Workshop I
Lipoproteins and the Pathogenesis of Atherosclerosis

Participants
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Professional and Public Education and Community Programs

Background. All patients with clinical coronary artery disease, and particularly those undergoing coronary artery bypass surgery or angioplasty, should receive special attention. Current National Cholesterol Education Program (NCEP) and American Heart Association (AHA) guidelines use a total cholesterol value of 200 mg/dl as a cut point, but an occasional patient may have, as an example, a total cholesterol value of 190 mg/dl and an elevated low density lipoprotein (LDL) level of 135 mg/dl because his or her high density lipoprotein (HDL) cholesterol is low (e.g., 30 mg/dl) and the very low density lipoprotein (VLDL) cholesterol is also low (e.g., 25 mg/dl). To avoid missing such patients, the panel strongly suggested that every patient with established clinical coronary heart disease have a complete lipid profile initially, regardless of total cholesterol level.

Research Initiatives

Background. Although the current NCEP-AHA recommendations are focused on the measurement of serum cholesterol levels and, in some cases, on triglyceride and HDL levels, current research indicates that measurement of specific subfractions of lipoproteins and apolipoproteins may prove more useful. The rationale for seeking more predictive markers of individual risk is that some persons for whom treatment would currently be prescribed may not actually need treatment; conversely, some of those who would not be treated under current guidelines should be treated.

Recommendations. The panel unanimously dissented from the position that triglyceride levels have been ruled out as an independent risk factor. The panel proposed that the epidemiologic evidence leading to the conclusion that hypertriglyceridemia is not an independent risk factor may be flawed. The issue of the potential atherogenicity of triglyceride-rich lipoproteins has not been resolved.

Dr. Robert Mahley introduced the discussion of atherogenicity of lipoproteins, stressing the important lessons that have been learned from the study of genetic disorders. Three genetic diseases of lipid-protein metabolism, type III hyperlipoproteinemia, familial hypercholesterolemia, and familial defective apolipoprotein B-100 (apo B-100) provide key insights into the role of specific lipoproteins in atherogenesis. The importance of understanding these disorders lies in the close parallels between lipoprotein profiles associated with these genetic disorders and those induced by consumption of diets high in saturated fat and cholesterol.

Chylomicron Remnants and Very Low Density Lipoprotein Remnants
Type III Hyperlipoproteinemia

Background. Type III hyperlipoproteinemia is associated with hypertriglyceridemia and hypercholesterolemia, which are characterized by the accumulation of cholesterol-rich remnants of chylomicrons and VLDL or intermediate density lipoproteins (IDL) (collectively known as β-VLDL) in the plasma. Patients with type III hyperlipoproteinemia develop accelerated atherosclerosis involving both coronary and peripheral arteries. In considering the potentially atherogenic lipoprotein levels in this disorder, it is important to note that type III patients usually have low levels of LDL and HDL and that the hyperlipidemia is associated exclusively with an elevation in the level of β-VLDL. The underlying genetic defect responsible for the lipoprotein abnormalities is the synthesis of an abnormal form of apo E, that is, a form that does not bind normally to either the apo B,E(LDL) receptors or, presumably, the remnant (apo E) lipoprotein receptors. (For a more complete discussion of type III hyperlipoproteinemia, see Mahley and Mahley et al.)

The abnormal apo E, defective in its ability to bind to the lipoprotein receptors, disrupts the normal metabolism of chylomicron and VLDL remnants (the β-VLDL). The impaired receptor-
mediated uptake of these lipoproteins results in their accumulation in the plasma. In addition, the presence of the abnormal form of apo E interferes with the normal formation of LDL from VLDL and IDL in type III patients. This process could contribute to the low level of LDL in patients with type III hyperlipoproteinemia and could further contribute to the accumulation of β-VLDL in the plasma. The cholesterol-rich remnants (the β-VLDL) that accumulate must be cleared from the plasma by an alternative pathway. The alternative route may well be through macrophages (scavenger cells), including macrophages of the arterial wall that participate in foam-cell production in atherosclerotic lesions.

