minireview

Low density lipoproteins in atherosclerosis

Lawrence L. Rudel, John S. Parks, Fred L. Johnson, and John Babiak

Arteriosclerosis Research Center and Department of Comparative Medicine, Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC 27103

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I. INTRODUCTION

Low density lipoproteins (LDL) have long been implicated in the development of atherosclerosis. Perhaps the most convincing evidence that these lipoproteins are causative factors in this disease is the genetic disorder, familial hypercholesterolemia, in which homozygous patients develop massive LDL concentrations and frequently die within the second decade of life from complications of coronary artery atherosclerosis (1). The reasons for the atherogenicity of LDL are incompletely understood. In epidemiologic studies, the concentration of LDL cholesterol in plasma has been shown to be highly correlated to the incidence of coronary heart disease (CHD) (2). Hence, factors that regulate LDL concentration through formation and catabolism are undoubtedly important in determining atherogenicity associated with LDL. Indeed, the massive LDL concentration that results from the absence of the LDL receptor in familial hypercholesterolemia is a graphic illustration of how a lack of control of LDL catabolism can lead to increased LDL concentrations and, subsequently, to premature complications of atherosclerosis (3). In addition, much new information has been developed about the heterogeneity in composition and character of plasma LDL particles. It now is apparent that many forms of LDL exist within and among individuals and it seems possible that some forms are more atherogenic than others. The purpose of this review will be to examine several aspects of low density lipoproteins that may be contributory to the development of CHD.

We will review the information about LDL heterogeneity and how this may relate to the development of atherosclerosis. Since LDL concentration is also important in atherosclerosis, we will also review information about LDL formation and catabolism. Nonhuman primate models of experimental atherosclerosis have been extensively studied in this laboratory, so we will include, where appropriate, the lessons learned from these experimental models. Diet-induced elevations of the average size and concentration of LDL have been shown to be highly correlated to the extent of atherosclerosis in the coronary arteries of nonhuman primates (4). It seems clear that the low density lipoproteins are important in initiation and/or exacerbation of coronary artery atherosclerosis in the experimental primate model as well as in man. For prevention and treatment of CHD, it will be important to learn what factors cause elevated LDL concentrations and which properties of LDL lead directly to the atherogenicity of these lipoproteins.

II. COMPOSITIONAL HETEROGENEITY OF LDL

A. Metabolic origin

Numerous recent studies have demonstrated that the plasma low density lipoproteins are a heterogeneous collection of particles with distinct physical and chemical characteristics. The metabolic basis and physiological significance of LDL subpopulations are unknown, although certain LDL subpopulations may be more atherogenic than others, a possibility first suggested by Gofman and coworkers in 1950 (5). Subsequently, LDL heterogeneity related to CHD has been described by several investigators. Gofman's analytical ultracentrifuge data showed that a large, lighter density LDL, defined as a fraction of S_f 12–20, was found in many patients with CHD, and in rabbits that were fed cholesterol to induce atherosclerosis (5, 6). Krauss and Burke (7) and Musliner, Giotas, and

Krauss (8) demonstrated heterogeneity among LDL by size analysis using polyacrylamide gradient gel electrophoresis, and suggested that an LDL-1 (IDL) fraction as well as the smaller, more dense LDL subfraction may be more prevalent in some patients with coronary heart disease. Several laboratories have studied the composition of the plasma LDL of patients with familial hypercholesterolemia (type II hyperlipoproteinemia) (9, 10). The data of Patsch et al. (11) showed that the LDL of patients from a kindred with familial hypercholesterolemia were larger and contained more cholesteryl esters and less triglycerides than the LDL of normal individuals within the same kindred. One report described an increase in the content of saturated cholesteryl esters in LDL of a familial hypercholesterolemic subject (12). Together, these studies in familial hypercholesterolemic individuals suggest that large, cholesteryl ester-enriched LDL may occur when LDL receptor deficiency is present. The increased size and altered composition of LDL, in addition to the elevated concentration, may be contributory to the dramatically increased incidence of atherosclerosis in familial hypercholesterolemia. It is possible that other individuals with enlarged LDL may also have suboptimal LDL receptor function, although this remains to be demonstrated.

