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DRUGS, LIPID METABOLISM, AND ATHEROSCLEROSIS

Edited by David Kritchevsky, Rodolfo Paoletti, and William L. Holmes

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Edited by

David Kritchevsky
The Wistar Institute
Philadelphia, Pennsylvania

Rodolfo Paoletti
Institute of Pharmacology and Pharmacognosy
University of Milan
Milan, Italy

and

William L. Holmes
Division of Research
The Lankenau Hospital
Philadelphia, Pennsylvania

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Preface

This volume comprises the proceedings of the Sixth International Symposium on Drugs Affecting Lipid Metabolism. Since the first of these symposia in 1960 these triennial meetings have been devoted to the exploration of new ideas, new data and new concepts related to lipid metabolism and atherosclerosis. The Sixth Meeting was particularly stimulating in this regard. The concept of the "protective" action of HDL was thoroughly explored within the framework of its molecular biology with data on its epidemiological as well as its in vitro mechanism(s) of action being discussed. The action of drugs on arterial and HDL metabolism was also discussed as were newer aspects of platelet aggregation, especially as related to prostaglandins. New ground was also broken in discussions of lipid mobilization and mechanisms of hypocholesteremia.

We are indebted to the many organizations who contributed generously to the support of this meeting. Among the sponsors, the assistance of the Lorenzini Foundation was especially helpful. As in all meetings of this type, the hard work of the local organizing committee was instrumental in its success. We are grateful to Mrs. Caroline Hyatt and Mr. Ralph Hollerorth for their invaluable help in the secretariat. We are also deeply indebted to Miss Jane T. Kolimaga for her expert assistance in the preparation of this volume.

David Kritchevsky
Rodolfo Paoletti
William L. Holmes

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LIPOPROTEIN METABOLISM - NEW INSIGHTS FROM CELL BIOLOGY

Daniel Steinberg, M.D., Ph.D.

Division of Metabolic Disease, University of California San Diego, La Jolla, California, U.S.A.

The primary purpose of this paper is to review some of the recent developments in our understanding of how lipoproteins are metabolized by peripheral cells. Studies utilizing cultured mammalian cells promise to enhance considerably our insights into factors regulating steady-state levels of lipoproteins in the plasma compartment and, at least potentially, our insights into the cellular basis for atherogenesis. Quite possibly we may see the development of new modalities of pharmacologic intervention based on a better understanding of how lipoproteins are degraded by or modified by interactions with peripheral cells. Research in this area is still at an early stage of development but progress is being made rapidly. Those of us interested in the role of lipids in atherogenesis and in the possibilities for preventive intervention should be aware of the opportunities to capitalize on the new findings as they come along. Before turning to the cellular level, however, we should establish the context by briefly reviewing current concepts of lipoprotein metabolism *in vivo*.

LIPOPROTEIN METABOLISM *IN VIVO*

The liver is generally accepted to be the major source of the plasma lipoproteins. Undoubtedly the intestine makes some contribution as well but in man that contribution is probably quantitatively minor. The fate of chylomicrons, well studied in the rat, has not been fully established in man. To what extent do they contribute to the very low density, low density and high density

lipoprotein fractions (VLDL, LDL and HDL)? Does chylomicron and/or VLDL clearance play a role in atherogenesis as proposed by Zilversmit (1)? To what extent is the intestine an important source of lipoprotein apoproteins? None of these questions has been satisfactorily answered in man. Clearly it would be premature to set aside the intestine as a source of materials of importance in overall lipoprotein metabolism and atherogenesis. The information available does not permit any firm conclusions and we shall not attempt to deal with this question further today.

The primary lipoproteins secreted by the liver are VLDL and HDL. In normal human subjects most or all of the circulating LDL can be accounted for as a product of VLDL metabolism i.e. relatively rapid degradation of VLDL by lipoprotein lipase giving rise to an intermediate density lipoprotein (IDL) and then LDL as a more slowly metabolized "end product" (2-6). In the rat, IDL (or VLDL "remnant") is rapidly removed by the liver (7) but its fate in man is uncertain. There is now direct and indirect evidence that the liver can under some circumstances secrete LDL directly into the plasma compartment. In patients with familial hypercholesterolemia the daily transport of apoprotein B in the LDL fraction (d 1.019 - 1.063) has been found to exceed the transport of apoprotein B in the VLDL fraction ($d < 1.006$) (8,9). As much as 50% of the LDL apo B may have an origin other than VLDL. Recently it has been reported that the isolated perfused liver of the pig may directly secrete LDL (10,11). It now appears that not all of the apo B in VLDL must obligatorily be converted to LDL prior to its disappearance from the plasma compartment i.e. the net daily transport of apo B in VLDL can exceed that in LDL (9). Another recent finding worth noting is that changes in transport of triglycerides in VLDL need not parallel changes in apo B transport in VLDL (12). During carbohydrate-induction the transport of triglycerides can increase (along with an increase in steady-state plasma levels of VLDL triglycerides) with either no increase or a much smaller increase in apo B transport. Evidently on a high carbohydrate diet the triglyceride:apo B ratio in the secreted VLDL is increased i.e. larger, triglyceride-rich particles are secreted (13).

