Lowering Cholesterol, 1988

Rationale, Mechanisms, and Means

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This past year, 1987, has seen publication of some important studies of the ability of cholesterol-lowering therapies to prevent complications of coronary heart disease (CHD)[1] and to modify atherosclerotic lesions. It has also seen the release of the first of a new class of potent cholesterol-lowering drugs that inhibit beta-hydroxy-beta-methylglutaryl CoA reductase. Physicians are evincing new interest in treating patients with hypercholesterolemia and there is rapidly increasing public acceptance of the importance of measuring plasma cholesterol levels and acting upon the results. Clinical investigators who have been studying and treating patients with hyperlipidemia may have felt neglected in the past, but now they are showered with the attention of colleagues and patients. This attention is merited by the new information on the effectiveness of cholesterol-lowering therapies and increasing evidence that such treatment can influence health status. The recent issuance of guidelines for cholesterol lowering by the National Cholesterol Education Program provides help for practitioners and represents a consensus among the majority of clinical lipidologists, but many issues regarding indications for specific interventions need to be clarified. Moreover, new insights into the relationship between the plasma lipoproteins and atherogenesis could modify currently recommended approaches.

Here I will briefly review the intervention trials that provide the basis for current recommendations and point out areas of continuing uncertainty and controversy about the application of the results of these trials to the prevention of CHD and the treatment of established disease. I will discuss the metabolic basis for cholesterol lowering and the mechanisms by which diet and drugs reduce cholesterol levels. Finally, I will give a perspective on intervention strategies and techniques.

Cholesterol-lowering intervention trials

There have now been at least 10 trials in which diet or drugs have been used to modify the course of established CHD and three trials in which drugs have been used to prevent the disease (1). Only in the drug trials were patients selected on the basis of high cholesterol levels. Most of these trials yielded reduced risk of new or recurrent CHD manifestations or reduced the rate of progression of established atherosclerotic lesions. In the well-known Lipid Research Clinics Primary Prevention Trial (2), carried out in men whose mean serum cholesterol level was 294 mg/dl, the reduction of risk (~ 2% for each 1% reduction in serum cholesterol level) was well within the range of risk reduction observed in all of these trials.

The reduction of CHD risk in the primary prevention trial of the recently reported Helsinki Heart Study (3) was even greater in terms of cholesterol lowering (~ 4% for each 1% reduction in serum cholesterol level), but this was achieved with a fibric acid derivative, gemfibrozil, which also reduced serum triglycerides by 35% and increased HDL cholesterol levels by 10%. The participants' initial mean serum cholesterol level was 289 mg/dl and mean triglyceride level was 197 mg/dl. Reduction of LDL cholesterol level (8%) was somewhat less than in the Lipid Research Clinic trial (11%), but in the latter, serum triglyceride levels were not reduced and the increase in HDL-cholesterol was less.

In both of these trials, total death rate was higher in the treated than in the control groups, mainly as a result of accidents or suicide. In fact, in only one study has there been any evidence for a reduction in death rate—this was observed in individuals who had been treated with niacin for 5 yr in the Coronary Drug Project, carried out in the early 1970s. These individuals were evaluated again 9 yr after the trial had been completed (4).

The reason that risk reduction was greater in the Helsinki Heart Study than in the Lipid Research Clinics study is unclear. The investigators of the Helsinki Heart Study have suggested that the increase in HDL-cholesterol contributed to the favorable outcome. The actual increase in HDL-cholesterol (~ 5 mg/dl) was in fact commensurate with the reduction of plasma triglycerides (from 175 to ~ 110 mg/dl), based upon the established curvilinear relationship between VLDL triglycerides and HDL-cholesterol (5). A similar relationship has been observed with another fibric acid derivative, clofibrate (6). The close coupling of HDL-cholesterol to plasma triglyceride transport makes it difficult indeed to evaluate the distinct risks of hypertriglyceridemia and of reduced HDL-cholesterol, and renders attempts to apply statistical techniques to assign an independent role for either of doubtful significance. In this connection, the effect of another fibric acid derivative, bezafibrate, on HDL levels in healthy young men (7) is of interest. This drug increased HDL-cholesterol by ~ 10% while reducing serum triglyceride levels from ~ 67 to 52 mg/dl. The increase in HDL-cholesterol appeared to be confined to the

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1. *Abbreviations used in this paper:* CHD, coronary heart disease; CLAS, Cholesterol-Lowering Atherosclerosis Study; LCAT, lecithin-cholesterol acyltransferase; WHHL, Watanabe heritable hyperlipidemic.