Sniderman and coworkers (13) found that LDL with a high protein (apoB) to cholesterol ratio were more prevalent in human beings with coronary heart disease. The higher apoB to cholesterol ratio may be due to the presence of a relatively cholesteryl ester-poor, triglyceride-rich LDL occurring concomitantly with elevated plasma triglyceride concentrations (14). In hypertriglyceridemic individuals, the plasma LDL have been found to be smaller in size (15) and to have a higher average hydrated density (16). Smaller, cholesteryl ester-poor LDL could arise through the mechanism suggested by Deckelbaum et al. (17) in which the lipid transfer protein-mediated exchange of LDL cholesteryl ester for VLDL triglyceride molecules occurs. Subsequent lipolysis of the LDL triglyceride molecules results in a decrease in LDL size and formation of smaller, relatively cholesteryl ester-depleted LDL particles. Although this mechanism may exist in the plasma of all individuals, it should be especially significant in circumstances where elevated VLDL concentrations and prolonged LDL circulation times occur, such as in patients with familial combined hyperlipidemia (18), and in other hypertriglyceridemic patients in which a negative correlation between LDL size and plasma triglyceride concentration has been described (15). Even though a working hypothesis for the mechanism of formation of small LDL in CHD patients is available, it is not certain that small LDL particles, per se, are atherogenic. An alternative explanation is that the factors associated with the formation of small LDL, including overproduction of VLDL apoB and slow LDL catabolism, may be responsible for the predisposition of some hypertriglyceridemic individuals to premature atherosclerosis.

A contrasting situation occurs in nonhuman primates. Many cholesterol-fed monkeys have large cholesteryl ester-enriched apoB-100-containing LDL in plasma, the size of which is positively correlated to the severity of coronary artery atherosclerosis (4, 19). It may be that properties of the enlarged monkey LDL, per se, are atherogenic, or it may be that the enhanced development of atherosclerosis is due to some aspect of LDL metabolism that, ultimately, results in the formation of enlarged LDL particles. For example, monkeys fed atherogenic diets consistently have low (<30 mg/dl) triglyceride concentrations (20). And yet, the apoB-containing lipoproteins secreted by the liver in monkeys are all triglyceride-rich lipoproteins although they are cholesteryl ester-enriched in cholesterol-fed monkeys (21). This suggests that rapid degradation and removal of triglyceride occurs upon secretion of these lipoproteins into the circulation. Therefore, in the nonhuman primate model of atherosclerosis, the resultant elevation in plasma concentration of cholesteryl ester-rich, triglyceride-poor, apoB-100-containing LDL particles may be due to hepatic overproduction and/or cholesteryl ester enrichment of LDL precursor particles that are subsequently converted to large LDL in the circulation.

A similar hepatic overproduction or cholesteryl ester enrichment could also occur in humans, e.g., many kinetic studies suggest an overproduction of VLDL apoB in hypertriglyceridemic patients (18), but the resultant plasma LDL particles in humans and monkeys would be different. In monkeys, limited cholesteryl ester for triglyceride exchange occurs due to the low VLDL triglyceride concentrations and large cholesteryl ester-rich LDL result. In humans with elevated VLDL concentrations and delayed LDL clearance, cholesteryl ester for triglyceride exchange would be facilitated and smaller LDL would result. In this way, the metabolism of hepatic precursor lipoproteins in humans leading to small, apoB-rich LDL may be analogous to that leading to production of large, atherogenic LDL in cholesterol-fed monkeys. In both cases, the increased concentration and circulation time of LDL presumably results in enhanced deposition of these particles in the arterial wall. The atherogenicity of the enlarged LDL in monkeys is even further enhanced by the enrichment of the large particles with saturated and monounsaturated cholesteryl ester that may form a liquid crystalline core at body temperature (22).

B. Consequences in arterial wall

Since LDL size is highly correlated with coronary artery atherosclerosis in nonhuman primates, there may be properties of the enlarged LDL particle related to size that promote atherogenesis. As just mentioned, one apparent difference in large versus small LDL is the

physical state of the cholesteryl ester core (22). In animals fed a saturated fat-rich diet, a selective increase in the number of saturated and monounsaturated cholesteryl esters occurs that is proportional to the LDL molecular weight increase (Ref. 22 and **Fig. 1**). Increase of these cholesteryl esters suggested accumulation of acyl CoA:cholesterol acyltransferase (ACAT)-derived esters in LDL; the lecithin:cholesterol acyltransferase (LCAT)-derived cholesterol linoleate was present in essentially fixed amounts in the saturated fat-fed animals. A proportional increase also occurred in the melting temperature (Tm) of the liquid crystalline to liquid transition of the LDL cholesteryl ester core (22), and this was also true for the saturated fat-fed monkeys of Fig. 1 (Parks, J. S., and L. L. Rudel, unpublished observations). The Tms of the enlarged LDL of saturated fat-fed monkeys are generally above body temperature, suggesting that the cholesteryl ester cores of LDL are in a liquid crystalline state in the circulation.

