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James F. Fixx Dies Jogging; Author on Running Was 52

NEW YORK TIMES, AUGUST, 1984

YOU CAN'T OUTRUN CHOLESTEROL

MEDICAL GRAND ROUNDS
PARKLAND MEMORIAL HOSPITAL
SEPTEMBER 20, 1984

MICHAEL S. BROWN, M.D.

Texas girl undergoes a double transplant

DALLAS TIMES HERALD, FEBRUARY, 1984

This year, dramatic events in two patients have focused attention on the relation between lipoprotein receptors, hypercholesterolemia and atherosclerosis. One event was the heart-liver transplantation operation in Stormie Jones, a six-year-old patient of David Bilheimer with homozygous familial hypercholesterolemia. Stormie's own liver was unable to remove cholesterol from plasma normally because she has a defect in the gene encoding a receptor for plasma lipoproteins. As a result, her plasma cholesterol was massively elevated and she suffered multiple myocardial infarctions in childhood.

The second event was the sudden death from myocardial infarction of a 52 year old jogger named Jim Fixx. Mr. Fixx's father died at age 42 of a myocardial infarction. At age 40 Jim Fixx was an obese smoker who did not like doctors. In an attempt to avoid the fate of his father Mr. Fixx became a dedicated jogger and physical fitness buff. He lost 60 lbs in weight and stopped smoking. He wrote several books on jogging and is credited with having popularized jogging in our country. After a decade of physical fitness Mr. Fixx died suddenly of a myocardial infarction while jogging in Vermont. Mr. Fixx avoided doctors, and his cholesterol level is unknown. However, for our purposes today I will assume that Mr. Fixx had a blood cholesterol level of about 225 mg per deciliter, which is the mean value in 53 year old men in this country.

Today I wish to present a hypothesis that Joseph Goldstein and I have developed. This hypothesis states that Stormie Jones and Jim Fixx both owe their troubles to the same underlying cause - namely, a deficiency of low density lipoprotein (LDL) receptors in the liver. In Stormie Jones the deficiency results from defective genes encoding the receptor. In James Fixx the receptor deficiency was not genetic but acquired as a result of his earlier life-style and eating habits. Stormie Jones has a complete defect that occurs in only one in one-million persons. Jim Fixx had a much more subtle defect that may be present in more than half of all Americans. In fact, this acquired defect is so frequent as to be considered normal. We believe that this subtle regulatory defect in LDL receptors is responsible for most of the heart attacks that occur in the United States.

Before presenting this thesis I will briefly describe the case of Stormie Jones, who is a patient of David Bilheimer and who is known to many members of our house staff and faculty who have participated in her heroic care. I will then tell you about the LDL receptor as we know it from studies in tissue culture and in the body. Then I will discuss two forms of receptor deficiency - genetic and acquired. Finally I will discuss the implications of the LDL receptor studies for the general problem of atherosclerosis in our society.

CASE REPORT

(Adapted from Lancet, v.i., June 23, 1984)

On September 1, 1983, Stormie Jones, aged 6 years and 4 months was admitted for metabolic studies to Parkland Memorial Hospital and the University of Texas Southwestern Medical School in Dallas. From the age of 3 months progressive xanthomas had developed on the contact areas of her buttocks and extremities. FH had been diagnosed when she was 6 years old. Culture of cutaneous fibroblasts indicated that she had the genetic deficiency of low density lipoprotein (LDL) receptors which is diagnostic of FH. On admission, her plasma cholesterol was 1225 mg/dl and her plasma triglyceride was 154 mg/dl. Liver function tests were normal and she had no cardiac symptoms.

In late September, she suffered a myocardial infarction followed by continuing angina pectoris and she required a double coronary artery bypass. A second myocardial infarction and recurrent angina led to the performance of a second bypass operation: and when she could not be weaned from the heart-lung bypass because of mitral regurgitation caused by previous papillary muscle necrosis, the mitral valve was replaced.

After these operations, during late December, 1983, Stormie had recurrent bouts of angina pectoris and heart failure. Liver transplantation was considered as a means of providing her with normal LDL receptors so as to allow cholesterol to be removed from her plasma. However, her heart disease had become too severe to permit liver transplantation, and for this reason concomitant heart replacement was suggested by the transplant team as the only realistic option. During late December, 1983, and in early January, 1984, this seemingly drastic proposal was considered and ultimately accepted by a consortium of physicians and surgeons from the University of Pittsburgh and UTHSCD. There was approval by the Human Rights Institutional Review Board of the Children's Hospital of Pittsburgh.

The heart and liver replacements were carried out on Feb. 14, 1984, during total cardiopulmonary bypass. Although the recipient was aged 6 years and 9 months, she weighed only 19.1 kg. Her blood type was A. The donor was a girl of 4 and one half of O blood type who weighed 16.2 kg. There was a total donor/recipient mismatch at the HLA A, B, and D_R loci. The removed heart had advanced atherosclerotic and valvular disease. The excised liver was normal by gross and microscopic examination.

Good cardiac and hepatic function were achieved from the grafts. Cyclosporin and steroids were used as immunosuppressants. A persistent increase in serum bilirubin concentration 5 days after surgery suggested rejection but this subsided without an increase in immunosuppression. Standard liver function tests have been normal since the second postoperative week. Immunosuppression with 300 mg/day cyclosporin and 7-5 mg/day prednisone is being continued.

LDL-Cholesterol and Atherosclerosis (Figure 1)

The most striking biochemical abnormality in atherosclerosis is the massive deposition of cholesteryl esters in the artery wall. This cholesteryl ester is derived primarily from cholesterol-carrying low density lipoprotein (LDL), which circulates in the bloodstream. The lipoprotein enters the artery wall and deposits its cholesterol in places where the cells that line the wall (the endothelium) have been injured. The rate at which LDL deposits its cholesterol in arteries is influenced by several so-called "risk factors" that have been defined by epidemiologic studies. Many of these risk factors damage the endothelium and thereby promote the penetration of plasma LDL into the wall. These include cigarette smoking, hypertension, diabetes mellitus, and poorly understood genetic influences that determine the vulnerability of an individual's vessels to cholesterol deposition. The more LDL in blood, the faster the development of atherosclerosis. Conversely, if the level of LDL is low enough, atherosclerosis is slow to develop, even in the face of all of the other risk factors.

Epidemiologic studies have revealed that more than half of all people in Western industrialized societies, including the United States, have levels of circulating LDL that are above the threshold at which atherosclerosis is accelerated. In the past these concentrations of LDL have been considered "normal" in the sense that they are the usual values found in such populations. However, they seem not to be normal for the human species in the sense that they lead to accelerated atherosclerosis.

Why do half of all Americans have concentrations of LDL that place them at high risk for developing atherosclerosis? Answers are emerging from studies of LDL receptors. Projecting outward from the surfaces of cells in the liver and other organs, these receptors bind circulating LDL. Binding initiates a process by which the LDL is taken up by the cells and degraded, yielding its cholesterol for cellular use. When the body produces abundant LDL receptors the plasma LDL level is kept low, When production of LDL receptors is reduced, the blood LDL level rises.

The cells of the body produce varying numbers of LDL receptors, depending on their needs for cholesterol. When the cells' needs are high, they produce large numbers of receptors, LDL is removed rapidly from the circulation, and the level of LDL in blood is kept below the threshold for rapid development of atherosclerosis. On the other hand, when cells accumulate excess cholesterol, they produce fewer LDL receptors and take up LDL at a reduced rate. This protects the cells against excess cholesterol accumulation, but at a very high price: the reduction in the number of receptors decreases the rate at which LDL is removed from the circulation, the level of LDL rises, and atherosclerosis is accelerated.

Joe Goldstein and I have proposed recently that the high level of LDL in many Americans is attributable to a combination of factors that lead to a diminished production of LDL receptors. One of these factors is a general tendency of humans to produce a relatively small number of LDL receptors, as compared with animals of other species. This tendency is aggravated by a diet that is rich in cholesterol and saturated fats derived from meat and dairy products. Such a diet causes cholesterol to accumulate in cells of the liver, leading the cells to further decrease their production of LDL receptors. Much of our insight into the consequences of diminished LDL receptor production has come from studies of familial hypercholesterolemia (FH), a human disease in which the receptors are diminished not as a result of a dietary excess, but as a result of a defect in the gene encoding the receptors. These studies have led to the suggestion that LDL

receptor deficiency, aggravated by a high fat diet, is a major cause of high blood cholesterol levels and atherosclerosis in the general population of the United States.