Several lines of evidence implicate β-VLDL in atherogenesis and suggest that macrophages are involved in the process. (For reviews, see Mahley,1 Mahley et al,2 Mahley and Innerarity,4 Brown and Goldstein,5 and Steinberg.6) Macrophages are capable of taking up chylomicron and VLDL remnants by way of their apo B,E(LDL) receptors. Chylomicron and VLDL remnants cause massive cholesteryl ester accumulation in cultured macrophages, whereas LDL and other cholesterol-rich lipoproteins (HDL cholesterol) do not.7 In addition, it has been shown that foam cells within the arterial wall have β-VLDL receptors.8

In further support of the postulate that β-VLDL may be atherogenic, animals fed diets high in fat and cholesterol have markedly elevated levels of β-VLDL (chylomicron and VLDL remnants) in their plasma and develop accelerated atherosclerosis.1,2 These diet-induced β-VLDL also cause cholesteryl ester accumulation in macrophages. Furthermore, lipoproteins resembling β-VLDL are seen in human plasma after consumption of a single high-fat, high-cholesterol meal, and it is reasonable to speculate that these transient lipoproteins may contribute to the atherogenic risk characteristic of populations consuming such diets.1,2

Unlike type III hyperlipoproteinemia, in which the accumulation of β-VLDL is due to the presence of an abnormal apo E that binds poorly to the receptor, the β-VLDL accumulation seen after fat and cholesterol feeding appears to be secondary to a reduction (down-regulation) in the number of apo B,E(LDL) receptors.4,9,10 Cholesterol feeding decreases the number of apo B,E(LDL) receptors in the liver, but such feeding may not decrease the expression of remnant (apo E) receptors.9 Presumably, lipoprotein overproduction induced by dietary fat and cholesterol, in association with a decrease in apo B,E(LDL) receptors, exceeds the ability of lipoprotein receptors to clear the extra particles from the plasma, and both chylomicron and VLDL remnants accumulate. Both conditions are associated with accelerated atherosclerosis.

**Low Density Lipoproteins**

**Familial Hypercholesterolemia**

**Background.** The role of LDL in the development of accelerated atherosclerosis is dramatically illustrated by studies of patients with familial hypercholesterolemia. As demonstrated by the classic studies of Goldstein and Brown and others,4,5,9,10 patients with this disorder lack or have defective apo B,E(LDL) receptors that prevent normal LDL uptake and catabolism. Patients homozygous for this disorder often die in the second decade of life from coronary artery disease. It should be kept in mind that these subjects also have abnormally high levels of VLDL remnants and IDL, which could contribute to the atherogenic process through the mechanisms discussed above.

Not only does LDL accumulate in the plasma of individuals with familial hypercholesterolemia, but LDL also accumulates in the plasma of animals fed diets high in fat and cholesterol.1,2 The increase in plasma LDL in various animal models is likely secondary to the down-regulation in expression of the apo B,E(LDL) receptors. The decrease in the number of these receptors would be expected to decrease LDL catabolism and to result in an increased plasma concentration of LDL. This may well be the mechanism whereby the consumption of high-fat, high-cholesterol diets raises plasma cholesterol levels in humans and predisposes them to an increased risk of developing coronary heart disease.

**Familial Defective Apolipoprotein B-100**

**Background.** Familial defective apo B-100 is a newly described genetic abnormality characterized by the occurrence of moderate hypercholesterolemia (250–300 mg/dl) and plasma LDL that are defective in binding to the apo B,E(LDL) receptors on cultured fibroblasts.11 Familial defective apo B-100 is inherited as a codominant trait. Several lines of evidence suggest that a mutation within apo B-100, possibly a point mutation, is responsible for the defect.12,13

Familial defective apo B-100 very likely represents a class of genetic abnormalities responsible for defective LDL binding secondary to altered apo B-100 structure. The exact frequency of familial defective apo B-100 requires further study. A series of such defects could, in fact, be responsible for the hypercholesterolemia in many subjects. In addition, this abnormality provides a unique opportunity to determine the effect of high levels of LDL on the pathogenesis of atherosclerosis in humans. The precise mechanism whereby LDL may be atherogenic is not clear.1,5,6

An intriguing hypothesis has been advanced to explain how macrophages in the developing fatty streak may become loaded with cholesterol in response to high levels of LDL. It has been shown that chemically modified LDL can be recognized by unique receptors on macrophages (referred to as the acetyl LDL receptor or the receptor for chemically modified LDL).1,4–6

In the laboratory, LDL altered by acetylation, acetoacetylation, malondialdehyde modification, or oxidation can be recognized by this receptor and
can cause massive cholesteryl ester accumulation within macrophages. Oxidation of LDL can be cell mediated by incubation of this lipoprotein with endothelial cells, smooth muscle cells, or macrophages. In addition, oxidative modification can be induced by metal ions, such as copper or iron. Such modifications of LDL could occur in plasma as lipoproteins circulate or in the arterial wall as they perfuse tissue.