For animals fed a polyunsaturated fat diet there was also an increase in monounsaturated cholesteryl esters proportional to the LDL molecular weight (Fig. 1) and there was also a positive relationship between Tm and LDL molecular weight (Parks, J. S., and L. L. Rudel, unpublished observations). However, the LDL of polyunsaturated fat-fed animals were enriched in polyunsaturated cholesteryl esters at all molecular weights and had considerably lower transition temperatures that were consistently below body temperature. We have completed one study in which the effect of dietary polyunsaturated and saturated fat on the development of atherosclerosis was measured in African green monkeys fed atherogenic diets for five years (23). The data of **Table 1** summarize the findings. The severity of atherosclerosis in the coronary arteries was significantly less in the polyunsaturated fat-fed animals in spite of a significant lowering of the HDL concentration by polyunsaturated fat. This emphasizes that the dietary fat effects on LDL, which were lower in concentration and size in the polyunsaturated fat-fed animals (Table 1) in addition to having a modified cholesteryl ester composition (Fig. 1), are likely to play an important role in moderating atherogenesis in these animals. Since several characteristics of LDL are modified by the type of dietary fat, more work will be needed to determine which may be the most important for atherogenesis; however, the data emphasize that dietary polyunsaturated fat effects on LDL are involved in slowing the development of atherosclerosis.

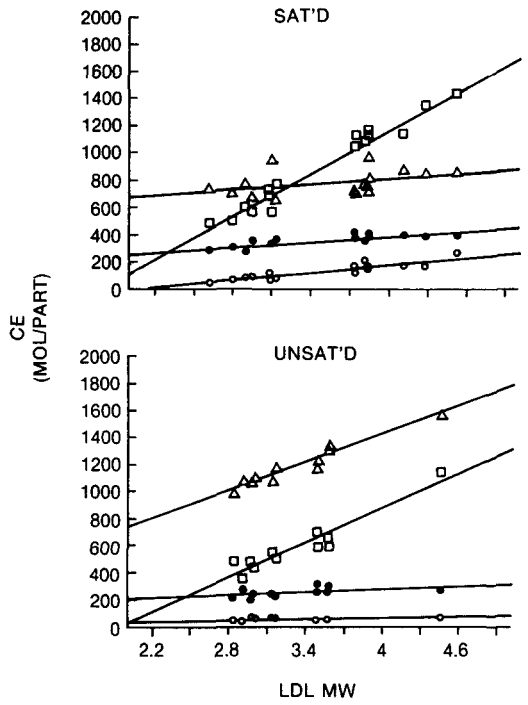

Fig. 1. Cholesteryl ester composition of LDL of varying molecular weight (MW in g/μmol) isolated from saturated (Sat'd) and polyunsaturated (Unsat'd) fat-fed African green monkeys. Each point represents one animal; all animals were fed their experimental diet for at least 1 year. The number of cholesteryl ester (CE) molecules per particle (mol/part) was determined for cholesteryl palmitate (●), cholesteryl stearate (○), cholesteryl oleate (□), and cholesteryl linoleate (△) after separation of cholesteryl esters by HPLC (81). LDL MW was measured by agarose column chromatography (82). Both diets contained 40% of calories as fat and 0.8 mg of cholesterol per kcal. The saturated fat diet has a polyunsaturated to saturated fatty acid ratio (P/S) of 0.3, while the saturated fat diet has P/S = 2.2. The lines are least squares regression lines.

Compositional heterogeneity of LDL may also affect binding of LDL to proteoglycans that make up the structural matrix of the artery wall (24). When proteoglycans are extracted from arteries and tested for binding reactivity with LDL from different human subjects, the more reactive LDL were enriched in free and esterified cholesterol and were relatively poor in triglycerides and protein (25). The highly reactive LDL were less dense (and presumably larger) and had a more basic isoelectric range than the less reactive LDL. In addition, the highly reactive LDL had X-ray scattering profiles indicative of core cholesteryl esters in the liquid crystalline state, while less reactive LDL appeared to have liquid cholesteryl ester cores. Binding of human LDL to chondroitin-6-sulfate increased the transition temperature of the cholesteryl ester core from 33° to 40°C, resulting in a liquid crystalline cholesterol core at body temperature (26). Alterations in the physical state of LDL upon binding to connective tissue components have also been suggested by pyrene fluorescence studies of LDL (27). Fusion of LDL particles upon binding to proteoglycans has been suggested (24) and may explain some of the reported changes in the LDL physical properties after binding. Together these data suggest that LDL that are less dense, enriched in cholesteryl esters, and that have liquid crystalline cores preferentially bind to arterial proteoglycans. Furthermore, the binding interaction of LDL with arterial matrix components may modify the composition of the LDL particles.