The LDL Receptor at the Cellular Level (Figs. 2 and 3)

LDL receptors were discovered in our laboratory, in 1973 during studies of human skin cells (fibroblasts) growing in tissue culture. These fibroblasts, like all cells, require cholesterol as a building block for the surface membrane that surrounds the cell. Although fibroblasts can produce their own cholesterol, they rarely use this capacity in their natural state. Rather, they supply themselves with cholesterol from the LDL in the fluid that bathes the cells.

LDL is a large spherical particle with a molecular mass of 3×10^6 daltons. It contains an oily core composed of 1500 cholesterol molecules attached to long chain fatty acids in ester linkage. Surrounding this core of cholesteryl esters is a polar coat that acts like a detergent and allows the LDL to be dissolved in the watery fluid of the blood. The polar coat is composed mostly of phospholipids and a large protein that is embedded in the lipid matrix. This protein is called apoprotein B-100.

To obtain the cholesterol of LDL, cells synthesize LDL receptors and place them on their outer surfaces. The LDL receptor is a protein that spans the lipid bilayer of the plasma membrane with its binding site extending outward into the extracellular milieu. The receptor binds LDL by attaching itself to apoprotein B-100. The binding reaction shows extremely high sensitivity (binding occurs when LDL is present at a concentration of less than 10^{-9} molar) and high specificity (the receptor binds only those lipoproteins that contain apoprotein B-100 or, as will be discussed below, another protein designated apoprotein E).

Our collaborator, Richard G.W. Anderson discovered in 1976 that LDL receptors are clustered in specialized regions of the cell's surface where the membrane is indented to form craters called "coated pits." Within minutes of their formation, the pits invaginate, or pouch inward, into the cell and pinch off from the surface to form membrane-enclosed sacs called coated vesicles. In this way LDL that is bound to the receptor is carried into the cell. Within the cell the LDL is delivered to a lysosome, which is a membrane-enclosed sac filled with digestive enzymes. The protein and phospholipid components of LDL are rapidly broken down by these enzymes, thereby exposing the core of LDL. The cholesteryl esters of this core are then hydrolyzed by acid lipase, an enzyme that clips off the fatty acid to liberate free (unesterified) cholesterol. The free cholesterol leaves the lysosome and is used by the cells in the formation of new surface membranes. In certain specialized cells the cholesterol delivered by LDL is used for other purposes. The adrenal glands and ovaries convert this cholesterol to the steroid hormones, cortisol and estradiol. The liver transforms the LDL-derived cholesterol into bile acids.

"Receptor-mediated endocytosis" is the term that we and Anderson first applied to the process by which cells take up LDL through coated pits and vesicles. This process is now recognized to be a general mechanism by which cells take up many different kinds of nutritional and regulatory macromolecules, each of which has its own specific receptor (see Dautry-Varsat and Lodish, Sci. Amer., May 1984). Through receptor-mediated endocytosis, cells take up protein hormones (such as insulin), growth-stimulating hormones (such as epidermal growth factor), and other plasma transport proteins (such as the transport proteins for iron and vitamin B_{12}). Certain viruses (such as Semliki Forest virus) and toxins (such as

diphtheria toxin) also enter animal cells by receptor-mediated endocytosis, apparently through opportunistic attachment to receptors whose primary job is to transport the host's own proteins. Each of the receptors is an independent molecule with its own distinct function. For example, the insulin receptor regulates the entry of glucose into the cell. The transferrin receptor supplies the cell with iron just as the LDL receptor supplies the cell with cholesterol.

After leaving the lysosome, the cholesterol liberated from LDL regulates the cell's cholesterol metabolism, thereby assuring a steady level of cholesterol within the cell. To accomplish this regulation, the incoming cholesterol modulates three events. First, it turns off the production of an enzyme, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), which catalyzes a crucial step in the biosynthesis of cholesterol. Suppression of this enzyme ensures that the cell will preferentially use external cholesterol derived from the receptor-mediated uptake of LDL; yet, if LDL were not available, the cells could synthesize new HMG CoA reductase molecules so as to produce their own cholesterol. Second, the LDL-derived cholesterol activates an enzyme called acyl-CoA: cholesterol acyltransferase (ACAT) that reattaches a fatty acid to excess cholesterol molecules and deposits the resultant cholesteryl esters in storage droplets in the cytoplasm.

The third regulatory action is perhaps the most important of all. In this reaction, the build-up of cholesterol within the cell causes the cell to shut off the synthesis of new LDL receptors. Through this "feedback" mechanism, cells adjust the production of receptors to supply sufficient cholesterol to meet their varying demands, but not enough to overload themselves with cholesterol. When cells are growing actively and producing new membranes, they synthesize a maximal number of LDL receptors (approximately 40,000 per cell) to supply the necessary amounts of cholesterol. On the other hand, when cells cease to grow, they have little demand for cholesterol. Consequently, the cholesterol begins to build up in the cell, and the production of LDL receptors is suppressed to levels as low as 10 percent of the maximum. In this way, intracellular accumulation of excessive cholesterol is prevented.

Within recent years the molecular structure of the LDL receptor has come under scrutiny. Wolfgang Schneider purified the receptor from the adrenal gland of the cow. The adrenal gland has a large number of LDL receptors because it uses these receptors to supply the cholesterol that is converted into steroid hormones such as cortisol. David Russell and Tokuo Yamamoto have isolated, through molecular cloning, a complementary DNA (so-called cDNA) that corresponds to the messenger RNA for the human LDL receptor. By determining the sequence of nucleotides in this cDNA and through knowledge of the genetic code, we have been able to deduce the linear sequence of amino acids in the receptor protein. The receptor contains a long protein backbone of 839 amino acids to which multiple sugar molecules are attached. The protein backbone of the receptor is synthesized in the rough endoplasmic reticulum, which is the cell's factory for production of proteins that reside in the surface membrane. Some of the sugar molecules are added to the protein in the endoplasmic reticulum, and the rest are added after the receptor has been transported to another membrane structure, the Golgi complex. Shortly thereafter the receptor appears on the surface of the cell where it clusters in coated pits, ready to bind and internalize LDL.

Functions of the LDL Receptor in the Body (Fig. 4)

Soon after the LDL receptor was discovered in cells grown in tissue culture, its presence was demonstrated in blood cells in the circulation of humans and in

cell membranes prepared from many tissues of rats, mice, cows, dogs, pigs, and humans. In whole animals and in humans, functioning of the receptors can be assessed by injecting radioactively-tagged LDL into the bloodstream and measuring how fast LDL is removed from the circulation. The rate at which the radioactivity disappears from plasma depends on the total number of LDL receptors produced by all the cells in the body. The more receptors, the faster the LDL is removed from the circulation. This can be verified by experiments in which, prior to injection, the apoprotein B-100 of LDL is modified chemically so that it can no longer bind to LDL receptors. James Shepherd and Christopher Packard at the University of Glasgow showed that modified LDL circulates much longer than normal LDL, confirming the requirement for receptor binding in removal of LDL from the circulation.

The studies of John Dietschy have shown that about 75 percent of the receptor-mediated removal of LDL occurs in the liver. This conclusion is supported by our own results in which the cell membranes from body tissues were isolated and the number of receptors measured. Although most tissues have some LDL receptors, the liver, the adrenal gland, and the ovary have the highest concentration. This distribution is logical since these are the three tissues with the largest requirements for cholesterol - for conversion to bile acids in the liver and for the production of cortisol in the adrenal gland and estradiol in the ovary.