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denser (HDL_3) subfraction, which is thought to be unrelated to CHD risk. This contrasts with its effect in hypertriglyceridemic individuals, in whom the predominant effect is to increase the concentration of the less dense (HDL_2) subfraction. Currently, there is no evidence that alterations in HDL level can by themselves influence CHD risk, and, as discussed below, there is little evidence that changing HDL level influences the process of reverse cholesterol transport, usually postulated to be the link between HDL levels and atherogenesis.

Regarding reduction of CHD risk, the results of the Helsinki primary prevention trial are in general agreement with the World Health Organization's trial of clofibrate (8), but in the latter trial clofibrate appeared to increase total mortality significantly (including an increase in gastrointestinal cancer). The reason for this difference between the results of the two trials is unclear. In both trials, treated patients had more cholelithiasis, an expected result of the increased saturation of bile with cholesterol caused by fibric acid derivatives, although the increase was not significant in the Helsinki trial.

The results of the Cholesterol-Lowering Atherosclerosis Study (CLAS), also reported last year (9), have aroused wide interest, in part because they have been considered to provide the first clear evidence that cholesterol-lowering therapy can reverse established plaque in human coronary arteries. This trial involved 188 men who had coronary artery bypass surgery. Participants had demonstrated a good response to treatment with colestipol (a bile acid–binding resin) and niacin in a pretrial test period. This drug combination, which had been shown to normalize LDL-cholesterol levels in compliant patients with heterozygous familial hypercholesterolemia (10), was used in the same dosage in one-half the patients in CLAS, together with a low-cholesterol, low-saturated fat diet. Total cholesterol level was reduced from 246 to 180 mg/dl, LDL-cholesterol from 171 to 97 mg/dl, and triglycerides from 151 to 110 mg/dl in treated patients, while HDL-cholesterol rose from 45 to 61 mg/dl (the disproportionate rise in HDL-cholesterol is an expected effect of treatment with niacin). Coronary angiograms, performed at the onset of treatment and after 2 yr, were evaluated by a blinded panel for change in lesions in native vessels and grafts. The number of native lesions that progressed was reduced from an average of 1.4 to 1.0 per subject, and 24% of treated as opposed to 39% of control subjects had new lesions or occlusions of bypass grafts. Importantly, the improvement with treatment occurred in subjects with entry serum total cholesterol levels between 185 and 240 mg/dl as well as in those with levels between 241 and 350 mg/dl. The CLAS investigators also concluded that 16.2% of treated subjects as compared with 2.4% of control subjects had "perceptible improvement" in overall coronary status, which was taken as evidence of regression of lesions. The random error of estimation of lesion change, however, would yield both apparent progression and apparent regression in individual lesions that in fact had not changed at all, so that a lower rate of progression in treated subjects could yield a greater number of lesions that "improved." At any rate, this study apparently provides the first unequivocal evidence that the progression of atherosclerotic plaques can be slowed with highly effective lipid-lowering therapy.