TABLE 1. Dietary fat and cholesterol effects on lipoproteins and atherosclerosis in African green monkeys

Diet Group[a]	N	Plasma Concentration: Total Cholesterol	Plasma Concentration: LDL Mass	Plasma Concentration: HDL Mass	LDL MW	Coronary Artery Atherosclerosis[b]: Intimal Area	
		mg/dl			*g/μmol*	*mm²*	
Control							
Polyunsat'd	9	131 ± 5[c]	147 ± 12	483 ± 27	2.98 ± 0.06	0 ± 0	(4)
Sat'd	8	150 ± 10	166 ± 20	536 ± 31	3.23 ± 0.08	0 ± 0	(4)
Test							
Polyunsat'd	11	174 ± 17	267 ± 50	406 ± 55	3.30 ± 0.13	0.02 ± 0.01	
Sat'd	11	277 ± 37	489 ± 106	526 ± 32	3.76 ± 0.09	0.30 ± 0.19	(10)[d]
Significance, two-way ANOVA							
Cholesterol level		< 0.01	< 0.01	NS	< 0.01		
Type of fat		< 0.05	< 0.05	< 0.05	< 0.05		
Interaction		NS	NS	NS	NS		

[a]Control diets were low cholesterol diets, 0.15 mg cholesterol/kcal. Test diets were high cholesterol diets, 0.8 mg cholesterol/kcal. All diets contained 40% of calories as fat, either safflower oil (polyunsat'd) or butter fat (sat'd).
[b]All animals were fed their diets for 5 years, after which atherosclerosis was evaluated morphometrically. Details are given in ref. 23.
[c]All values, mean ± SEM; parentheses indicate n when different from column 2.
[d]Significantly different, $P < 0.02$, from polyunsaturated test group; two-tailed Student's *t*-test.

The mechanism by which the physical state of the LDL cholesteryl ester core moderates the ability of the LDL particles to interact with cells or components of the arterial matrix is unknown. One potential explanation is that the nature of the core affects the surface properties of the particle (28, 29) and, possibly, the conformation of apoB on the surface. Studies using monoclonal antibodies have suggested that the conformation of apoB changes with LDL particle size (30). In other studies, delipidation of apoB resulted in loss of reactivity to epitopes exposed on intact LDL (28). Binding of detergent-solubilized apoB to cholesteryl ester microemulsions, but not cholesterol-phospholipid liposomes, restored the binding reactivity of the monoclonal antibodies to the epitopes, suggesting that the cholesteryl ester core influences the conformation of apoB (28). Circular dichroism studies of LDL also have suggested a temperature-induced change in apoB conformation, although it is unclear whether the change was related to core cholesteryl ester transitions (31–33). These data suggest that lipoprotein surface–core interactions can occur and that LDL apoB conformation may be influenced by these interactions. However, it is not clear whether core size and the physical state of the core independently influence LDL apoB conformation.

Binding of LDL to cell receptors or proteoglycans may be altered by a difference in LDL surface charge or by addition of soluble peptides to the surface of the particle. LDL that bind with proteoglycans have a more basic isoelectric range (34). Chemical modifications of apoB that result in charge differences affect the binding of LDL to the LDL receptor (35). Although LDL contains predominantly apoB, smaller apoproteins are also present and have been shown to increase in amount relative to apoB with incubation of plasma in vitro (17). St. Clair, Mitschelen, and Leight (36) have demonstrated that cholesteryl ester accumulation in cells in culture is enhanced by larger molecular weight LDL from cholesterol-fed monkeys, and the larger LDL of cholesterol-fed monkeys are generally enriched in apoE and apoC (37).

