review

Metabolic origins and clinical significance of LDL heterogeneity

Kaspar K. Berneis and Ronald M. Krauss[1]

Donner Laboratory, Lawrence Berkeley National Laboratory, University of California, Berkeley, CA 94720

Abstract LDLs in humans comprise multiple distinct subspecies that differ in their metabolic behavior and pathologic roles. Metabolic turnover studies suggest that this heterogeneity results from multiple pathways, including catabolism of different VLDL and IDL precursors, metabolic remodeling, and direct production. A common lipoprotein profile designated atherogenic lipoprotein phenotype is characterized by a predominance of small dense LDL particles. Multiple features of this phenotype, including increased levels of triglyceride rich lipoprotein remnants and IDLs, reduced levels of HDL and an association with insulin resistance, contribute to increased risk for coronary heart disease compared with individuals with a predominance of larger LDL. Increased atherogenic potential of small dense LDL is suggested by greater propensity for transport into the subendothelial space, increased binding to arterial proteoglycans, and susceptibility to oxidative modification. Large LDL particles also can be associated with increased coronary disease risk, particularly in the setting of normal or low triglyceride levels. Like small LDL, large LDL exhibits reduced LDL receptor affinity compared with intermediate sized LDL. Future delineation of the determinants of heterogeneity of LDL and other apoB-containing lipoproteins may contribute to improved identification and management of patients at high risk for atherosclerotic disease.—Berneis, K. K., and R. M. Krauss. **Metabolic origins and clinical significance of LDL heterogeneity. *J. Lipid. Res.* 2002.** 43: **1363–1379.**

Supplementary key words atherosclerosis • low density lipoprotein • lipoprotein subclasses • insulin resistance • intermediate density lipoprotein • very low density lipoprotein

Physicochemical heterogeneity

Distinct VLDL, IDL, and LDL subpopulations have been identified and defined on the basis of a number of characteristics, including particle buoyant density, size, charge, and lipid and apolipoprotein content [reviewed in (1–3)] (**Table 1**). In normolipidemic humans there are at least two physically distinct species of VLDL: larger VLDL (VLDL-1, S_f 60–400), and smaller (VLDL-2, S_f 20–60), as well as two subspecies of IDL (1, 2, 4, 5). The larger form of IDL, designated IDL-1, appears to form a continuum with VLDL-2, such that together, these represent a spectrum of particles of S_F 14–60 and $d < 1.010$ g/ml (1). Most of the differences in size among VLDL and IDL are due to the esterified lipid core since the phospholipid/cholesterol/apolipoprotein coat is of constant thickness in these lipoproteins (6, 7). Compared with larger VLDL, smaller VLDL and IDL are enriched in cholesteryl ester, depleted in triglyceride, and have a lower ratio of apoE and apoC to apoB (6, 7).

Nondenaturing gradient gel electrophoresis has identified as many as seven distinct subspecies of LDL (8, 9) that have been grouped, based on density, into four major subclasses designated LDL-I through -IV, from the largest, most buoyant to the smallest, most dense (1, 2, 8). Therefore LDL do not comprise a population of particles with a continuously variable size, but there are a number of subclasses of particles with relatively discrete size and density. Subspeciation of LDL particles appears to involve differences in surface lipid content and conformational changes in apoB-100, including increased exposure on the particle surface (10). Features of apoB structure that may contribute to stepwise change in LDL particle diameters have been described recently (10). Mass concentrations of subfractions across the VLDL-IDL-LDL spectrum can be determined by chromatographic and ultracentrifugal techniques (1, 2, 11, 12). In addition, methods have been described for evaluating size distribution of apoB containing lipoproteins by NMR spectroscopy (13).

Apolipoprotein heterogeneity

Subspeciation of apoB-100-containing particles also has been described as a function of differing content of other apolipoproteins (3). In plasma of normolipidemic subjects, the most abundant particles contain only apoB-100 with lesser concentrations of particles containing apoB-

Manuscript received 13 March 2002 and in revised form 20 May 2002.
DOI 10.1194/jlr.R200004-JLR200

Abbreviations: GGE, gradient gel electrophoresis; HL, hepatic lipase; LpL, lipoprotein lipase; S_f, Svedberg flotation rate.