Genetic Defects in the LDL Receptor in FH Patients (Figs. 5 and 6)

As is often the case in medicine, the importance of the LDL receptor in normal physiology was first appreciated when its absence was shown to produce a severe disease, in this case familial hypercholesterolemia (FH). As a disease, FH has a rich clinical history. It was described in 1939 by Carl Müller, a clinician at the Oslo Community Hospital in Norway, as a so-called "inborn error of metabolism" that produced high blood cholesterol levels and heart attacks in young people. Müller pointed out that FH is transmitted as a single gene-determined autosomal dominant trait. In the 1960's Avedis K. Khachadurian, then at the American University in Beirut, Lebanon, and Donald S. Fredrickson, then at the National Institutes of Health, showed that the disease exists clinically in two forms: the less severe heterozygous form and the more severe homozygous form. Heterozygotes, who inherit one mutant gene, are quite common, accounting for 1 out of every 500 persons among most ethnic groups throughout the world. These heterozygotes have plasma LDL levels that are 2-fold above normal even before birth, and they begin to have heart attacks as early as age 35 years. Among people under age 60 who suffer heart attacks, 5 percent have the heterozygous form of FH - a 25-fold enrichment over the prevalence in the general population.

Ordinarily, each FH heterozygote passes a single copy of the mutant gene to half of his or her offspring, and these offspring then have heterozygous FH. If two FH heterozygotes marry, as occurs in one out of 250,000 marriages, each offspring has a one in four chance of inheriting two doses of the mutant gene - one from each parent. Such an offspring is an FH homozygote. FH homozygotes number about 1 in one million persons in the population. They have LDL levels that are more than six times above normal. Heart attacks can occur as early as age 2 and are almost inevitable by age 20. It is notable that these children have no risk factors for atherosclerosis other than an elevated level of LDL. They have normal blood pressure, they do not smoke, and they do not have an elevated blood glucose level. The homozygous form of FH is a vivid experiment of nature that demonstrates unequivocally the causal relationship between elevated LDL levels and atherosclerosis.

Eleven years ago, we began to study FH with the hope of learning the mechanism for the elevation of LDL in these patients. We soon discovered that cultured skin

fibroblasts and circulating blood cells from FH homozygotes produce few or no functional LDL receptors. They are therefore unable to bind, internalize, and degrade LDL with normal efficiency. The receptor deficiency arises because FH homozygotes inherit two defective copies of the gene for the LDL receptor, one from each parent. They have no copy of the normal gene and therefore can produce no normal LDL receptors. Cells from their parents (and from other FH heterozygotes) have one normal gene and one mutant gene for the receptor. They synthesize half the normal number of LDL receptors and are therefore able to bind, internalize, and degrade LDL at one-half the normal rate. All individuals with FH so far studied have mutations in the gene encoding the LDL receptor, but all of the mutations are not the same. The mutations fall into several different classes, depending on the site in the gene at which the mutation has occurred.

How does the defect in the LDL receptor gene cause the level of LDL to rise in the bloodstream? Answers to this question have come from studies of the rate at which intravenously administered radioactive LDL is removed from the circulation. These studies have shown that LDL survives about 2.5 times longer in the circulation of FH homozygotes than it does in normal subjects. Eventually, the LDL is degraded by less efficient alternate pathways for removal of lipoproteins from the circulation.

Reducing Plasma LDL By Raising LDL Receptors (Fig. 7)

The recent insights into the functions of the LDL receptor have suggested new treatments for the one-in-500 people who have the heterozygous form of FH. These subjects have one normal gene turning out functional receptors and one faulty gene turning out nonfunctional receptors. Might it not be possible to stimulate the normal receptor gene to produce twice its normal number of messenger RNA molecules so that the FH heterozygote will produce a normal number of functional LDL receptors?

The possibility of such a treatment was first raised by our studies in cultured skin fibroblasts which demonstrated that synthesis of LDL receptors is subject to "feedback" regulation by cholesterol. When a cell's demand for cholesterol is increased, the cell synthesizes more LDL receptors. On the other hand, when cells begin to accumulate excess cholesterol, the production of LDL receptors is shut down. This regulation is mediated through changes in the level of the messenger RNA that encodes the receptor protein: cholesterol deficiency stimulates production of this messenger RNA, and cholesterol excess inhibits the production. In response to cholesterol deficiency, tissue culture cells from FH heterozygotes can be made to produce a normal number of LDL receptors by turning out increased numbers of messenger RNA molecules from the normal gene.

So the therapeutic problem resolved itself to this: how could we create a condition of cholesterol deficiency in cells of FH heterozygotes in the body so as to stimulate them to produce increased LDL receptors? The answer to this question came from two fronts: 1) from an understanding of the normal physiology of cholesterol metabolism in the liver and 2) from the discovery of a remarkable enzyme inhibitor by Akira Endo at Tokyo Noko University in Japan.

The liver is the only organ that can break down large amounts of cholesterol and excrete it from the body. The liver does this by converting cholesterol into bile acids in a reaction that involves removal of part of the side chain of the cholesterol molecule, with oxidation of the terminal carbon to a negatively charged carboxylic acid. The bile acids are excreted into the upper intestine, where they act as detergents to emulsify dietary fats. When the bile acids reach the lower

part of the small intestine, their work has been accomplished. However, the bile acids are not allowed to escape into the feces; rather, about 90 percent of the bile acids are reabsorbed from the intestine and returned to the bloodstream. The reclaimed bile acids are taken up by the liver and again secreted into the bile. This is the so-called "enterohepatic circulation" of bile acids. Because of this circulation, the liver is called upon to convert relatively little cholesterol into bile acids. If this enterohepatic circulation could be interrupted so that the bile acids could not be reused, the liver would be called upon to convert more cholesterol into bile acids. This would increase the liver's demand for cholesterol and should lead to an increased production of LDL receptors by liver cells.

A class of drugs that interrupt the enterohepatic circulation are the bile acid binding resins, which are gritty polymers with multiple positively charged chemical groups. When taken by mouth, these resins bind the negatively charged bile acids in the intestine. Since the resins cannot be absorbed from the intestine, they pass into the feces, carrying their bound bile acids with them. The first bile acid binding resin, called cholestyramine, was synthesized more than 20 years ago. When given to humans, cholestyramine raises LDL receptors and lowers plasma LDL levels by an average of 10 percent. A recent 10-year prospective study by the National Institutes of Health has shown that this 10 percent drop in LDL is sufficient to reduce the incidence of heart attacks by 20 percent. While this result is encouraging, it is not ideal: a more profound fall in plasma LDL levels is desirable.

How can we improve the ability of bile acid binding resins to stimulate the production of LDL receptors? This is where Endo's discovery comes in. The limited effectiveness of the bile acid binding resins is due to the dual response of the liver to cholesterol deficiency. In addition to producing more LDL receptors, the liver increases its production of HMG CoA reductase, the enzyme that controls cholesterol synthesis, and by this means it manufactures increased amounts of cholesterol. The increased cholesterol synthesis partially satisfies the increased demand for cholesterol and so prevents the liver from maximally increasing the number of LDL receptors.

Several years ago, we tested the hypothesis that an inhibition of cholesterol synthesis might force the liver to rely more on LDL uptake and thereby stimulate greater production of LDL receptors. To block cholesterol synthesis, we took advantage of Endo's discovery of a natural inhibitor of HMG CoA reductase, which he isolated in 1976 from a strain of penicillin mold. This chemical, called compactin, seems to have been designed by nature to be a perfect inhibitor of this enzyme. In the United States, Alfred W. Alberts and colleagues at the Merck, Sharp, and Dohme Research Laboratories have isolated from a different mold a structural relative of compactin, called mevinolin, which is even more potent.

In collaboration with Petri Kovanen, we administered a bile acid binding resin to dogs either alone or together with an HMG CoA reductase inhibitor. After two weeks, we obtained biopsies from the livers of the dogs and assessed the number of LDL receptors by measuring the ability of the liver membranes to bind radioactive LDL. We found that the bile acid binding resin produced a modest increase in the number of LDL receptors. When the HMG CoA reductase inhibitor was given together with the bile acid binding resin, there was a much greater increase in the number of receptors. At the whole body level this was reflected by a marked increase in the rate of removal of LDL from plasma, as determined from intravenous injections of radioactive LDL. As a result, the two drugs produced a remarkable 75 percent decline in the LDL level in these normal dogs.