In summary, available data have suggested that several properties of LDL are important to the development of atherosclerosis, including LDL molecular weight, the surface charge of LDL, the physical state of LDL core cholesteryl esters, the conformation of apoB, and the presence of other apoproteins. These factors may function in atherosclerosis by altering the binding of LDL to cell receptors and to proteoglycans. The data show that LDL are heterogeneous among and within individuals and several aspects of composition and metabolism may interact to determine the relative atherogenicity of LDL in any one individual.

III. LDL FORMATION

A. VLDL conversion to LDL

LDL formation is a dynamic process that occurs intravascularly and involves remodeling and delipidation of precursor very low density lipoproteins (38). However, evidence from VLDL turnover studies indicates that only a portion of the plasma VLDL become LDL, the actual proportion of which differs among individuals and among pathological conditions (18, 39, 40). Stalenhoef et al. (41) have followed the metabolism of VLDL obtained from the plasma of patients with lipoprotein lipase deficiency, a

state in which the circulating VLDL particles were postulated to be relatively nascent when compared to those obtained from the plasma of normal subjects. When injected into normal individuals, these large VLDL were rapidly removed from the circulation and only a small proportion were converted to LDL. Packard et al. (42) have demonstrated metabolic heterogeneity for plasma VLDL. In their studies of normal individuals, the production of LDL from larger triglyceride-rich VLDL was low (10% of that injected) whereas a much greater portion (>40%) of small VLDL became LDL after injection into the circulation. Even the metabolic fate of the small VLDL particles was heterogeneous. These investigators concluded that only small VLDL secreted into the circulation are destined to become plasma LDL; the small VLDL formed from larger plasma VLDL are removed from the circulation without conversion to LDL.

The specific characteristics of VLDL that determine whether the particle will be removed from the circulation or undergo conversion to LDL are just beginning to be recognized. The role of apoE has been extensively examined because apoE is bound to specific, high affinity receptors in the liver (43). Havel (44) has reported that apoE is found in high amounts on large VLDL and the catabolic remnants produced from them, but only one or two apoE molecules may be present on the remnants from small VLDL. There may also be a relationship between the cholesteryl ester content and the apoE content of the lipoprotein particles. VLDL that contain more cholesteryl ester than LDL may contain more apoE and be more efficiently cleared from plasma by the hepatic LDL receptor. The ratio of apoC to apoE may also be an important determinant of the fate of the particle, with particles containing high amounts of apoC being more likely to become LDL since they would more effectively escape uptake and catabolism in the liver (45). Relative size may also be an important determinant of the potential of VLDL to become LDL (42). The difference in size of the plasma VLDL is largely due to the triglyceride content of the particles. Since the apoB content per VLDL particle appears to be constant (46), the rate of triglyceride synthesis relative to the rate of apoB synthesis may determine the size of the VLDL particle produced by the liver and, consequently, the proportion of VLDL particles converted to LDL.

Numerous laboratories have investigated the enzyme-mediated modifications of plasma VLDL necessary for conversion to LDL. The importance of lipoprotein lipase and hepatic triglyceride lipase for the hydrolysis of VLDL core triglycerides and some surface phospholipids is well established (47), but the role of neutral lipid transfer proteins present in the plasma of many animal species is only recently being evaluated (reviewed in 48). Nichols and Smith (49) first demonstrated the exchange of VLDL triglyceride for HDL cholesteryl ester during in vitro incubations. Deckelbaum and coworkers (17) have shown that it is possible to exchange LDL cholesteryl ester for VLDL triglyceride in vitro in a reaction that results in a net transfer and a significant enrichment of the LDL with triglyceride. Subsequent addition of lipoprotein lipase to the system results in hydrolysis of a significant amount of the LDL triglyceride and a significant decrease in LDL particle size. Eisenberg (50) has shown that VLDL are heterogeneous and vary in ability to accumulate cholesteryl esters by exchange. The larger VLDL accepted more cholesteryl ester than the smaller particles. Plasma VLDL that have a cholesteryl ester content higher than that of plasma LDL are believed not to become plasma LDL through the usual delipidation pathway (51). Presumably, these particles are cleared directly from plasma by the liver, perhaps due to their apoE content as discussed above.