Metabolic basis of cholesterol-lowering therapy

The lipoproteins that carry potentially atherogenic lipoproteins in blood plasma are derived largely, if not entirely, from the liver (11). The liver synthesizes and secretes triglyceride-rich VLDL, which serve as vehicles for transport of triglycerides from liver to extrahepatic tissues. The formation and initial metabolism of VLDL resembles that of chylomicrons, which transport dietary triglycerides and cholesterol in the blood. With the removal of the major component of the core of VLDL particle (triglycerides) in extrahepatic tissues by lipoprotein lipase and the accompanying transfer of surface lipids (mainly phospholipids) and apolipoproteins to HDL, smaller VLDL remnant particles are produced. Some VLDL remnants, which retain apo E, are thereby recognized by LDL receptors on the surface of hepatocytes, leading to endocytosis and lysosomal catabolism of all remaining components of the particles. Other VLDL remnants are further metabolized, probably by lipase action on cell surfaces in the liver, to yield still smaller particles (LDL) that lack apo E and contain little triglycerides, but mainly cholesteryl esters in their cores. Each newly secreted VLDL particle contains one molecule of a large apolipoprotein (B-100) that is retained in remnants and the final product LDL. Apo B-100 in LDL is also a ligand for the LDL receptor and most LDL particles are also eventually taken up into hepatocytes by endocytosis and catabolized in lysosomes. VLDL remnants containing apo E are taken up into the liver in a matter of minutes to hours, presumably because they contain several molecules of the E protein and hence are able to bind multivalently and with very high affinity to LDL receptors. LDL, by contrast, bind to the receptor monovalently via the single molecule of apo B-100. The affinity of binding is, accordingly, much lower, accounting for the longer lifespan of LDL in the blood (normally $\sim$ 3 d). The ligand-binding domain of the LDL receptor is composed of seven repeating units of about 40 amino acids, each of which contains a cluster of negatively charged amino acids. The receptor-binding sites on apo E and B-100 contain clustered arginyl and lysyl residues that are positively charged. These residues must be properly exposed on the lipoprotein surface for the particle to bind to the LDL receptor. Nascent VLDL secreted by the liver contain one molecule of apo B-100 and a variable number of apo E molecules, but their binding regions are hidden. Apo C, present in large numbers on nascent VLDL, appear to have a role in the accessibility of these binding regions, preventing premature uptake of VLDL particles by the liver. As remnants are formed, the apo C are largely transferred to HDL and the binding domain of apo E, and eventually, that of apo B-100 becomes exposed.

The cholesteryl esters found in LDL as well as their VLDL precursors are not synthesized to any extent in the liver, but are transferred by the plasma cholesteryl ester transfer protein to the various apo B-containing particles from species of HDL, to which the enzyme lecithin-cholesterol acyltransferase (LCAT) is bound (12). LCAT esterifies cholesterol with a fatty acyl moiety derived from HDL-lecithin, yielding both lysolecithin and cholesteryl ester. The cholesterol substrate for LCAT is derived from the surface of various lipoproteins or from the plasma membrane of cells. That which is transferred from extrahepatic cells to HDL and then after esterification to VLDL and LDL can be taken up by the liver via the LDL receptor. This pathway of cholesterol transport from extrahepatic cells to the liver is thought to be the major route of reverse cholesterol transport.

In general, the higher the number of LDL receptors on hepatocytes, the more efficient the uptake of VLDL remnants,

so that fewer remnants remain in the blood to form LDL. At the same time, the higher the number of hepatocytic LDL receptors, the more efficient the removal of LDL. Hence modulation of the number of hepatocytic LDL receptors exerts a powerful dual effect on LDL levels in the blood. This effect has been well demonstrated in studies of normal and Watanabe heritable hyperlipidemic (WHHL) rabbits. WHHL rabbits have a mutation of the LDL receptor gene which results in an abnormal receptor protein lacking four amino acids in one of its ligand-binding domains (13). In normal rabbits, ~ 92% of VLDL remnants are taken up directly into the liver and only ~ 8% remain to form LDL (14). In WHHL homozygotes, ~ 60% of remnants are taken up directly and 40% are converted to LDL (chylomicrons, transporting dietary fat and cholesterol, and some large VLDL particles yield remnants that are metabolized normally by WHHL homozygotes) (15). The severe receptor deficiency does not alter the rate of secretion of nascent VLDL particles by the liver. Furthermore, as in normal rabbits, apo B-100 is secreted only as a component of nascent VLDL (16).