[1] To whom correspondence should be addressed.
e-mail: rmkrauss@lbl.gov

TABLE 1. Physicochemical properties of apolipoprotein B containing lipoproteins subspecies

	Peak S_f	Density Peak (gm/ml)	Diameter (Å)	%PR	%CE	%UC	%TG	%PL
VLDL (4)								
VLDL-1	60–400	<1.006	330–700	11	8	6	58	17
VLDL-2	20–60	1.006–1.010	300–330	18	24	9	29	22
IDL (4)								
IDL 1	12–20	1.008–1.022	285–300	17	35	10	16	21
IDL 2	10–16	1.013–1.019	272–285	17	37	11	13	21
LDL (11, 12, 127)								
LDL-I	7–12	1.019–1.023	272–285	18	43	9	7	22
LDL-II	5–7	1.023–1.028	265–272	19	45	10	4	23
		1.028–1.034	256–265	21	45	9	3	22
LDL-III	3–5	1.034–1.041	247–256	22	46	8	3	21
		1.041–1.044	242–247	24	44	7	3	21
LDL-IV	0–3	1.044–1.051	233–242	26	42	7	5	19
		1.051–1.06	220–233	29	40	7	6	18

PR, protein; TG, triglycerides; CE, cholesteryl ester; PL, phospholipids; UC, unesterified cholesterol.

100 in conjunction with apoC-III and apoE, singly and in combination (14, 15). ApoB only particles are most commonly found in LDL, whereas most particles with apoC-III and apoE are triglyceride-rich particles. There is, however, evidence for the presence of these apolipoproteins in LDL, particularly at the extremes of the density ranges (16), (La Belle, Krauss et al., unpublished observations). VLDL subspecies lacking apoE appear to comprise one third of total VLDL (17), and have been shown to have increased content of phosphatidylethanolamine (18).

Isolation and definition of lipoprotein subfractions due to apolipoprotein composition is challenging as all apolipoproteins are exchangeable with the exception of apoB. Using anti apoE and apoC-III immunoaffinity chromatography in sequence and then ultracentrifugation, it has been demonstrated recently that VLDL with apoC-III is increased in hypertriglyceridemic patients and carries most of the apoE. The concentrations of particles without apoE and without apoC-III were similar between a hypertriglyceridemic and a normolipidemic group, but distributed more to VLDL and IDL than to the LDL density range. In contrast, concentrations of particles without apoC-III and apoE were increased in hypercholesteremic patients (19). These findings are consistent with evidence as described below that VLDL particles with apoC-III have decreased clearance and may therefore promote atherosclerosis.

Metabolic influences on LDL heterogeneity

Production of LDL subclasses. It has been suggested (**Fig. 1**) that there is metabolic channeling within the VLDL-IDL-LDL delipidation cascade such that parallel processing pathways generate different IDL and LDL products from different triglyceride-rich lipoprotein precursors (20–24). Very large VLDLs normally yield small amounts of LDL (25, 26) since their delipidation generally ceases in the VLDL or IDL density range with the formation of remnants that may be cleared from the plasma or persist in the circulation. Variation in plasma triglyceride is principally a function of changing VLDL-1 levels (27). The fractional conversion rate from VLDL-1 to VLDL-2 is reduced 90% in LpL deficiency (20, 26), indicating that a substantial portion of VLDL-2 represents lipolytic remnants of larger particles. Lipolysis of VLDL is not affected by hepatic lipase deficiency (28), by homozygous familial hypercholesterolemia (29), or by homozygosity for apoE-2 (30). A portion of VLDL-2 also arises by direct hepatic production, indicating further biochemical heterogeneity within the VLDL particle spectrum. While the rate of VLDL-1 secretion is dependent on triglyceride availability (31–33), VLDL-2 secretion may be more dependent on cholesterol synthesis (34), cholesterol ester availability (34) and microsomal transfer protein activity (35).

Smaller VLDLs are more effective ligands than their larger counterparts for LDL receptors (36). Studies in LDL receptor deficient and VLDL receptor transgenic mice suggested a role for the VLDL receptor in peripheral uptake of VLDL (37). Since apoB seems not to be in a receptor-competent conformation on large triglyceride-rich VLDL (38), the ligand for the putative receptor is likely to be apoE, while apoC-III may play an inhibitory role by interacting with or displacing apoE (39–42). The LRP receptor is involved in uptake of chylomicrons (43) and VLDL remnants (44). The role of heparan sulfate proteoglycans in LpL (45) and apoE-mediated lipoprotein uptake (46, 47) has been demonstrated. Recently it has been shown that apoB-48 may mediate the binding of triglyceride rich lipoproteins to a human monocyte-macrophage receptor (48).