The role of lecithin:cholesterol acyl transferase (LCAT) in the formation of LDL has not been an active area of research. Rather, attention has been focused on HDL since apoA-I, the major apoprotein of HDL, and, to a lesser degree, apoE (52), are the primary activators of LCAT (53). However, it is generally believed that the majority of the cholesteryl ester molecules in the circulation of normal individuals are LCAT-derived (54). After formation by LCAT, the cholesteryl ester molecule is transferred by lipid transfer proteins to LDL and other lipoproteins. Lower amounts of IDL material, but larger LDL particle sizes, were noted after in vitro incubation of monkey whole plasma relative to plasma incubated with an LCAT inhibitor, DTNB (Carroll, R. M., and L. L. Rudel, unpublished observations). Concomitantly, LDL incubated with active LCAT lost surface lipids (free cholesterol and phospholipids) but gained soluble apoproteins, in particular apoE and apoA-I. These data suggest that LCAT may indirectly enhance the transfer of excess surface lipids from IDL or VLDL remnant particles to HDL presumably after depletion, by LCAT, of HDL free cholesterol and phospholipid. The HDL cholesteryl esters produced by LCAT can then be transferred to LDL in exchange for triglycerides by lipid transfer proteins in a reaction that has been shown to decrease LDL particle size while modifying its core composition (Babiak, J., and L. L. Rudel, unpublished observations). Therefore, it appears that LCAT may play an indirect role in the intravascular formation of LDL and is a factor to be considered in the development of LDL subpopulation heterogeneity.

B. VLDL-independent LDL formation

Recent in vivo kinetic studies of VLDL and LDL metabolism in humans and in nonhuman primates indicate that LDL arise not only from VLDL but also from direct secretion of LDL into the circulation (39, 40, 55,

56); furthermore, the proportion of LDL produced by direct secretion versus by conversion of VLDL also differs among individuals (56). The studies of VLDL and LDL apoB metabolism by Soutar, Myant, and Thompson (39) indicate that, in patients with homozygous familial hypercholesterolemia, the absolute synthetic rate of LDL apoB was about twice that of VLDL apoB such that VLDL apoB could not be the sole precursor to LDL apoB. Following protocaval shunt in one patient, the rate of LDL apoB synthesis dropped and could be totally accounted for on the basis of VLDL apoB-synthesis, implicating the liver as a source of newly synthesized LDL. The studies in type III hyperlipoproteinemia of Berman et al. (40) using radiolabeled VLDL apoB turnover indicated that a portion of the LDL apoB pool is not derived from VLDL or IDL and that an alternate mechanism of LDL production must occur in these individuals. By blocking VLDL catabolism in squirrel monkeys by injection of Triton WR 1339 and monitoring the incorporation of [^{14}C]leucine into LDL apoB, Illingworth (55) demonstrated that 10–19% of LDL apoB was directly secreted into the circulation. A similar conclusion was made based on studies in cynomolgous monkeys by Goldberg et al. (56) in which the catabolism of radiolabeled plasma VLDL and LDL was measured. Depending upon the animal, 25% to 75% of plasma LDL production could be accounted for by plasma VLDL conversion, the remainder presumably being derived from direct secretion of LDL into the plasma.

Direct hepatic secretion of lipoprotein particles within the LDL density range (1.019–1.063 g/ml) has been described in liver perfusion studies in pigs (57) and in nonhuman primates (21, 58). In primates, these particles did not have the composition of plasma LDL but were apoB-100-containing particles with an excess of surface constituents and with a triglyceride-rich instead of the cholesteryl ester-rich core typical of plasma LDL. ApoB-100 was the major apoprotein, but a significant amount of apoE was also present, and trace amounts of small molecular weight apoproteins (presumably mostly apoC) were also detected. All of these apoproteins were radiolabeled when [^{14}C]leucine was present in the perfusion medium, in both recirculating and in nonrecirculating perfusion systems (Johnson, F. L., and L. L. Rudel, manuscript in preparation). Approximately 25% of the perfusate lipoprotein cholesterol was present in the 'LDL-like' fraction, indicating that it represented a significant hepatic secretion product. In preliminary studies, some of this LDL-like material from primate liver perfusions has been labeled and reinjected into recipient monkeys. Significant portions of the labeled apoB-100 in the plasma lipoproteins of the recipients were found to be similarly distributed in density gradients and have a pattern of plasma decay similar to that of labeled plasma LDL, suggesting that this fraction contains LDL precursor lipoproteins (Marzetta, C. A., L. A. Zech, and L. L. Rudel, unpublished observations). These data provide evidence to support the concept that a precursor to plasma LDL of a density similar to LDL is secreted in significant amounts by the primate liver, and the data support the conclusions derived from the kinetic turnover studies cited above. Based on their lipid and protein composition, the LDL-like liver perfusate particles of our experiments in primates appear to represent the metabolic equivalents of small VLDL. However, the fact that they would be isolated from plasma in the 1.019–1.063 g/ml density range would mean that the presence of such LDL precursors would be missed in kinetic studies where plasma VLDL are isolated, labeled, and reinjected.