Subsequent studies have confirmed that humans have a response that is comparable to that of dogs. David Bilheimer and Scott Grundy administered a bile acid binding resin and mevinolin to humans with heterozygous FH. The LDL level fell by approximately 50 percent, reaching the normal range. Injection of radioactive LDL into these individuals revealed that the drop in LDL levels was caused by an increase in LDL receptors. Thus, the normal gene in FH heterozygotes was made to work twice as hard as it usually would, and it therefore produced sufficient receptors to allow LDL to be removed from plasma at a normal rate.

There is one aspect to these studies that was predicted by the theory, but is nevertheless disappointing. FH homozygotes, who have no normal gene for the LDL receptor, do not respond to this combined drug regimen. Indeed, when a bile acid binding resin and mevinolin are administered to these children, there is no significant fall in LDL levels and no apparent increase in the rate of removal of LDL from plasma, a result that is to be expected from their complete genetic defect. Another approach must be found to treat the FH homozygotes.

The new approach was first applied to Stormie Jones, the six-year-old girl with homozygous FH who was discussed above. Thomas Starzl and his associates at the University of Pittsburgh removed the liver from a child who had died suddenly of an accident and transplatned it into Stormie Jones. Six months after this operation, Stormie was able to maintain a total plasma cholesterol level in the range of 300 mg/dl, whereas she previously had total cholesterol levels in the range of 1200 mg/dl. While liver transplantation is obviously not an ideal treatment for this disease, it nevertheless dramatically illustrates the ability of LDL receptors on the transplanted liver cells to lower LDL levels in such a patient.

Widespread Atherosclerosis in the Western World: The Problem of Jim Fixx (Figs. 8 and 9)

The foregoing analysis seems a plausible explanation for the high incidence of heart attacks in people who have genetic defects in the LDL receptor. But what about the vast numbers of people like Jim Fixx who have heart attacks or strokes but do not have a genetic defect in the LDL receptor? Have we learned anything from our studies of FH that may be of relevance to this large number of people? Accumulating data have led us to advance the LDL receptor hypothesis. This hypothesis states that much of the atherosclerosis in the general population is caused by dangerously high plasma levels of LDL, which result from a failure to produce sufficient LDL receptors to keep the LDL level in a healthy range. The inadequate production of receptors is attributable to environmental factors that limit production of receptors even in people with normal receptor genes. One of the environmental factors is a high dietary intake of cholesterol and saturated fats derived from animal tissues.

The starting point for our reasoning comes from epidemiologic surveys performed in many countries over the past 30 years. These surveys have uniformly shown that atherosclerosis becomes more severe as the mean level of LDL in the population rises. The LDL level rises, in turn, when the dietary intake of cholesterol and saturated fats is high.

How can we integrate this epidemiologic information with the experimental knowledge that the LDL level is controlled by LDL receptors? A likely explanation is suggested by the studies of cultured fibroblasts. As mentioned above, these studies show that a buildup of cholesterol within cells turns off the production of LDL receptors. If a high fat intake were to cause cholesterol to accumulate in the

liver - even in small amounts - it would partially suppress production of LDL receptors, and this might lead to an increase in the overall LDL level of a whole population.

Experiments in animals, carried out by our group and by Mahley and Innerarity in San Francisco, support the hypothesis that ingestion of a high fat diet reduces the production of LDL receptors in the liver. In animals such as baboons, rabbits, and dogs that are maintained on low fat diets, the concentration of LDL in plasma is much lower than it is in humans. In these animals the number of LDL receptors is high, and therefore these animals rapidly degrade intravenously injected radioactive LDL. When rabbits and dogs are fed diets high in cholesterol, the production of receptors in the liver is suppressed by as much as 90 percent, and this causes a buildup of IDL and LDL in the plasma.

At the time of birth, humans have concentrations of LDL in plasma that are similar to those of the other animal species, implying that newborn humans produce a high number of LDL receptors. However, during childhood and early adulthood in industrialized societies, the LDL level rises 3 to 4-fold. Studies of the metabolism of intravenously injected LDL show that this age-related rise in plasma LDL is attributable to a progressive decrease in the number of LDL receptors with age. A rise in plasma LDL levels with age is also seen in certain non-human primates, but it is not nearly so dramatic as in humans.

The causes of the acquired receptor deficiency in humans are not known in their entirety. Although a high dietary intake of animal fats is one important factor, it is not the sole factor. Even in humans who are raised on extremely low fat diets, plasma LDL levels tend to be above those in other animal species - although they are not so high as to be above the threshold for accelerated atherosclerosis. LDL receptors are known to be regulated by other factors in addition to diet. These include hormones such as thyroid hormone and estradiol, both of which stimulate production of receptors in the liver. Whether subtle abnormalities in these or other hormones contribute to the age-related fall in LDL receptors is unknown. Considered together, the data suggest that there is a general tendency for LDL receptors to decline with age in humans, and this tendency is aggravated markedly by the ingestion of the type of high fat diet that is customarily consumed in the United States and other Western countries. The concentration of LDL that is eventually reached in middle-aged adults seems to be far above the level that is conducive to a healthy arterial system.

A definitive test of the LDL receptor hypothesis would require a comprehensive and well-controlled study of the rate of metabolism of intravenously administered VLDL and LDL in members of populations who habitually consume low and high fat diets and who have varying LDL levels. Unfortunately, such studies have not yet been carried out on any systematic basis. However, William P. Hazzard and his associates at the Johns Hopkins University Hospital in Baltimore have recently shown that the ingestion of a high cholesterol diet (3 egg yolks per day) does lead to a decrease in the number of LDL receptors as measured directly in circulating blood cells, a finding that is consistent with the LDL receptor hypothesis.

If the LDL receptor hypothesis does prove to be correct, it would suggest that the human LDL receptor system is selected by evolution to function in an environment in which the intake of animal fats is extremely low. Unfortunately, the type of diet that is necessary to maintain such low LDL levels, and by inference high levels of LDL receptors, is markedly different from the customary diet in Western societies. Such a diet requires the total elimination of dairy

products and eggs as well as a severe limitation in the intake of meat and other sources of saturated fat such as coconut oil and palm oil. It is much more stringent than the modest limitation imposed by moderately low-cholesterol diets, such as those recommended by the American Heart Association.

For several reasons, we believe that an extreme dietary change, i.e., the total elimination of meat and dairy products, is not warranted for the whole population. First, such a radical change in diet would have severe economic and social consequences. Second, such a radical change in diet might well expose the population to other diseases that are now prevented by a moderate fat intake. Third, experience shows that most Americans will not adhere to an extremely low fat diet voluntarily.

But the most compelling argument against a radical dietary change for the whole population relates to the genetic variability among people. Among those who consume the current high fat diet of Western societies, only 50 percent will die of atherosclerosis. The other 50 percent are resistant to this disease. Some of these individuals resist atherosclerosis because their LDL level does not rise dangerously, even though they consume a high fat diet. These individuals may inherit genes that somehow keep their LDL receptors turned on despite a high fat diet. A whole population with such genes is the Pima Indian tribe of Phoenix, Arizona. Barbara Howard at the NIH Clinical Research Center in Phoenix showed that despite a high fat diet these Indians continue to produce relatively large numbers of LDL receptors and maintain low LDL levels. As a result, the Indians have a low incidence of atherosclerosis despite the presence of other risk factors such as diabetes mellitus, hypertension, and smoking.

Another source of genetic variation derives from the resistance of certain individuals to the damaging effects of high LDL levels on arteries. Even when LDL is elevated, not all people develop severe atherosclerosis. For example, 20 percent of men with heterozygous FH will not have a heart attack before age 60, despite their high LDL levels. Presumably, their arterial systems are somehow able to withstand bombardment by high levels of LDL. For those individuals who can withstand a high LDL level, the elimination of meat and dairy products would be a needless sacrifice and might well expose them to other diseases from which they are now protected.

Given these constraints, how can we rationally approach the prevention of atherosclerosis? One way is to individualize dietary recommendations. A diet that is moderately low in animal fats, such as that proposed by the American Heart Association, would seem prudent for most people. This diet will reduce blood cholesterol levels by up to 15% and should lessen the incidence of heart attacks somewhat. People who can maintain low LDL levels on such a moderate fat intake would not need to lower their fat consumption further. An exception to this rule might be made in people who have a strong family history of heart attacks and/or strokes. Clearly, these individuals may be especially susceptible to the damaging effects of LDL. Such individuals might be encouraged to maintain a very low dietary fat intake even though they might have an LDL level that is near the mean of the population as a whole. Hopefully, additional research will identify those factors that protect or sensitize people to the ill effects of LDL so that the ones at highest risk can be so advised. In the meantime, everyone in the population should be informed that they can also ameliorate atherosclerosis if they diminish other risk factors, such as smoking and high blood pressure.