These observations suggest that the activity of hepatocytic LDL receptors does not influence the rate of production of potentially atherogenic lipoproteins, but does directly affect the removal of VLDL as remnants, the formation of LDL, and the efficiency of LDL removal. Higher receptor activity thus reduces the concentration of VLDL remnants and greatly reduces the concentration of LDL. In rats, stimulation of hepatocytic LDL receptor synthesis by administration of pharmacological amounts of ethinyl estradiol reduces LDL levels and plasma levels of apo B and E by > 80% (17). Transgenic mice recently have been produced in which the number of LDL receptors in the liver has been increased severalfold. The transfected construct contains the metallothionine promoter. When expression of the gene is increased by dietary cadmium, apo B and E virtually disappear from the blood (18).

Modulation of hepatocytic LDL receptor activity by diet and drugs

In their classical studies of the regulation of cholesterol homeostasis in cultured fibroblasts from normal individuals and familial hypercholesterolemia homozygotes lacking functional LDL receptors, Brown and Goldstein provided the basis for our current understanding of the mechanism by which diet and certain drugs affect plasma cholesterol levels (19). They showed that the availability of unesterified cholesterol within the cell regulates the number of LDL receptors on the cell surface. When cholesterol is delivered to regulatory sites via lipoproteins or by other means, the receptors are down-regulated. Conversely, when cellular cholesterol is depleted, the receptor is up-regulated. The rate of turnover of the receptor is sufficient to regulate lipoprotein uptake into the cell within a few hours both in cultured cells (19) and in the liver in vivo (20).

Dietary cholesterol, taken into the liver with chylomicron remnants, tends to down-regulate hepatocytic LDL receptors. The extent of down-regulation depends upon the ability of the liver to excrete this cholesterol, mainly into the bile. In the rat, dietary cholesterol is rapidly excreted, mainly as bile acids (21). In rabbits, bile acid formation is limited, hepatic cholesterol stores rise rapidly, the receptor is down-regulated, and hypercholesterolemia ensues (abetted by secretion of cholesterol-enriched VLDL from the liver) (22). Humans evidently vary in their capacity to excrete cholesterol into the bile and to convert cholesterol to bile acids, but in general humans resemble rats more closely than rabbits in this respect (23). Hence, dietary cholesterol has a variable and usually limited effect on human plasma cholesterol levels, presumably because the liver has relatively little need to alter receptor number to maintain cholesterol homeostasis (24).

Recent studies in hamsters have shown that dietary saturated fat also down-regulates hepatocytic LDL receptors (25). The mechanism of this effect remains to be determined. In humans, saturated fats raise plasma LDL levels and also tend to increase those of HDL (26). This important dietary component thus may have effects on cholesterol or lipoprotein metabolism that extend beyond hepatocytic LDL receptors.

That LDL receptors are potent regulators of plasma LDL levels in humans, is, however, clearly evident from the doubling of LDL levels in familial hypercholesterolemia heterozygotes. Moreover, two important classes of drugs that alter LDL levels effectively in humans appear to increase hepatocytic LDL receptors by depleting hepatic cholesterol. Bile acid–binding resins do this by increasing the conversion of cholesterol to bile acids, consequent to interruption of the enterohepatic circulation of bile acids (27). 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors reduce hepatic cholesterol biosynthesis competitively by binding to the active site of the enzyme (28). As used clinically, the reductase inhibitors are almost twice as effective in reducing LDL-cholesterol levels as the resins (29), indicating the importance of cholesterol synthesis in maintaining hepatic cholesterol homeostasis despite a substantial intake of cholesterol in the diet. Whereas the effect of the resins is limited, presumably by the large compensatory increase in cholesterol biosynthesis (27), studies in animals suggest that increasing doses of reductase inhibitor can increase receptor number to exceedingly high levels, reducing LDL levels drastically (30).