While most remnants of large VLDL are rapidly cleared from plasma without undergoing further intravascular metabolism (25, 26), not all large VLDL are metabolized by this route. In Watanabe heritable hyperlipidemic (WHHL) rabbits, a relatively high proportion of IDL and LDL production results from catabolism of a minor subpopulation of large apoE-containing VLDL particles that are cleared slowly from plasma (49). A metabolic relationship of large VLDL with small dense LDL in humans is suggested by recent stable isotope kinetic studies indicating increased transport of large VLDL through a metabolic cascade to small IDL in subjects with a predominance of small dense LDL (50). The finding that VLDL-1 and small dense LDL are metabolically related is also described in the model by Packard et al. (51) in which CETP is required for the formation

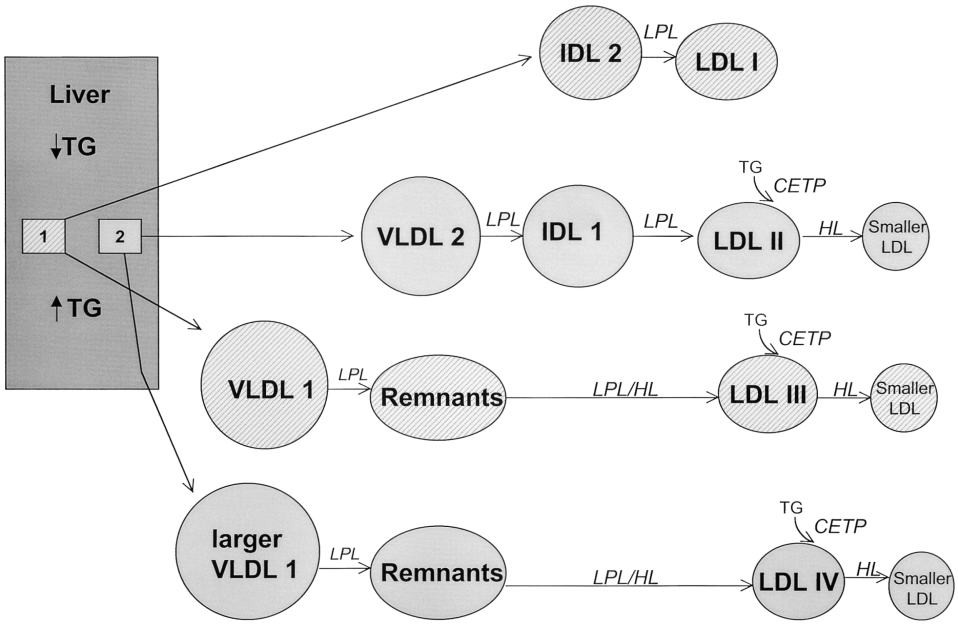

Fig. 1. Hypothetical metabolic scheme incorporating proposed pathways for the production of the major LDL subclasses I, II, III, and IV. As described in the text, a key hypothesis is that the properties of triglyceride-rich lipoprotein species secreted by the liver are determined in large part by the operation of multiple pathways (e.g., those labeled 1 and 2) as well as by hepatic triglyceride availability. Pathway 1: The coordinate production of triglyceride-rich VLDL-1 and triglyceride-poor IDL-2 is based on the reciprocal relationship that we have observed between their hypothesized catabolic products LDL-I and LDL-III (Fig. 2). We propose that production of VLDL-1 results from loading of a discrete quantity of lipid on a precursor particle. Lipolysis of VLDL-1 yields remnants, which in turn yield LDL-III by the action of hepatic lipase. Further remodeling of these particles may occur by CETP mediated triglyceride enrichment and hepatic lipase mediated lipolysis. Pathway 2: We hypothesize that this pathway, which results in production of VLDL-2, is distinct from pathway 1 and gives rise to IDL-1 and LDL-II by lipolysis (68). Further processing by CETP mediated transfer of triglycerides into LDL-II and lipolysis by hepatic lipase yields smaller and denser LDL products. Finally, we propose that in an analogous manner to pathway 1, there is loading of a discrete quantity of lipid in hepatic VLDL in pathway 2 (larger VLDL-1) that includes precursors of the smallest and densest LDL IV. TG, triglycerides; CE, cholesteryl esters; LpL, lipoprotein lipase; HL, hepatic lipase; CETP, cholesterol ester transfer protein.

of small dense LDL. However, as described further below, there is evidence that CETP is not required for this process.