IV. LDL CATABOLISM

Most of the tissues in the body have been demonstrated to have the capacity to express LDL receptors; however, measurements in rats suggest that most cells satisfy their needs for cholesterol by synthesizing the small amount required without expressing the LDL receptors (59). The liver is a tissue that processes a lot of cholesterol daily and it is the organ of the body that demonstrates the highest level of LDL receptor activity (60, 61). In recent studies in rats and rabbits, it has been suggested that at least two-thirds of the LDL cleared from plasma each day is taken out of the circulation by the liver (62), and 80–90% of this is via the LDL receptor. It seems likely that the same is true in human beings (63). The LDL receptor in the liver has been shown to be regulated in rabbits and dogs so that when hepatic cholesterol accumulates, as in cholesterol feeding, the receptor level goes down (64), and when the animals are fed cholestyramine, the LDL receptor level increases (65). In young, rapidly growing dogs, the hepatic LDL receptor levels have been shown to be higher than in adults (43). On the other hand, Spady and Dietschy (66) have demonstrated in hamsters that hepatic LDL receptor levels are expressed somewhat independently of the hepatic cholesterol level, depending rather on the type of fat present in the diet. Receptor-mediated transport of LDL was suppressed by about 30% in hamsters fed dietary cholesterol with polyunsaturated fat. In contrast, it was suppressed by about 90% in animals fed hydrogenated coconut oil with an equivalent amount of cholesterol, even though the saturated fat-fed animals had lower liver cholesterol concentrations than the polyunsaturated fat-fed animals. If the same situation occurs during polyunsaturated fat feeding in humans (67) and nonhuman primates (68), it is possible that the degree of receptor insensitivity to down-regulation by cholesterol during polyunsaturated fat feeding could be part of the explanation for the lower plasma LDL cholesterol concentrations, in spite of higher liver cholesterol concentrations as seen in polyunsaturated fat-fed monkeys (58).

Results of studies performed in rabbits have suggested that the LDL receptor may play a role in LDL formation from IDL and VLDL precursors, in addition to its role in LDL clearance from plasma (69). In Watanabe heritable hyperlipidemic (WHHL) rabbits that do not possess a functional LDL receptor in the liver, IDL were seen to accumulate in addition to LDL during the delayed clearance of VLDL (69). The accumulation of IDL was believed to be due to the inability of this lipoprotein to interact with a liver LDL receptor in the receptor-deficient animals. IDL is a lipoprotein that contains apoE, in addition to apoB-100, and since more of it was converted into LDL in WHHL rabbits than in normal rabbits, it has been postulated that its normal pathway for clearance from the circulation is via the interaction of the apoE on the particle with the LDL receptor. The presence of apoB-100 on the particle appeared to prevent this particle from being cleared by the apoE or remnant receptor since the clearance of chylomicron remnants, containing apoB-48 (and apoE) was not delayed in WHHL rabbits (70). Therefore, it appears that the LDL receptor functions to permit LDL formation (via IDL) and to facilitate catabolism (62). Such a possibility is consistent with the early studies in familial hypercholesterolemic patients in which an overproduction of LDL as well as an impaired LDL clearance was described during turnover studies (71).

The degree of LDL receptor regulation in the liver appears to vary widely from species to species. The rabbit has been shown to down-regulate the LDL receptor upon cholesterol feeding (64), whereas the rat does not appear to down-regulate the LDL receptor until thyroid suppression has been induced (72). The hamster will down-regulate the LDL receptor in response to cholesterol feeding, but not as efficiently when polyunsaturated fat is fed (66). This degree of variability in species responsiveness of the LDL receptor has prompted Goldstein and Brown (73) to suggest that the degree to which the plasma cholesterol concentrations of individual animals are hyperresponsive or hyporesponsive to dietary cholesterol may well be due to the degree of down-regulation of the LDL receptor. We have noted that, in any group of monkeys to which dietary cholesterol is fed, a wide range of serum cholesterol concentration responsiveness occurs (74), suggesting the possibility that LDL receptor regulation might vary among these animals and account for at least a part of the heterogeneity of serum cholesterol responsivity among individuals. The extent to which the differential in this response is due to differences in hepatic LDL receptor activity is only a matter for speculation at the present time. However, the possibility that there are important differences among individuals in LDL receptor activity that lead to elevated plasma LDL concentration and size seems worthy of further evaluation.