Both the reductase inhibitors and resins reduce cholesterol levels in virtually all individuals who have functioning hepatocytic LDL receptors (i.e., in all but familial hypercholesterolemia homozygotes with absent functional receptors on the surface) (27, 28). They have quite different effects, however, on VLDL levels: resins tend to increase VLDL levels (27), whereas reductase inhibitors reduce them (31, 32). The effect of resins is thought to reflect increased production of VLDL, which is coupled in some way to the increased cholesterol synthesis that they induce. Reduction with reductase inhibitors may reflect increased removal of VLDL remnants, but an effect on VLDL synthesis has not been excluded. With both classes of drug, efficiency of LDL removal from the blood is usually increased (27, 33), but sometimes this is not observed —rather, reduced LDL formation appears to underlie the reduction of LDL levels (34, 35). Interpretation of such data is difficult because of the kinetic heterogeneity among LDL particles (35), but the variability could reflect differing effects of receptor induction among individuals on the removal of VLDL remnants. In some cases, increased uptake of remnants may competitively inhibit LDL removal despite increased LDL receptor number. When resins and reductase inhibitors are given together in the usual dose to humans, an effective complementary effect is observed, reflecting a blunting of the resin-induced increase in cholesterol synthesis (36–38).

The addition of a reductase inhibitor (lovastatin) to the binary combination of resin and niacin has yielded further

reduction of LDL levels in familial hypercholesterolemia heterozygotes by as much as 75% (39). Since niacin also effectively lowers LDL by reducing VLDL secretion and raises HDL levels (40), patients on this ternary regimen usually have greatly reduced VLDL and LDL levels, whereas HDL levels are increased substantially. Current studies suggest that the amount of niacin required to achieve optimal reduction of lipoprotein levels (with serum cholesterol levels < 200 mg/dl) is considerably lower than that needed with the standard binary regimen. These and other studies of drug combinations suggest that such rational treatment regimens may lead to increased compliance and, possibly, lower toxicity.

Bile acid–binding resins have been used for many years to lower LDL-cholesterol levels and are known to be safe and effective, but they are difficult for many patients to tolerate in large doses (tolerance can be optimized by starting with small doses taken with meals and by treating constipation with bran or stool softeners). Some physicians have long used niacin successfully to lower LDL-cholesterol levels, but careful education of patients, gradual increase in dosage, and close monitoring for toxicity are required. By contrast, reductase inhibitors are easy to take and rarely produce any symptoms, but they have been used for only a few years, so that their long-term safety remains to be established. Effects of reductase inhibitors on CHD risk or atherosclerotic lesions also remain to be demonstrated. Only two toxic effects of these drugs have been observed to date. First, they produce myopathy with substantial elevations of serum creatine phosphokinase levels in ~ 0.5% of individuals (30). This seems to be more common in patients taking fibric acid derivatives (which can have similar effects) with reductase inhibitors and it has also been observed in cardiac transplant patients taking cyclosporin, sometimes with rhabdomyolysis (41, 42). The effect with fibric acid derivatives is particularly unfortunate because this combination could be quite useful in patients with combined elevations of LDL and VLDL. Second, reductase inhibitors can occasionally cause substantial elevations of serum transaminases, perhaps reflecting hepatotoxicity (slight elevation of transaminases are seen with a number of lipid-lowering drugs, including resins) (28). It is not yet known whether this effect is dose related, but it may be more common in patients who have preexisting liver disease and in alcohol abusers. Other toxicities have been observed in animals given very large doses of reductase inhibitor. A number of these seem to be mechanism based, because they are prevented by administration of mevalonate (30). In dogs, lenticular opacities have been observed with very large doses of lovastatin, but these occur only when plasma cholesterol levels have been reduced by 80% or more. Even under these conditions, tissue concentrations of important nonsterol products of 3-hydroxy-3-methylglutaryl CoA, such as ubiquinone and dolichol are reduced only in certain sites and species. Unless additional toxicity is found with wider use in humans taking therapeutic doses, reductase inhibitors will probably become the primary class of drugs used to lower cholesterol levels. Reductase inhibitors are also teratogenic in some animals given large doses; they are not currently recommended for women who are likely to conceive.