Two recent observations support the hypothesis that chemical modification of LDL may be physiologically relevant. First, the studies of Kita et al and Carew et al have demonstrated that probucol, a hypolipidemic agent with the properties of an antioxidant, retards atherosclerosis in Watanabe heritable hyperlipidemic (WHHL) rabbits. These studies have been interpreted as suggesting that the drug, which is actually incorporated into the LDL, prevents LDL oxidation and thus retards atherosclerosis. A second observation suggests that modified LDL, specifically malondialdehyde-LDL presumably produced by lipid peroxidation, can be identified by antibodies within the lesions of WHHL rabbits. An immunochromatographic colocalization of protein modified by malondialdehyde and the extracellular deposit of apo B-100 was found in WHHL rabbit atheroma. Despite the attractiveness of these postulated mechanisms, more data are required to establish the importance of chemical modification of LDL in the pathogenesis of atherosclerosis. Even the demonstration of modified lipoproteins in the artery wall does not establish the cause of atherosclerosis.

High Density Lipoproteins

Background. The very strong negative correlation between HDL cholesterol and coronary heart disease risk is universally accepted. A low HDL cholesterol level (below 35 mg/dl) is considered a risk factor under current NCEP-AHA guidelines. However, there is still no clearly established mechanism by which a low level of HDL enhances risk and a high level of HDL is protective against such risk. It is often stated that HDL removes cholesterol from cells of peripheral tissues. However, that function remains to be tested in vivo. Moreover, data now suggest that at least the major part of HDL in native plasma may not participate actively in such a function in vitro. The extreme complexity and heterogeneity of HDL in native plasma and the lability of the functional complexes directing free and ester cholesterol traffic have become apparent.

Most prospective correlations are based simply on total HDL cholesterol. Some studies suggest that HDL₃ may correlate with the presence of disease better than does HDL₁, a denser subfraction. Fielding and others have shown that even greater complexity emerges when the apolipoprotein composition of HDL particles is examined by newer methods. Some HDL molecules (about 5%) contain only apo A-I and have pre-β mobility on electrophoresis. This subfraction, which itself is internally heterogeneous, may be particularly important in reverse cholesterol transport because it contains most of two proteins believed to be involved in that process—lecithin:cholesterol acyltransferase (LCAT) and the cholesterol ester transfer protein. This “A-I only” subfraction is also the most active fraction in vitro in transferring free cholesterol from the surface of cultured cells to plasma. Other subfractions of HDL contain apo C or apo E. Whether these serve only as reservoirs for the apolipoproteins or whether they confer special properties on the HDL particles remains uncertain. Of course, most HDL particles contain both apo A-I and apo A-II, and it seems probable that these particles play a role in the protective function. However, investigators are still not able to identify which components of HDL are crucial in protection or what the precise mechanism may be.

The most attractive hypothesis concerning the protective mechanism of HDL is certainly the reverse cholesterol transport hypothesis advanced by Glomset, which has since been supported by a great deal of in vitro evidence. However, direct in vivo demonstration and quantification of reverse transport in either experimental animals or humans is still lacking. There are alternative hypotheses. For example, HDL inhibits the binding of LDL to matrix connective tissue elements; HDL inhibits oxidation of LDL, which could protect against the cytotoxic effects of LDL; and HDL also inhibits the accelerated receptor-mediated uptake of oxidized LDL by way of the acetyl LDL receptor. Additionally, subfractions of HDL containing apo E can compete with LDL for uptake by way of the LDL receptor. Finally, it should be noted that a high HDL level may not mean that HDL is itself directly involved in protecting against atherogenesis. Instead, a high HDL level may only be a marker for some metabolic process that somehow interferes with atherogenesis and only incidentally leads to a rise in HDL steady-state levels.

The finding that cholesteryl esters can be directly transferred to cells from HDL or LDL without endocytosis confounds earlier beliefs that the relative importance of these lipoproteins in carrying cholesterol esters in plasma is predictable from the ratio of LDL and HDL cholesterol. At present, the proportion of LCAT-derived cholesteryl esters transferred to HDL in vivo in any syndrome remains unknown. In a number of syndromes where the risk of vascular disease is increased, there is a lower net transfer of cholesteryl esters to LDL in vitro than in normal plasma. Whether this reflects proportional changes in transfer in vivo is not known.