These findings should help to rationalize some seeming paradoxes in the hyperlipoproteinemias. As long as we assumed that all VLDL must be converted to LDL and that all LDL had its origin in VLDL, it was difficult

to explain hyperlipoproteinemic patterns with widely different ratios of VLDL to LDL and to explain some of the complex responses to dietary and drug interventions. If LDL can be directly secreted we can see how LDL levels can be increased without changes in VLDL levels or rates of VLDL secretion. If not all VLDL apo B must be converted to LDL we can see how VLDL levels can be increased as a result of overproduction (including overproduction of VLDL apo B) without necessarily affecting LDL levels or LDL transport. Finally, if VLDL triglyceride transport can be increased without concomitant increase in VLDL apo B transport, we can see how VLDL (triglyceride) levels can be increased as a result of overproduction without affecting LDL levels or LDL transport.

Interest in HDL metabolism and its regulation has increased considerably with the accumulation of epidemiologic evidence that the risk of coronary heart disease varies inversely with HDL cholesterol levels (14-17). As shown by Hamilton and coworkers (18), HDL is secreted from perfused rat livers in the form of a disc-shaped particle with a high ratio of free cholesterol to ester cholesterol and a high content of arginine-rich protein (apo E). It is presumably converted to the form found at steady-state through the action of plasma lecithin-cholesterol acyltransferase (19) and shifts in apoprotein composition effected by interchanges with other lipoprotein fractions. It should be stressed that the designation "HDL" can be ambiguous. The fraction isolated in the density range 1.063-1.21 is not homogeneous. Subfractions can be recognized by analytic ultracentrifugation (20). More importantly, the fraction includes subpopulations of molecules with different apoprotein patterns. Mahley and coworkers have shown that the heterogeneity is further increased in cholesterol-fed animals (21), a population of molecules rich in cholesterol and in apo E increasing. This is especially relevant, as discussed below, to the interactions between LDL and HDL metabolism and possibly to the "antiatherogenic" role of HDL.

The major apoproteins in the HDL fraction in man are apo A-I and apo A-II and the occurrence of apo E has just been discussed. In addition HDL binds the several classes of C apoproteins, serving as a reservoir from which these can be readily exchanged into VLDL or chylomicrons (22,23). Since apo A-I and C-I are activators for lecithin-cholesterol acyltransferase (24) and apo C-II an activator for lipoprotein lipase (25), HDL-

associated apoproteins could play a regulatory role in overall lipoprotein metabolism. The precise role of this "apoprotein reservoir" function in normal subjects and in hyperlipoproteinemic subjects remains to be established.

EXTRAHEPATIC DEGRADATION OF LIPOPROTEINS

It has been the general view that LDL must be degraded in the liver. At first glance this seems an unavoidable conclusion because extrahepatic tissues (with the quantitatively minor exceptions of the adrenal cortex and the gonads) do not have any significant capacity to degrade or eliminate cholesterol. All that is absolutely required, however, is that the cholesterol moiety of the LDL ultimately make its way back to the liver for excretion. The apoprotein moiety of the LDL (and probably the other lipid components) could in principle be degraded outside the liver. In fact there is now evidence from several directions that, at least in some species, most of the degradation of LDL apoprotein takes place extrahepatically.

The first evidence for this was provided by the work of Sniderman et al.(26), who showed that the rate of the degradation of intravenously injected ^{125}I-LDL in swine was not reduced by total hepatectomy. In intact swine the injected LDL showed a biphasic disappearance, the initial more rapid phase being attributable to equilibration with an extravascular pool of LDL. Postmortem tissue distribution studies indicated that much of this extravascular pool resided in the liver. If the liver were the exclusive or predominant site of degradation, there should be little or no disappearance of labeled LDL after hepatectomy. Instead the disappearance rate was as great as or greater than the rate in the same animal studied prior to hepatectomy. Similar results were obtained in hepatectomized dogs. Furthermore, in swine the net plasma level of LDL protein fell progressively after hepatectomy and at the same rate as ^{125}I-LDL, eliminating the possibility that the labeled LDL might not be a valid tracer and, additionally, indicating that under the conditions of the experiment there was little or no extrahepatic contribution to the plasma LDL fraction. These results do not rule out some hepatic contributions to LDL apoprotein degradation but strongly suggest that, in the species studied, it is small.