Treatment of combined hypercholesterolemia and hypertriglyceridemia

Moderate to severe hypertriglyceridemia occurs most commonly with abdominal (centripetal) obesity occurring in a susceptible host, and usually responds to restriction of caloric intake, restriction of alcohol, or both (43). Moderate hypertriglyceridemia (levels < 500 mg/dl), occurring in thin individuals who have a normal or reduced LDL-cholesterol level, is not ordinarily thought to increase risk of CHD and hence does not require treatment (43). Many individuals with combined moderate elevations of LDL and VLDL, as in familial multiple type hyperlipoproteinemia, are at risk for premature CHD. Their problem may not be adequately addressed by restriction of saturated fats and cholesterol or by drugs that stimulate hepatocytic LDL receptors. Niacin, either alone or in combination with resin or reductase inhibitor, usually reduces the levels effectively (40). Reduction of LDL-cholesterol levels in such individuals who receive a fibric acid derivative, as in the Helsinki Heart Study trial, may be suboptimal, but a trial with one of these drugs is appropriate because responsivity varies considerably. When drug treatment is judged to be appropriate, an empirical approach thus is justified, beginning with an easily tolerated agent, but it must be borne in mind that the risk of precipitating gout or diabetes mellitus with niacin, or of cholelithiasis with a fibric acid derivative, is probably increased with obesity. Drug combinations can also be used, but (as stated earlier) there appears to be an increased risk of myopathy when fibric acid derivatives are given with a reductase inhibitor.

Reduction of CHD by cholesterol lowering

The report of the National Cholesterol Education Program's expert panel on detection, evaluation, and treatment of high blood cholesterol in adults now provides detailed guidance to physicians (44). By its standards, most Americans should be restricting their intake of saturated fats and cholesterol. A committee of the National Research Council on Diet and Health is currently considering dietary recommendations aimed at increasing the health of the nation by reducing the burden of chronic disease (45). This committee has been charged with the task of relating the intake of specific foods and dietary patterns to the incidence of heart disease, cancer, osteoporosis, and other chronic disorders. The National Cholesterol Education Program's expert panel recommends major dietary alterations for individuals judged to be at increased risk for CHD, including, if necessary, reduction of daily intake of saturated fat to < 7% of total caloric intake and of cholesterol to < 200 mg. This recommendation applies to all those with LDL-cholesterol levels > 160 mg/dl (equivalent, on the average, to a total serum cholesterol > 240 mg/dl) and, for those with CHD or two or more other CHD risk factors, > 130 mg/dl (serum cholesterol equivalent > 200 mg/dl). To comply with such a dietary program and to make it effective, much effort will be needed on the part of clinical chemists to provide reliable estimates of LDL-cholesterol, from physicians, dieticians, and other health professionals to guide patients, from educators to provide improved tools to help patients understand the guidelines, and from the agricultural and food industries to provide appropriate foods that are palatable and simple to prepare.

The National Cholesterol Education Program is patterned after the National High Blood Pressure Education Program, which has contributed to the improved treatment of hypertension during the last 15 yr. The recommendations are not conditioned by the considerable increase in LDL-cholesterol levels

with age, but rather are consistent with the hypothesis that risk is a function of the absolute level of LDL cholesterol, not of the ranking of the individual's level among peers. In this respect the new recommendations represent a clear improvement over those of the Consensus Conference on Hypercholesterolemia in 1985, which were based on age-specific cutpoints (46).

The new program is much more ambitious and will be more difficult to implement than the National High Blood Pressure Education Program, depending as it does primarily on dietary change rather than drugs to lower cholesterol. The recommendations are accepted by many, but not all, experts in the cholesterol field. In particular, the dietary recommendations for adults with LDL-cholesterol levels between 130 and 160 mg/dl (equivalent to average serum cholesterol levels between 200 and 240 mg/dl) are not accepted by all. This recommendation relies heavily on the observation made in more than 300,000 white men in the Multiple Risk Factor Intervention Trial that CHD mortality increases continuously above ~ 180 mg/dl (47). The relationship observed in that study is quite close to that found in subjects of the Framingham study and the control subjects in the Lipid Research Clinics Intervention Trial (1). The recommendation also recognizes that even though the slope of the risk relationship rises much more rapidly with cholesterol levels > 240 mg/dl, (so that the relative and absolute benefits of cholesterol lowering are less in those in the 200–240 mg/dl range), such individuals account for most of the mortality from CHD in the United States. Arguments against intervention in this range are based in part upon evidence that a threshold exists, below which the relationship is undetectable (48), and to the limited benefit to be derived from modest lowering of LDL-cholesterol levels (49). The recent CLAS investigation addresses this issue for those with established CHD. Those treated in that study with LDL-cholesterol levels < 170 mg/dl appeared to benefit as much as those treated with higher levels (9).