Summary and Recommendations

Recommendations. The biochemical basis for the statistical relation between HDL and atherosclerosis remains unknown. Knowledge of the functions of HDL, even in normal plasma, is still fragmen-
Hypotheses based mostly on in vitro studies need to be confirmed by appropriate studies in vivo. The panel therefore recommended that the AHA encourage 1) studies on the metabolic role of HDL in cholesterol transport, especially in vivo studies of transport from peripheral tissues back to the liver (reverse cholesterol transport); 2) studies with animal models in which HDL levels may be selectively modified (by inbreeding, identification of appropriate natural mutants, or transgenic techniques); 3) studies of the alternative ways in which HDL may be protective through the use of animal models; and 4) studies that may identify which fractions of HDL are most relevant to its protective function.

The panel proposed that further research is needed to test the assumption that a lipoprotein causes cholesteryl ester accumulation in cultured macrophages in vitro to confirm that such a lipoprotein is atherogenic in vivo. Steps that are necessary to determine if remnant particles are atherogenic in vivo include determining if and how remnant lipoproteins interact with artery wall cells. The panel decided that it was particularly important to develop methods for measuring remnant lipoproteins in vivo.

Related to these questions are questions regarding the ability of particles larger than LDL to enter the normal artery wall, that is, before a cellular response has been evoked. It was decided that the issue of extracellular cholesterol in the artery wall is important. It was noted that LDL is preferentially retained in the artery wall at sites destined to be lesions even before there is evidence of cellular infiltration.

The panel suggested that the interrelation of growth control of artery wall cells, regulation of artery wall matrix composition, and lipoprotein interaction with the matrix were areas in need of further research. The specific details of lipoprotein-matrix interactions need to be defined. Moreover, those processes that may modify lipoproteins retained in the artery wall need to be further defined (e.g., aggregation, oxidation, and antigen-antibody complex formation). The mechanisms for these modifications in the microenvironment of the artery wall must also be elucidated. The mechanisms of monocyte recruitment-retention at lesion-susceptible sites need to be determined. The mechanisms of cholesterol efflux from these sites also need further study.

Interactions of other risk factors such as diabetes, hypertension, and smoking with lipoproteins must be considered in understanding the complex process that results in an atherosclerotic lesion. The lesion itself must be studied. How does HDL, which readily enters and exits the artery wall, interact with apo E? How does the artery wall interact with apo E? How does the artery wall remodel with age?

The panel proposed that a stronger commitment to defining lipoprotein heterogeneity is required. Prospective studies on the value of apo A and apo B measurements are needed. The relative atherogenicity of lipoproteins of different composition needs to be established (e.g., dense LDL with less cholesterol and more apo B). The precision and accuracy of tests for plasma apo A and apo B need to be established and corrected for apo B in VLDL. A prospective study of the predictive value of these tests compared with the predictive value of total cholesterol, triglycerides, and HDL cholesterol is needed.

Plasma concentrations of lipoprotein (a) (Lp[a]) are positively associated with coronary heart disease risk, but the basis for this correlation has not been established. Reliable tests for Lp(a) measurement are needed for application to the general population.

It was agreed that epidemiologists should rapidly incorporate the new biologic information into their data bases and test against established risk factors in a prospective fashion. Additionally, the need to understand the relation between lesions and thrombosis was reemphasized.

The panel also concluded that a concerted effort is needed to create animal models for the study of lipoprotein physiology and atherogenesis. Molecular biology may allow the construction of transgenic animals that overproduce particular lipoproteins or receptors. Some models may also allow the study of deficiency states. The candidate gene approach is strongly favored in searching for genetic factors that may induce or modify atherosclerosis.

The panel recommended research on mild and moderate hypercholesterolemia (e.g., due to structurally abnormal apo B-100). A need for better in vivo modeling was also noted. More sophisticated means of measuring lipoprotein and cholesterol flux in vivo are required to translate results of in vitro studies to humans.

There is a need to understand the effects of specific fats on specific lipoproteins. In addition to the effects on total cholesterol levels, there may be other more subtle effects that either inhibit or promote atherogenesis.

The panel recognized that it has recommended a diverse and broad range of research initiatives. However, it was concluded that the importance of knowing who and how to treat amply justifies this enormous effort.

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Circulation. 1989;80:719-723
doi: 10.1161/01.CIR.80.3.719

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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