IDL and LDL represent discrete, thermodynamically stable particle configurations that are reached sequentially during the course of intravascular catabolism of VLDL. The transformations from VLDL to IDL and LDL are enabled by the presence of sufficient triglyceride to sustain lipolysis. This process also appears to depend on an as yet uncharacterized apoE-dependent mechanism (21), resulting in loss of apoE from most LDL particles. A portion of the IDL fraction is catabolized directly from plasma, probably via the LDL receptor since the rate of this process is dramatically reduced in FH homozygotes (29, 52). ApoE phenotype influences the conversion of IDL to LDL. In normolipidemic apoE-2 homozygotes, a 60% reduction in the rate of transfer of IDL to LDL was observed while direct catabolism of the fraction, presumably mediated by its apoB component, was normal (30).

Although in vivo kinetic studies have not definitively established the specific precursor-product pathways for the generation of individual LDL subclasses, studies in animal models have indicated that separate pathways may be responsible for production of differing forms of LDL. In rats, kinetic studies have shown that larger LDLs (S_f 5–12) are derived via a VLDL-IDL metabolic cascade, but small dense LDLs (S_f 0–5), which comprise 65% of total LDL mass, do not appear to derive from this pathway (53). In monkeys, it has been reported that the metabolic behavior of LDL derived from endogenously radiolabeled hepatic lipoprotein precursors often differs from that of radiolabeled autologous plasma LDL (54, 55). Kinetic analysis and studies involving nascent lipoproteins from perfused livers (55, 56) suggested that plasma LDL in these monkeys may be derived from a variety of precursors, with each source giving rise to metabolically (and possible physically) distinct LDL particles. In a spontaneously hypercholesterolemic strain of pigs, two metabolically distinct LDL subclasses have been characterized: the larger, more buoyant species appears to accumulate as a results of both increased production and reduced receptor clearance resulting from an apoB mutation (57). This subclass does not appear to arise either from catabolism of plasma VLDL, or from enlargement of smaller LDL (58), although an LCAT-induced increase in buoyancy of the denser LDL species in pigs has been reported (59).

A number of kinetic studies in animals and humans

have suggested that hepatic apoB is secreted throughout the VLDL-IDL-LDL particle spectrum (60–62). Direct production of LDL has been reported in kinetic analyses in humans (60), in perfused livers of experimental animals (55, 63–65), and cardiac tissue (66). Using a trideuterated leucine tracer and analysis with a multicompartmental model which allowed input into each fraction, Packard et al. found substantial input of apoB into IDL and LDL, which was inversely related to plasma triglycerides (67). Therefore, it may be, as first postulated in 1977 (62), that the whole range of apoB containing particles can be secreted from the liver. At low levels of triglyceride, a significant portion of apoB is released as IDL or LDL (60), whereas at normal or high triglyceride levels more than 90% of apoB is secreted in the form of VLDL (30, 60). Thus, despite differences in the characteristics of lipoprotein metabolism in humans, rats, monkeys, and pigs there is evidence in all these species for independent pathways resulting in production of differing forms of LDL.

However, the metabolic determinants of these parallel pathways are not understood. Studies in our laboratory have focused on the role of IDL heterogeneity in metabolic pathways leading to discrete LDL subspecies (1, 2, 68). The results have indicated that the small VLDL-2/IDL-1 particle spectrum includes precursors of mid-sized LDL-II, while IDL-2, which overlaps the size and density distribution of large LDL (LDL-I), also includes metabolic precursors of these particles (Fig. 1). This is also consistent with recent findings that IDL-1 is positively related to plasma triglycerides, whereas the smaller subfraction IDL 2 falls as triglycerides increase, suggesting that IDL 1 is part of the delipidation cascade, whereas IDL 2 arise from a separate source, possibly direct liver production (4, 5).