Techniques with which to estimate the role of the LDL receptor in dietary cholesterol responsiveness are now available. Particularly important among these would appear to be the techniques that permit measurement of LDL receptor function in vivo. LDL receptor activity has been estimated using radiolabeled LDL in which chemical modifications of LDL have been used to block the interaction of the labeled ligand with the LDL receptor (75). This type of study may not be easily interpretable because the LDL used in these studies is usually autologous, whole LDL. Given the degree of LDL heterogeneity that is now apparent within and among individuals, the turnover rate derived from such a study will be an averaged rate, depending on the heterogeneity of the individual's LDL. Several studies, including ours in monkeys in 1978 (76), have now shown that LDL subpopulations exhibit separate kinetic behavior (77, 78). A probe for the LDL receptor that does not suffer from these limitations is a monoclonal antibody that binds to the LDL receptor but does not interfere with the binding of LDL or the internalization and recycling of the receptor. Studies using such a probe have been attempted in the rabbit (79). The monoclonal antibody to the LDL receptor was radiolabeled and reinjected together with a labeled nonimmune mouse IgG so that its rate of disappearance could be used to monitor the rate of LDL receptor function. WHHL rabbits were studied in addition to normal controls, and the rate of disappearance of the monoclonal antibody from the circulation was faster in the control animals than in the WHHL rabbits that have no LDL receptors (79). Since the LDL receptor sequence is now known (80), it should be possible to make a variety of monoclonal antibodies to further examine LDL receptor activity in vivo.

V. CONCLUSIONS

Low density lipoproteins are a class of lipoproteins that can be heterogeneous in size and composition, even within a single individual. In addition, the concentration of LDL in plasma varies widely among individuals. Heterogeneity and concentration are factors that may act together to determine atherogenicity. A hypothetical scheme for how this may occur follows.

Several laboratories have demonstrated an association in human beings between large LDL and increased incidence of CHD. It is possible that large LDL are also present when LDL receptor function is impaired, as in familial hypercholesterolemia where some overproduction of LDL may also occur. Increased concentration of LDL would appear to be the primary atherogenic feature in these cases, and modified LDL particle composition may be an additional factor. Other laboratories have found an

association between small LDL and increased incidence of CHD. The small LDL may result from the following sequence of events. Increased VLDL production and delayed LDL clearance occur together. LDL clearance is delayed due to elevated liver cholesteryl ester content and lowered hepatic LDL receptor levels. Lipolysis is inadequate to prevent hypertriglyceridemia which then occurs in the presence of normal intravascular cholesteryl ester for triglyceride exchange. Because LDL circulation time is increased, intravascular metabolism can reduce LDL size and the LDL in the circulation become smaller.

This scenario in humans may bear important similarities (and differences) to LDL metabolism in hypercholesterolemic monkeys. In these models, hepatic VLDL cholesteryl ester secretion is higher during hypercholesterolemia and LDL clearance is delayed. However, lipolysis is high and low plasma triglyceride concentrations are maintained, resulting in a minimal exchange of cholesteryl esters for triglycerides. Large, cholesteryl ester-rich LDL result. Many of the cholesteryl esters remaining in the large LDL of nonhuman primates are more saturated, suggesting that they were ACAT-derived. The presence of these more saturated cholesteryl esters in the large LDL modifies the transition temperature of the core such that, at body temperature, an ordered, liquid crystalline state occurs. This change enhances the atherogenicity of the LDL over and above that due to increased concentration and size, perhaps by promoting enhanced proteoglycan binding. Therefore, maintenance of higher plasma triglyceride concentrations, as occurs in humans compared to monkeys, may prevent accumulation in plasma of large, cholesteryl ester-rich LDL. In monkeys, enhanced hepatic cholesteryl ester secretion together with decreased cholesteryl ester for triglyceride exchange leads to formation of enlarged, relatively atherogenic LDL.

It is concluded that the interplay between hepatic production of LDL precursors of varying composition, intravascular metabolism of cholesteryl esters and triglycerides, and catabolism of apoB-containing particles can produce a wide variety of LDL compositions and concentrations. Increased LDL atherogenicity may result from several different combinations of modified production, intravascular metabolism, and catabolism, as suggested by the variety of associations between LDL heterogeneity and premature CHD.

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