The use of drugs to lower cholesterol is almost certainly more risky than dietary alterations. The guidelines of the National Cholesterol Education Program therefore tailor the intensity of treatment to the level of risk, as judged from the LDL-cholesterol level and the presence of two more additional risk factors (male sex is one of these and family history of CHD before age 55 is another). The drugs recommended as first-line agents are resins and niacin. With the results of the Helsinki Heart Study, gemfibrozil might now be added. Drugs are recommended for all adults with LDL-cholesterol levels > 190 mg/dl on a saturated fat and cholesterol-restricted diet. These are formidable recommendations for drug treatment, which could result in millions of Americans taking one or another cholesterol-lowering agent. Individuals taking these drugs will need close medical supervision throughout their lives.

Cholesterol lowering with dietary measures is generally recommended after age 2 (46) and with drugs after age 5 (50). This recommendation is based on observations that cholesterol levels track as individuals age (51), that children with high cholesterol levels tend to come from families with a high prevalence of CHD (52), and evidence that advanced atherosclerotic plaques in the coronary arteries are not rare in individuals under the age of 30 (53). Dietary modification can be achieved even in young children provided that this is adopted by the family unit, but experience has shown that many children fail to adhere to diets, and especially to prescribed drugs, when they enter adolescence. It is therefore reasonable to defer drug treatment until early adult life in all but the most severe cases of hypercholesterolemia.

Is there an age after which cholesterol-lowering therapy becomes pointless? Certainly there must be, but there is little basis for making arbitrary distinctions based on age alone (44). A clear answer to this issue will probably have to await improved indices of risk or the ability to evaluate the status of the coronary vasculature noninvasively. Given a history of long-lived forebears, vigorous cholesterol lowering may be inappropriate, however, in octagenarians and those older.

Many of those who respond to cholesterol-lowering treatment are likely to benefit in terms of years of continued health and longer lifespan, but it is also clear that many will not. There is nothing new about this observation, which also applies to the treatment of hypertension. It is pertinent in this context, however, to consider the mechanism by which LDL may cause CHD. There is increasing evidence that LDL may not be intrinsically atherogenic. Familial hypercholesterolemia homozygotes inevitably develop atherosclerotic disease, but these unfortunate individuals have not only extraordinarily high LDL levels, but also a substantially increased concentration of VLDL remnants, which are rich in cholesterol and apo E (54). Such lipoproteins (so-called beta-VLDL) account for most of the hypercholesterolemia in cholesterol-fed rabbits, which invariably develop cholesterol-rich atheromata and eventually lesions resembling advanced human atherosclerotic plaques (55). Familial hypercholesterolemia heterozygotes may also have some increase in VLDL remnants but the great majority of their hypercholesterolemia is accounted for by elevated LDL levels. Recent angiographic studies of asymptomatic adult heterozygotes have shown that an appreciable number have no demonstrable abnormality of coronary arteries even beyond age 50 (Malloy, M. J., et al., unpublished observations).

Familial hypercholesterolemia heterozygotes do not accumulate cholesterol indiscriminately in cells or tissues. This is well understood in terms of the role of the LDL receptor in cholesterol homeostasis (56). The receptor is a component of a well-regulated system which maintains cellular cholesterol concentrations within narrowly defined limits (most cellular cholesterol is contained within the phospholipid bilayer of the plasma membrane). Down-regulation of the receptor and inhibition of cholesterol synthesis, together with egress of cellular cholesterol in the pathway of reverse cholesterol transport, all contribute to cellular cholesterol homeostasis.