The model described in Fig. 1 suggests that variation in hepatic triglyceride availability determines properties of primary lipoprotein secretory products. Specifically, the hypothesized hepatic pathway (pathway 1) for the coordinate production of triglyceride-rich VLDL-1 and triglyceride-poor IDL-2 in Fig. 1 is based on previously described reciprocal relationships involving their proposed lipolytic products LDL-III and LDL-I, respectively (**Fig. 2**) (69). Similarly, based on reciprocal relationships between LDL II and LDL IV (Fig. 2) (69), we hypothesize that VLDL-2 and larger forms of VLDL-1 (pathway 2 in Fig. 1) are precursors of LDL-IV and LDL-II, respectively.

The scheme shown in Fig. 1 is based on discrete transitions occurring between hepatic production of smaller triglyceride poor and larger triglyceride-rich particles. This is consistent with the current concept of two stage hepatic assembly of triglyceride-rich VLDL involving the fusion of a lipid droplet with a core apoB-containing particle (70). The scheme is also consistent with recent evidence described above that there is increased "direct" hepatic secretion of IDL and LDL particles in individuals with lower plasma triglyceride levels (67). In addition, the discrete transitions in the metabolic pathway associated with greater hepatic triglyceride, availability may provide a metabolic framework for understanding the origin small dense LDL

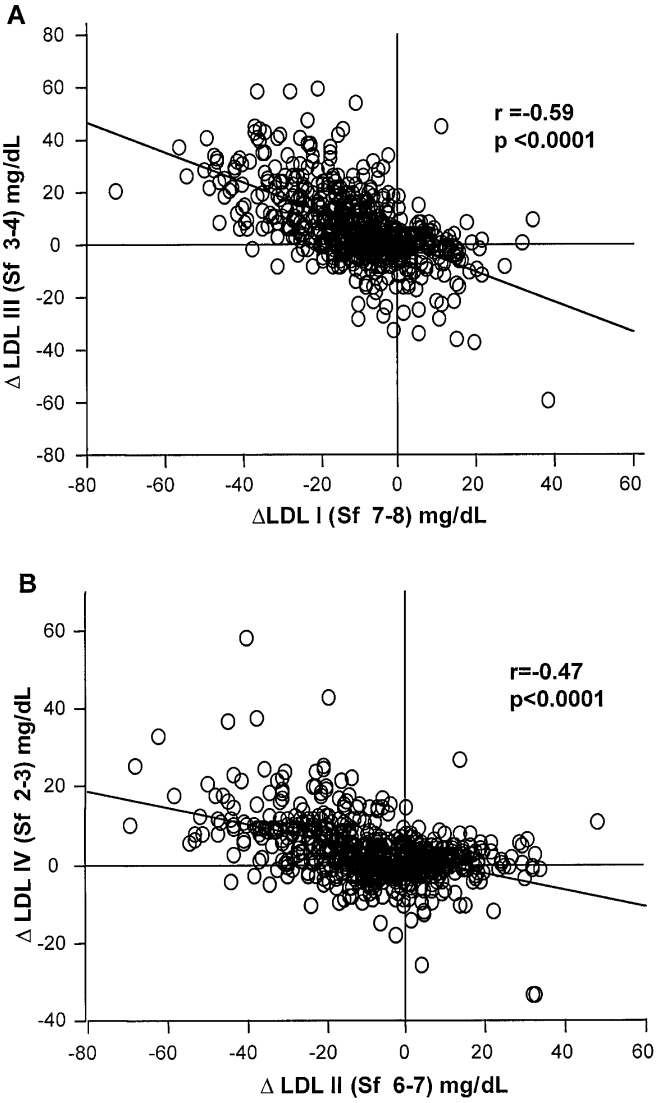

Fig. 2. Reciprocal relationships between diet induced changes in LDL subclasses as measured by analytical ultracentrifugation. Fasting blood samples were obtained from 686 healthy non-smoking Caucasian men and women aged >20 years and after 4–6 weeks of consuming diets containing high fat (35–46% energy) and low fat (20–24% energy). Inclusion criteria were plasma total cholesterol concentrations <260 mg/dl, triacylglycerol <500mg/dl, body weight <130% of ideal. A subset of 501 subjects in this population is described in reference (211). Differences in lipoprotein mass between the high and low fat diets were calculated and relationships between subclasses were analyzed by linear regression. LDL subclasses were narrowed to single flotation intervals to optimize statistical power. A: Relationship between the change in LDL I (S_f 7–8) and LDL-III (S_f 3–4). B: Relationship between the change in LDL-II (S_f 6–7) and LDL-IV (S_f 2–3).

