer and Scott Grundy of the University of Texas Health Sciences Center at Dallas, working with Brown and Goldstein, have found a way to trick these heterozygote cells into making a greater number of normal LDL receptors. Moreover, these investigators speculate that their method may also apply to persons who have high cholesterol levels but are not heterozygotes for familial hypercholesterolemia.

Goldstein and Brown had already learned several years ago that LDL receptors in cultured cells are under feedback regulation—give a cell more LDL and fewer receptors are made. Give it less and more receptors appear. The idea for treating heterozygotes was to deprive the liver of cholesterol. The liver will then make more LDL receptors. A drug, cholestyramine, that is commonly used to lower cholesterol levels, acts in just this way. It essentially drains the liver of cholesterol.

But cholestyramine alone is not sufficient to lower LDL levels to a normal range in heterozygotes for familial hypercholesterolemia. It lowers LDL levels by only 15 to 20 percent so that patients with cholesterol concentrations of 350 milligrams per deciliter reach about 300 milligrams per deciliter—which is still elevated. A more normal cholesterol concentration is 215 milligrams per deciliter.



Rabbit heart attack

This Watanabe rabbit ate a cholesterol-free diet and yet has a totally obstructed coronary artery. [Source: Maximillian Buja, University of Texas Health Science Center at Dallas]

The reason why cholestyramine is not fully effective, says Brown, is that "the liver makes a dual response. It takes up cholesterol but it also starts to synthesize more cholesterol." What is needed is a second drug to block cholesterol synthesis.

Two new drugs, now in an early experimental stage of testing, seem to fit the bill. Both were isolated from molds and both act by inhibiting an enzyme necessary for cholesterol synthesis. The first of these drugs, called compactin, was discovered by Akira Endo of the Sankyo Drug Company in Tokyo. The second, an even more potent drug called mevinolin, was discovered independently by Endo and by scientists at Merck Sharp & Dohme.

When given alone, either compactin or mevinolin will cause a decrease in cholesterol synthesis and a consequent increase in liver LDL receptors. But, says Brown, "The most dramatic effect is when one of these drugs is given with cholestyramine." As first reported by Hiroshi Mabuchi and his associates at Kanazawa University in Japan, the two-drug combination causes a 50 to 55 percent decrease in plasma LDL levels in heterozygotes for familial hypercholesterolemia—which puts them in the normal range. Says Brown, "The important scientific point is that here is a genetic disease that is manifested in heterozygotes and we can stimulate the expression of the normal gene." The immediate clinical importance is that this drug combination may stave off heart disease in the heterozygotes who have, Brown notes, "a grim prognosis now and no alternative treatments."

But the question that is of central interest to the majority of people in this country is: What about persons who have high cholesterol concentrations—say 280 milligrams per deciliter—and a strong family history of heart disease and yet are not affected by familial hypercholesterolemia? They are at high risk of developing heart disease. Should they take these cholesterol-lowering drugs? These questions, of course, extend the findings of the recent research to a much more speculative realm.

It is generally believed that many factors can cause high cholesterol levels in humans. For example, a high cholesterol diet may do it in some people. Dietary cholesterol is carried by a different system than the LDL, which carries endogenous cholesterol made by the body. But Robert Mahley of the University of California in San Francisco, working with Brown and Goldstein, found that when animals are fed cholesterol it accumulates in their livers. The liver's LDL receptors then decrease in number. This causes an increase in the LDL in the blood. It is likely that similar effects occur in humans.

Certain hormones can also affect LDL receptors in humans. For example, Gilbert Thomason and Nicholas Myant of the MRC Laboratory of Lipid Research at Hammersmith Hospital in England

found that a lack of thyroid hormone causes high LDL levels in the blood and a decrease in LDL receptors whereas too much thyroid hormone has the opposite effect.

But even though the causes of most cases of high cholesterol levels in humans are only dimly understood, Brown and Goldstein argue that the two-drug method of lowering cholesterol concentrations may be worth trying. "We feel that it will work," Brown says. And by using these drugs, it may be possible to answer at last the question of whether lowering cholesterol levels lowers the risk of heart disease. For the first time, a treatment is available that lowers cholesterol enough that a difference in heart disease risk, if it occurs, should be readily apparent in a clinical trial.