Finally, it is possible that therapy with drugs that raise LDL receptors such as bile acid binding resins and HMG CoA reductase inhibitors may be appropriate for

many of the people in the population who do not have FH, but whose receptors are suppressed by diet or other environmental factors. If these drugs can be shown to prevent diet-induced suppression of LDL receptors and if they prove safe for long-term use, it may be possible for many of us to "have our steak and (live to) eat it too."

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LEGENDS TO FIGURES

Fig. 1. Atherosclerotic plaque develops over many years, as illustrated in this diagram by Russell Ross (Arteriosclerosis, 1, 293-311, 1981). The initiating event is damage to the endothelium, the single layer of cells that lines the artery wall. Endothelial injury is initiated by sheer forces at areas of turbulence in the bloodstream. Some damage occurs inevitably as a result of normal wear-and-tear. The damage is more severe when the blood pressure is elevated and when the level of blood sugar is high, as in diabetes mellitus. It is also increased by substances in cigarette smoke and may be accelerated by certain viral infections. The damaged endothelium becomes leaky so that blood constituents, including low density lipoproteins or LDL, penetrate into the tissue of the artery wall. Blood platelets adhere to the sites of damage where they release growth-promoting hormones, including the platelet-derived growth factor. In response to these hormones, smooth muscle cells, which reside in the layer underneath the endothelium, multiply and migrate into the area of damage in an apparent attempt to strengthen the wall. At the same time, circulating blood monocytes invade the damaged area, where they are activated to become tissue macrophages or scavenger cells. Together, the macrophages and the smooth muscle cells ingest and degrade the plasma constituents that entered the wall through the damaged endothelium. Experiments in animals show that if the damage stops at this point the wall will heal and the artery will be left with a small scar that does not obstruct blood flow. However, if the concentration of LDL in blood is elevated above a threshold value, the lipoprotein-bound cholesterol penetrates through the damaged endothelium in amounts that overwhelm the digestive capacity of the scavenger cells. In order for this cholesterol to be transported out of the artery wall, the cholesterol must be attached to a new protein carrier called high density lipoprotein or HDL. As the plaque grows in size, the availability of the HDL carrier becomes limiting and the cholesterol cannot be excreted from the wall. After multiple episodes of injury over many years, the wall is left with a collection of cholesterol, cells, and debris that obstruct the channel, eventually leading to blood clot formation and infarction.

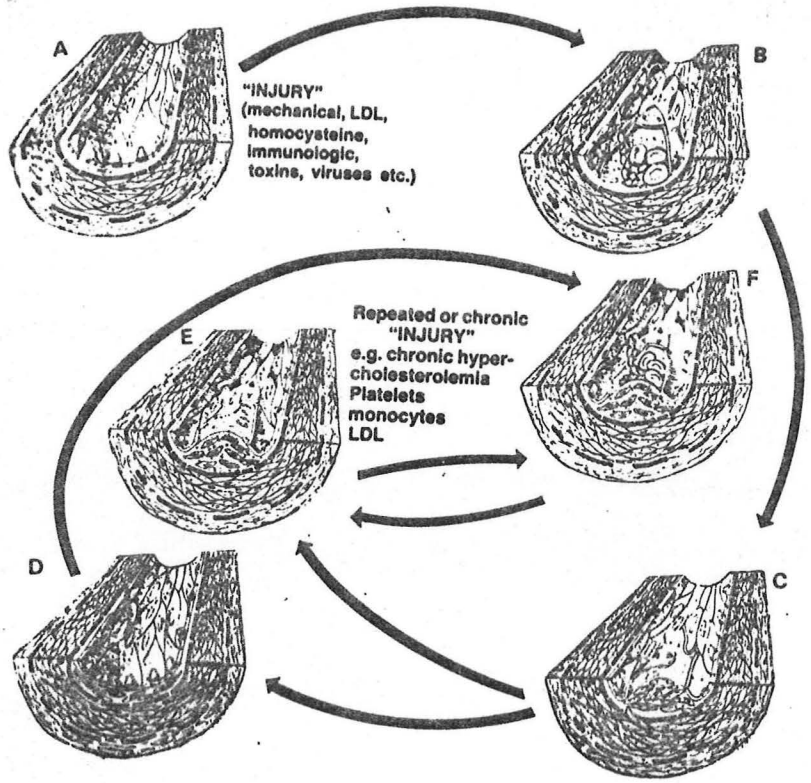


Fig. 2. Uptake of LDL by receptor-mediated endocytosis. LDL receptors are synthesized on ribosomes attached to the rough endoplasmic reticulum. Here, sugar chains are attached to certain amino acids of the receptor protein. Next, the receptor is carried from the endoplasmic reticulum to the Golgi complex, where the sugar chains are rearranged and lengthened to form mature receptors. The mature receptors are incorporated into vesicles that carry them to the cell surface, where they are inserted into the plasma membrane at random sites. On the surface the receptors cluster in pits that are coated on their cytoplasmic surfaces with the protein clathrin. Circulating LDL binds to an LDL receptor in a coated pit and is taken into the cell together with the receptor when the coated pit invaginates and pinches off to form a coated vesicle. The vesicle rapidly sheds its clathrin coat and fuses with other vesicles to form an endosome. Proton pumps in the membrane of the endosome cause the internal fluid to become more acidic and this causes LDL to dissociate from the receptor. The unoccupied LDL receptor cycles back to the plasma membrane where it again migrates to a coated pit, binds LDL, and delivers the LDL to endosomes. This cycle is repeated every 12 minutes in a remarkable process called receptor recycling. After its deposition in endosomes, the LDL is delivered to lysosomes, which are sacs that contain multiple digestive enzymes. These enzymes break down the apoprotein B-100 of LDL to its amino acid building blocks and cleave the ester bond of the cholesteryl esters to yield long-chain fatty acids and free cholesterol. The free cholesterol is transported out of the lysosome and is used by cells for the synthesis of membranes and other purposes.