Cholesterol in developing atheromas is now thought to accumulate mainly in intimal macrophages (57). Macrophages express LDL receptors (58), but although these receptors are poorly down-regulated with cellular cholesterol accumulation, they also recognize LDL poorly (as compared with beta-VLDL). Incubation of macrophages with beta-VLDL regularly produces foam cells, but incubation with LDL does not. In recognition of this problem, investigators began several years ago to investigate the possibility that chemical modification of LDL would yield particles that are taken up in a poorly regulated manner and produce foam cells resembling those found in early atheromas. The first of these was modification of lysyl residues to produce acetylated LDL (59), and several others, including some that might conceivably occur in the artery in vivo, have since been found (60). All of these modified LDL particles are recognized by a distinct receptor, internalized via coated pits, and catabolized in lysosomes to release

cholesterol that is subsequently esterified and stored in cytoplasmic fat droplets. This scavenger receptor is not down-regulated when the cells store cholesterol, and it is thought that eventual rupture of engorged foam cells is a major mechanism for the accumulation of extracellular cholesterol in mature atherosclerotic plaques.

LDL that are incubated with arterial extracts in the presence of a divalent cation, such as copper, are oxidatively modified with formation of lipid peroxides and partially degraded forms of apo B-100 (60). These modified LDL are taken up into macrophages by the scavenger receptor. Recently, it has been shown that administration of probucol, a cholesterol-lowering drug whose mechanism of action is poorly understood, to WHHL rabbits substantially reduces the rate of formation of atherosclerotic lesions, despite little cholesterol lowering (61, 62). Probucol also causes disappearance of cutaneous xanthomas in familial hypercholesterolemia homozygotes, accompanied by only moderate cholesterol lowering (63, 64). In vitro, probucol, a lipophilic antioxidant, prevents the oxidative modification that leads to uptake of LDL by the scavenger receptor (65). Indeed, probucol was synthesized as an analogue of butylated hydroxytoluene. These observations, most of them quite recent, are consistent with the hypothesis that LDL accumulate in macrophages and thereby become atherogenic only when they are oxidatively modified. Modified LDL are rapidly removed from the blood via scavenger receptors on hepatic sinusoidal endothelial cells or Kupffer cells (66). Modification, therefore, is likely to occur within the arterial intima. Recently, with specific antibodies, modified apo B has been demonstrated in atherosclerotic plaques of WHHL rabbits (67).

In the future, it may be necessary to consider the relationship of plasma lipoproteins to atherosclerosis, not only in terms of the concentration of atherogenic (or antiatherogenic) lipoprotein particles, but also in terms of the tendency of lipoproteins to be modified. The large variation in human LDL levels that occurs in populations eating similar diets cannot be explained by environmental influences, but, indeed, seems to be largely genetically determined. The incidence of familial hypercholesterolemia, as clinically defined, is so low ($\sim$ 1:500) that it can account for little of this variation. Recently, it has been shown that the common polymorphisms of apo E (including not only the E-2 isoform, which is poorly recognized by LDL receptors, but also the E-4 isoform, which is preferentially associated with VLDL particles rather than HDL) contribute to $\sim$ 7% of the variation in LDL-cholesterol levels (68). It seems likely that polymorphisms of apo B-100, one of the largest proteins in nature (4,536 amino acids in a single chain), will account for additional genetic variation. A mutation of this protein that interrupts receptor binding has recently been identified (69) and a restriction fragment length polymorphism that does not affect the coding region of the apo B gene is reportedly associated with increased LDL levels (70). Given our current knowledge of the regulation of LDL levels by the LDL receptor (admittedly little is known of the regulation of VLDL synthesis), it is difficult to see how the classification of individuals with respect to protein polymorphisms will greatly alter our current approach to cholesterol lowering. Perhaps discovery of genetic determinants of the propensity toward oxidative modification of LDL within the arterial wall will provide an independent and more useful marker for atherogenic and CHD risk. Indeed, it seems possible that prevention of LDL-modification may supplement or, in at least some cases, obviate the need to lower the levels of potentially atherogenic lipoproteins.

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