The most fundamental question about cholesterol and heart disease is still unanswered, however. How does LDL cause heart disease in the first place? But, once again, the Watanabe rabbit may provide the necessary clues. Several groups of researchers have established that the atherosclerosis that develops in these animals is similar to human atherosclerosis. Thus it is possible to use these animals to study how LDL interact with platelets, endothelial cells, scavenger cells, and the smooth muscle cells that line the artery wall. All of these cells seem to be damaged by high levels of LDL. Carew and Steinberg have recently developed methods to study in whole animals which cells are degrading LDL and how. "We just really have the methodology now in hand—within the last few months," says Carew.

So the unanswered questions in the cholesterol-heart disease story no longer seem so unanswerable as they did in the recent past. The stage is set for dramatic changes in the diagnosis and treatment of heart disease.—**GINA KOLATA**

Additional Reading

1. J. Goldstein, T. Kita, M. Brown, "Defective lipoprotein receptors and atherosclerosis," *N. Engl. J. Med.* **309**, 288 (1983).

Where Was the Moon Eons Ago?

Where was the moon in the early days of the solar system? Experts will only agree that it was not orbiting at its present distance of 380,000 kilometers; it was much closer to Earth in the past. Today the moon is moving outward at about 4 centimeters per year. A new study of the gravitational interaction of the moon and Earth over geologic time suggests that the moon was never closer than 225,000 kilometers, which would avoid the apparent problem of a close encounter of the two bodies only 2 billion years ago. It also argues against Earth originally calving the moon while still young and hot, or capturing the moon as it made a near miss.

Why the moon is receding has been clear for a long time. It raises tides in Earth's seas that in turn raise the moon to a higher orbit and slow Earth's rotation. The day and the month are becoming longer. The problem is that it is happening so quickly. Tidal currents dragging across the bottom of shallow seas are dissipating tidal energy and lengthening the day so fast that, if the present rate were extrapolated into the past, the moon and Earth would have been so close 1.5 to 2 billion years ago as to melt surface rocks. That did not happen.

To avoid the nonexistent Earth-moon encounter, celestial mechanicians have been looking for a way to calculate a smaller past rate of tidal dissipation. Since the advent of plate tectonics, they have usually done it by moving the continents around or changing sea level, which changes the shape and depth of ocean basins. It appears that the shape, orientation, and depth of today's oceans are particularly well suited to the efficient dissipation of tidal energy. The present 12.5-hour period of tidal forcing produces a resonant response in these particular basins that amplifies the rate of dissipation. Unfortunately, no one knows what the configuration of continents and oceans has been for most of the history of Earth.

Kirk Hansen of the University of Chicago (now at Shell Development Company, Houston) suggested recently* that it is not so much the changing ocean basins but the changing rotation rate of Earth that determines the rate of tidal dissipation over geologic time. To make his point Hansen calculated the dissipation rate in a simplified model of an unchanging ocean and linked the calculations to the changing rotation rate of Earth.

As the model moves back in time, Earth spins faster, as it regains the energy lost to the moon, decreasing the period of tidal forcing and breaking the resonances with the great ocean basins. In the absence of these resonances, the rate of tidal dissipation decreases and the model moon's march inward is slowed. During such a backward extrapolation, the moon does not approach closer than 290,000 kilometers by 4 billion years ago, says Hansen. The closest that the moon can come in the model is 225,000 kilometers. New resonances would form as the day and thus the tidal forcing period shortened, but they would involve smaller basins. These resonances would be less efficient in dissipating tidal energy and could not make up for the loss of the larger scale resonances, Hansen says. The apparent dominance of rotation rate over basin configuration argues against a fission origin for the moon, a recently revived theory, or capture by Earth during a near miss (*Science*, 20 July 1979, p. 292). The alternative is simultaneous accretion of Earth and the moon from the solar nebula as a "double planet."

The coupling of tidal calculations and Earth's rotation has been well received, but it does not settle the question entirely. One concern is the exclusion of any changes in the area of shallow seas that could change the rate of dissipation. Hansen argues that a series of highly unlikely coincidences would be required to preserve the kinds of resonances that produce today's high rate of dissipation. Hansen concedes that tides raised in the hot, more plastic rock of the young Earth might contribute additional dissipation, but the moon's orbital inclination as well as its distance at that time are not consistent with a fission or capture origin, he notes. In addition, other processes not included in his model, such as solar tides, would tend to reinforce the dominance of the rotation rate.

These and other assumptions need to be investigated, but this century-old field looks more interesting than it has in a while—it may not be so intractable as it seemed.

—**RICHARD A. KERR**

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