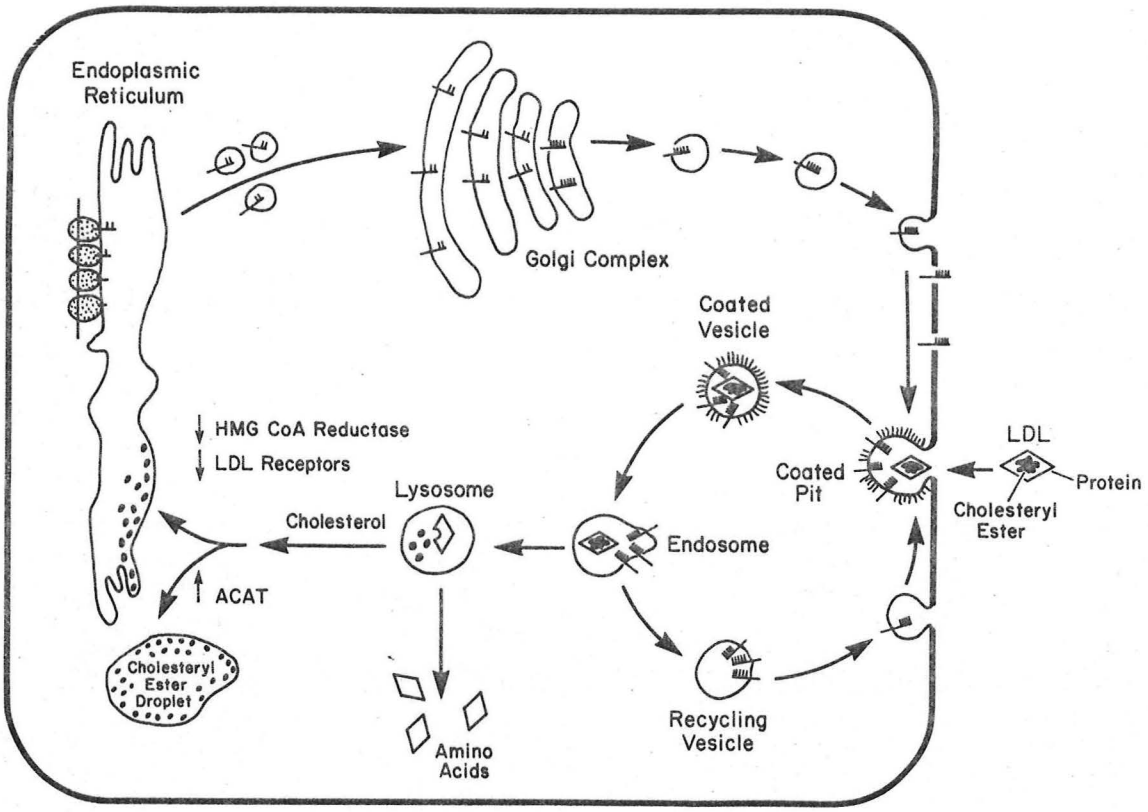


Fig. 3. The LDL receptor is composed of a single protein chain of 839 amino acids. Although the linear sequence of amino acids has recently been determined, the actual shape of the receptor is not known and is thus shown here in a highly schematic form. A short segment of 50 amino acids on the inner side of the plasma membrane is believed to interact directly or indirectly with clathrin, the protein that lines the inner surface of coated pits. Just above the carboxy terminal domain of the receptor is a stretch of 27 noncharged amino acids that traverse the lipid bilayer of the plasma membrane. Just outside of the plasma membrane is a short segment that is enriched in the amino acids serine and threonine; this is the site of attachment of a cluster of 9 to 18 O-linked carbohydrate chains, each chain consisting of four sugar molecules. In addition to the O-linked chains, the receptor has 1 or 2 N-linked carbohydrate chains that are attached to the amino acid asparagine. The amino terminal domain of the receptor contains a configuration of amino acids that binds to the apoprotein B-100 of LDL.

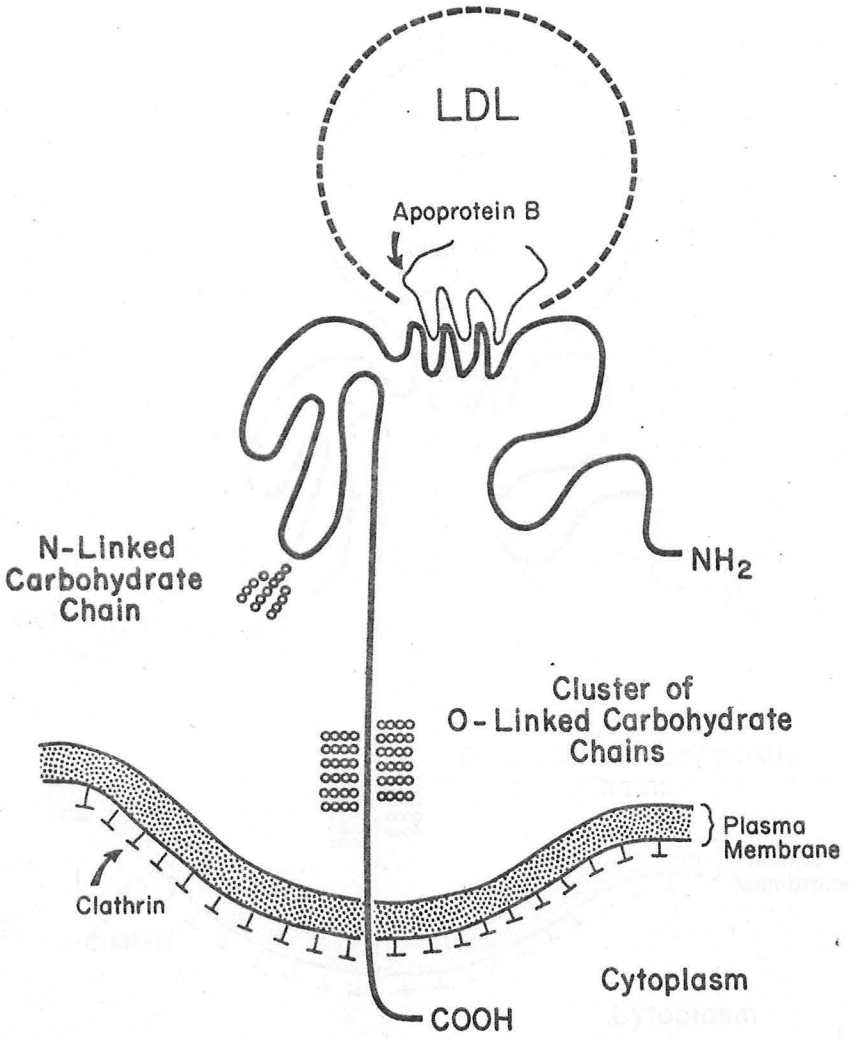


Fig. 4. Pathways of plasma cholesterol transport. Exogenous (i.e., dietary) cholesterol is absorbed in the intestine and packaged into chylomicrons, which contain triglyceride (three long chain fatty acids attached in ester linkage to glycerol) and cholesteryl ester. In the capillaries of body tissues, chylomicrons bind to an enzyme, lipoprotein lipase, which cleaves the ester bond in the triglycerides and allows the liberated fatty acids to escape from the lipoprotein and enter fat cells for storage. By this means chylomicrons are converted from triglyceride-rich particles to smaller cholesteryl ester-rich particles, called chylomicron remnants. The chylomicron remnants dissociate from the lipoprotein lipase and leave the capillaries. When they reach the liver, the remnants bind to specialized chylomicron remnant receptors and enter liver cells by receptor-mediated endocytosis. The liver disposes of the cholesterol by secreting it into the intestine in the form of free cholesterol or bile acid, or by secreting it back into plasma in newly synthesized lipoproteins of the endogenous cholesterol transport pathway.

Endogenous cholesterol transport begins when the liver secretes cholesterol into plasma together with triglycerides in a lipoprotein called very low density lipoprotein or VLDL. The triglycerides of VLDL are removed by lipoprotein lipase in the capillaries and the VLDL is converted into a smaller cholesteryl ester-rich particle that is called intermediate density lipoprotein or IDL. The IDL particles dissociate from the endothelium and return to the circulation. Some of the IDL particles bind to LDL receptors on liver cells and rapidly enter the liver cells by receptor-mediated endocytosis. Other IDL particles escape uptake by the liver and remain in the circulation where they are converted to LDL. Most of the LDL particles are removed from the circulation by receptor-mediated endocytosis after binding to LDL receptors on the surfaces of liver and other body cells (extrahepatic cells). As the membranes of cells are remodeled, cholesterol is continuously being released. When cholesterol leaves cells, it is immediately bound to high density lipoprotein or HDL. HDL interacts with a plasma enzyme called lethicin:cholesterol acyltransferase (LCAT). This enzyme converts the cholesterol to cholesteryl esters, which are transferred to IDL and then to LDL. This establishes a cycle by which IDL and LDL deliver cholesterol to tissues and in which cholesterol constantly leaches out of tissues onto HDL and is ultimately returned to new IDL and LDL particles.

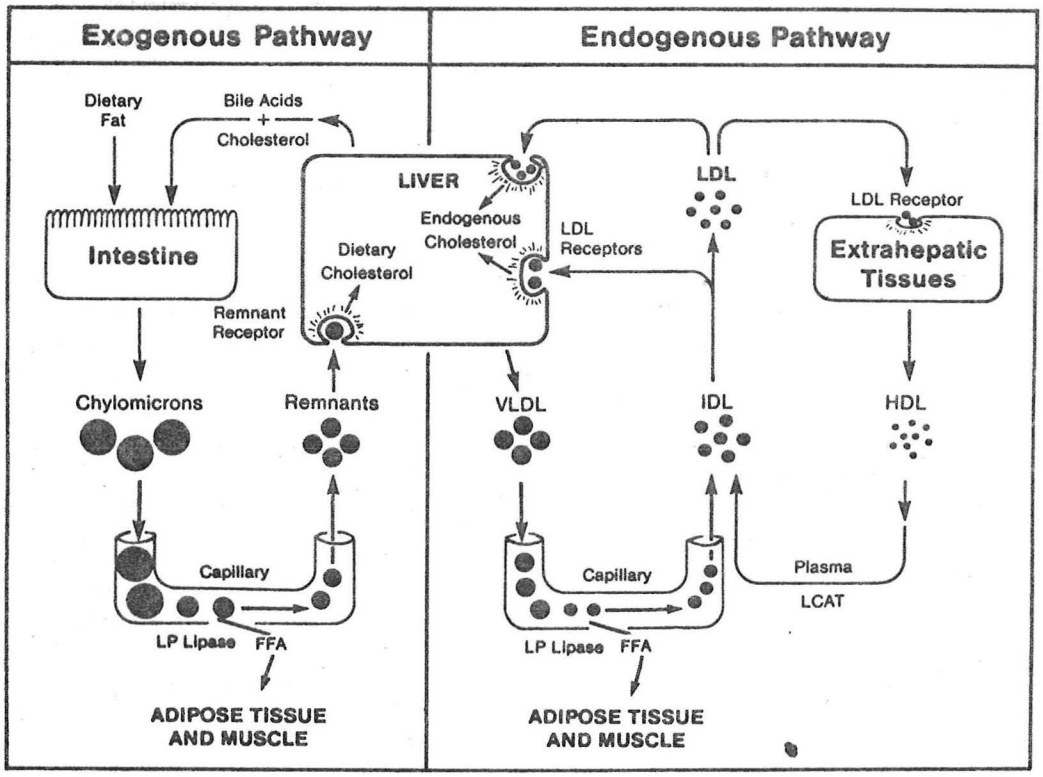


Fig. 5. The number of LDL receptors can be measured in human blood cells and in the whole body. For the cell assay, we isolate lymphocytes from the bloodstream and incubate them in a flask with radioactive LDL. After a few hours we measure the amount of radioactive LDL that has been degraded in lysosomes. This value reflects the amount of LDL that has entered the cell and is thus a measure of the number of LDL receptors. Cells from normal subjects have the highest number of functional receptors and take up and degrade LDL at the highest rate. Cells from FH homozygotes have few receptors and take up LDL at a very low rate. Cells from FH heterozygotes have an intermediate number of receptors and take up LDL at about half the normal rate. In the whole-body assay, a tracer amount of radioactive LDL is injected intravenously, and the radioactivity remaining in the circulation over the next several days is measured in small samples of blood. The higher the number of LDL receptors in the body, the faster the removal of LDL from the bloodstream. Normal individuals have the highest number of receptors and remove LDL from the circulation most rapidly. FH homozygotes have the lowest number of receptors and retain LDL in the circulation for the longest period of time. FH heterozygotes have a removal rate intermediate between normals and FH homozygotes.

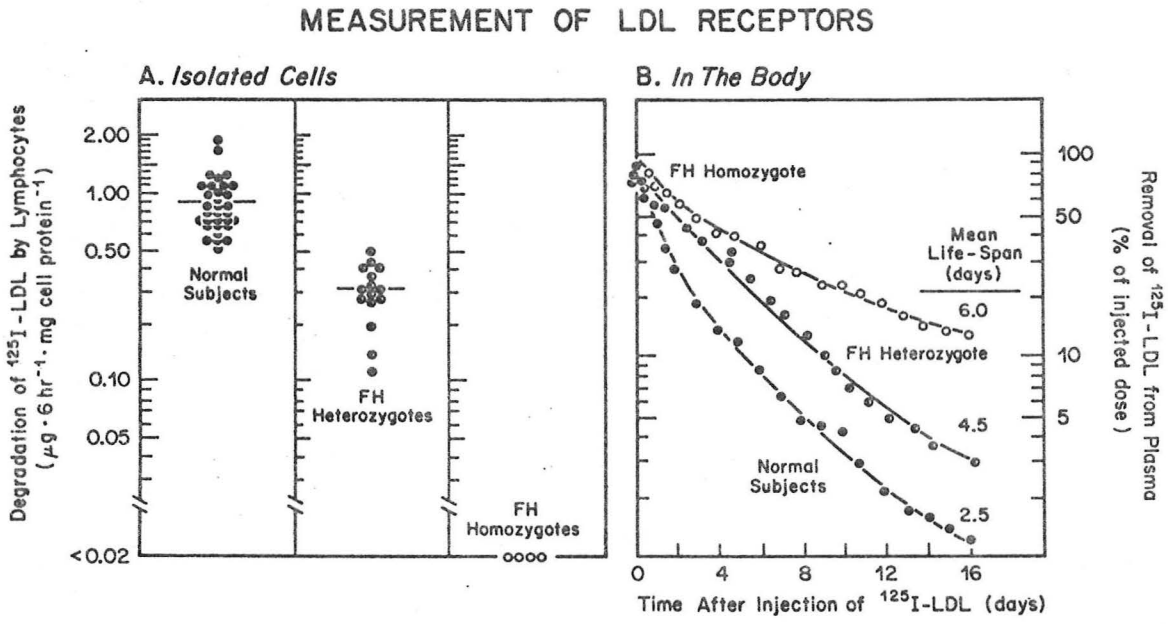
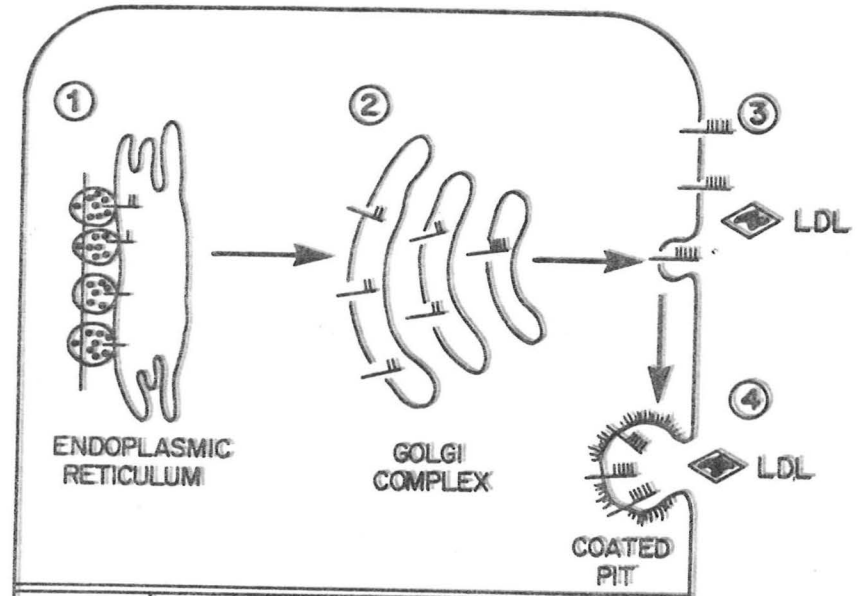


Fig. 6. Four classes of mutations in the LDL receptor gene have been identified in individuals with FH. Each mutation affects a different region in the gene. Mutations of Class 1 preclude the gene from directing the synthesis of any receptor protein. Class 2 mutations lead to the production of receptors that cannot be transported out of the endoplasmic reticulum. Class 3 mutations lead to the synthesis of receptors that are processed normally in the Golgi complex and reach the plasma membrane but are unable to bind LDL. Class 4 mutations lead to production of abnormal receptors that reach the plasma membrane and bind LDL normally, but fail to cluster in coated pits and therefore cannot carry the bound LDL into the cell. The genetic analysis that produced this classification was based on studies of cultured skin fibroblasts from 98 FH homozygotes and their heterozygous parents. These skin biopsies were sent to us by physicians in 25 countries around the world. WHHL rabbits, an animal model for homozygous FH, have a mutation in the LDL receptor gene that resembles the Class 2 mutation of humans.



Class of Mutation	Synthesis	Transport from ER to Golgi	Binding of LDL	Clustering in Coated Pits
1	X			
2	→	X		
3	→	→	X	
4	→	→	→	X

Fig. 7. Combined drug therapy with a bile-acid binding resin (such as cholestyramine) and an inhibitor of HMG CoA reductase (such as compactin or mevinolin) has been used in the treatment of FH heterozygotes in three medical centers, two in the United States and one in Japan. In all three studies the combined drug therapy lowered the plasma LDL level by about 50 percent. The Japanese study was carried out by Hiroshi Mabuchi at Kanazawa University. The study in Portland was carried out by D. Roger Illingworth at the Oregon Health Sciences Center University. The study in Dallas was carried out by David W. Bilheimer and Scott Grundy. In the Dallas study the fall in LDL levels was shown to be attributable to a more efficient removal of LDL from plasma, owing to stimulation of the production of LDL receptors. Compactin and mevinolin are in the early stages of drug development and are not yet available for widespread use. Their safety in humans over the long-term has not yet been tested.

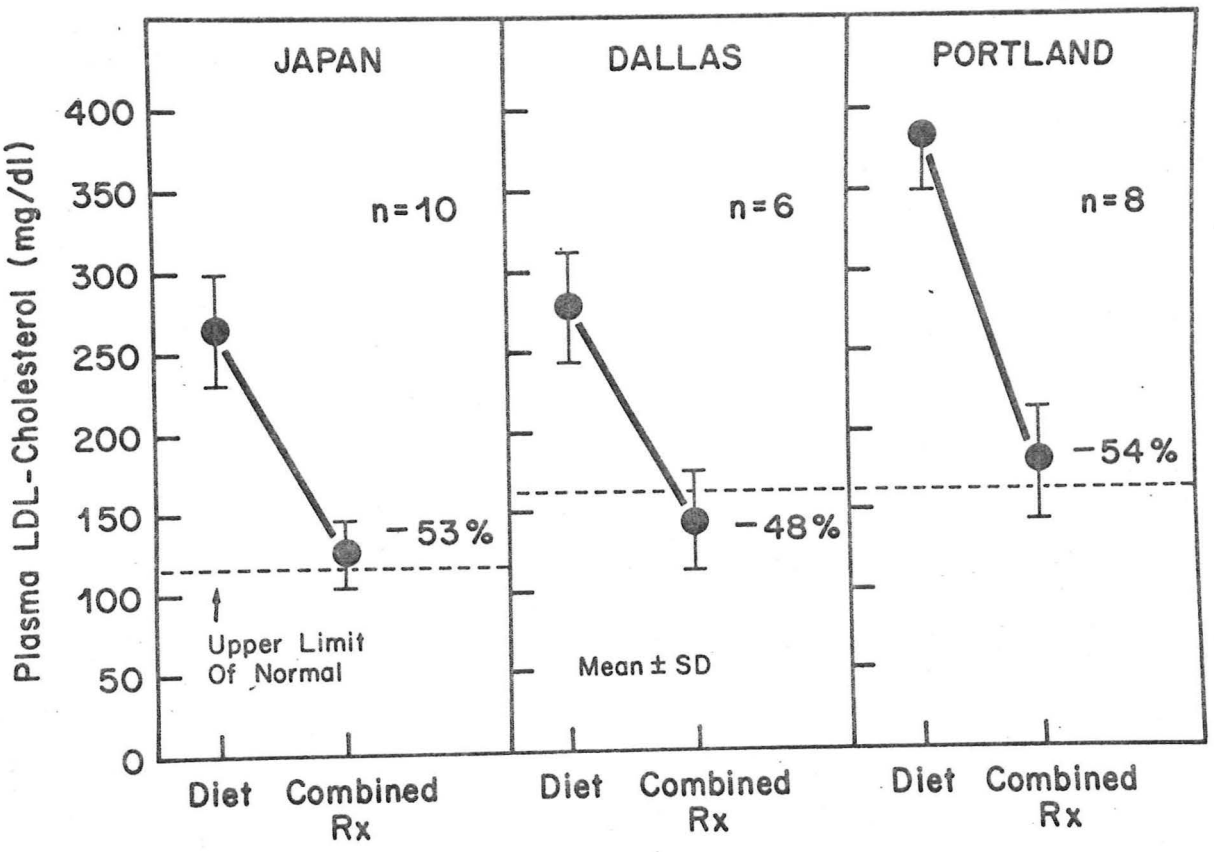


Fig. 8. Genetic defects and high fat diets reduce the number of LDL receptors in the liver, and this in turn leads to a combined overproduction and under-catabolism of LDL. In normal subjects VLDL is secreted by the liver and converted to IDL in body tissues. About half of the plasma IDL particles are taken up rapidly by binding to LDL receptors in the liver. The remainder of the IDL particles escape uptake in the liver and are converted to LDL. In individuals with familial hypercholesterolemia (FH), the number of LDL receptors in the liver is diminished owing to a defect in the gene encoding the receptors. An analagous (although less complete) deficiency can be produced in normal animals and humans by the ingestion of diets rich in cholesterol and saturated fats. By filling the liver with cholesterol, these diets cause the liver to cut back on its production of LDL receptors. The deficiency of receptors, whether genetic or acquired, has the same consequences for LDL metabolism: IDL particles can no longer enter the liver at a normal rate, and so they remain in the circulation where they are converted to LDL in increased amounts. The LDL, in turn, is removed slowly from the plasma. Thus, a receptor deficiency, either genetic or acquired, elevates the LDL level by two mechanisms: an increased rate of LDL production owing to increased conversion from IDL and a decreased rate of LDL catabolism owing to the slow removal of LDL from the circulation.

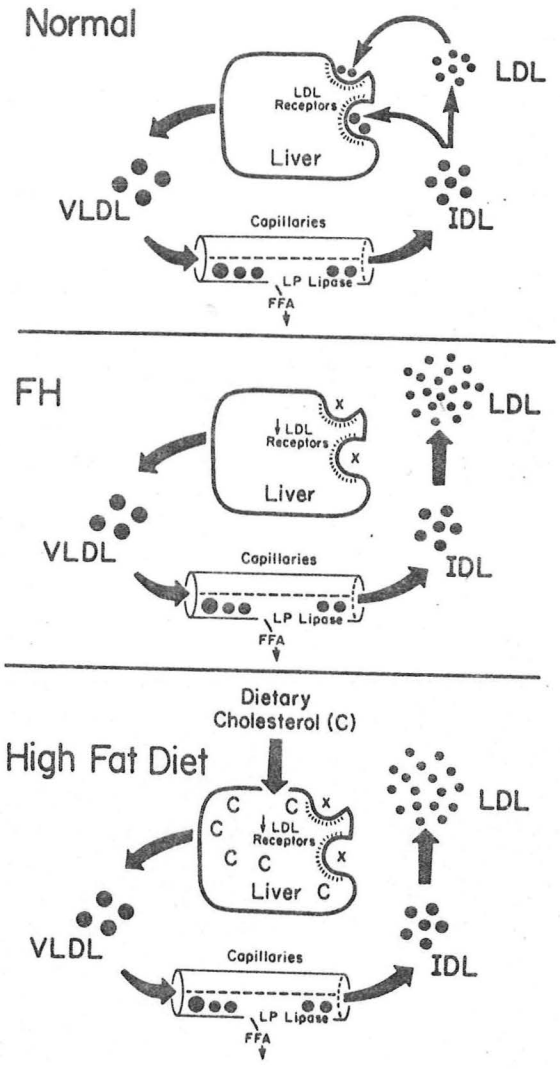


Fig. 9. More than half of the adults in the United States have plasma LDL levels that place them above the threshold for increased risk of atherosclerosis. The LDL level in these adults is much higher than the level in other animal species and in humans who consume extremely low fat diets. Studies of LDL catabolism suggest that this elevation can be attributed in large part to a decreased production of receptors in adult humans as compared with the lower animal species. Newborn humans, on the other hand, have LDL levels that are comparable to those of other animals, suggesting that they produce abundant LDL receptors. If human newborns are maintained on an extremely low fat diet, LDL receptors fall only slightly with age and LDL levels do not rise to the same extent as that seen in American adults. When a genetic defect in LDL receptors is present, as in FH heterozygotes, FH homozygotes, and in WHHL rabbits, the reduction in receptors is more severe and LDL levels rise profoundly and in a fashion that is independent of dietary fat intake.