(pattern B) phenotype and relation to plasma triglyceride levels, as described in greater detail below. Another aspect of LDL heterogeneity illustrated in Fig. 1 is the overlap of lipolytic remnants of larger VLDL with the size spectrum of directly secreted VLDL-2, as described above.

LDL catabolism and plasma clearance. LDLs are metabolically heterogeneous with some components being removed more rapidly than others. The initial rapid plasma decay is

due to both intra-extravascular exchange and catabolism of LDL (71). In studies of hypertriglyceridemic human subjects, increased LDL fractional catabolic rate was associated with a concomitant rise in the fractional catabolic rate for cylohexanedione-treated LDL (72), while receptor mediated clearance remained unchanged over the range 2.5 mmol/l to 5.0mmol/l of plasma triglyceride, suggesting that small dense LDL in these subjects are cleared from plasma to a large extent by receptor-independent pathways (72). Enhanced binding to LDL receptor-independent binding sites in (e.g., the arterial wall), a process mediated, in part, by cell surface proteoglycans, may be responsible for the enhanced atherogenic potential of small dense LDL particles as described further below (73). Using ^{13}C-NMR, it has been suggested that differences in the conformation of apoB-100 and surface charge between LDL subspecies are major determinants of their catabolic fate (74). These authors also suggested that the intermediate size LDL subspecies constitute the optimal ligand for the LDL receptor among human LDL particle subpopulations.

Role of plasma lipase activities in production of LDL from triglyceride-rich precursors

Since plasma lacks significant cholesteryl esterase activity, lipolytic catabolism of apoB containing lipoproteins in plasma requires triglyceride hydrolysis. Numerous studies have demonstrated strong correlations of plasma triglycerides and VLDL levels with increasing density and decreasing size of the predominant LDL subspecies (9, 69, 75). This may be related in part to the pathway relating VLDL to LDL heterogeneity shown in Fig. 1, but is also likely to reflect triglyceride enrichment and remodeling of LDL by lipase activities. LDL size and density in turn are inversely related to levels of plasma HDL, particularly the HDL-2 subclass (69, 76). By exchange of cholesteryl esters with triglycerides, LDL and HDL can become triglyceride enriched and can be processed further by lipases. Deckelbaum et al. described profound changes in the core and surface domains of both LDL and HDL particles with increasing triglyceridemia, including progressive depletion of core cholesteryl esters and replacement by triglyceride molecules (77). These processes can lead to further reductions in size and increases in density of the LDL species that arise by the pathways shown in Fig. 1.

Postheparin plasma lipoprotein lipase (LpL) activity is associated with levels of both larger LDL (78) and HDL 2 (79) and this may be due at least in part to transfer of surface lipids and apolipoproteins in the course of chylomicron and VLDL triglyceride hydrolysis. We have found significant inverse relationships of postheparin LpL activity with plasma levels of triglyceride, apoB, large VLDL mass, and small dense LDL (LDL-III) (80). Further increases in LpL activity induced by a high fat diet were found to be significantly positively correlated with increases in small IDL and large LDL I mass, and inversely related to changes in small LDL-III mass. These results coupled with reciprocal changes between LDL-I and LDL-III (69, 80) (Fig. 2), are consistent with the hypothesis that LpL contributes to the coordinate regulation of these LDL subfractions. Moreover, it has been reported (81) that in vitro lipolysis of VLDL by LpL generates IDL and large lipid and apoE-enriched LDL. Finally, in vivo conversion of VLDL and LDL are completely inhibited when LpL activity is blocked in the monkey (82). From studies of VLDL composition (83), human mutants (84), and genetically altered mice (85) it has been shown that apoC-III can impair VLDL lipolysis. While this could result in part from direct inhibition of activation of lipoprotein lipase by apoC-II (86, 87), apoC-III may also inhibit LpL mediated lipolysis of triglyceride-rich lipoproteins by interfering with lipoprotein binding to the cell-surface glycosaminoglycan matrix where lipolytic enzymes and lipoprotein receptors reside (88). ApoC-III can also inhibit hepatic clearance of triglyceride-rich lipoproteins (42). Therefore, both lipid and apolipoprotein composition of VLDL may be important regulators of lipolysis in vivo. Studies in apoC-III transgenic mice have suggested that excess plasma apoC-III interferes with the apoE-mediated clearance of lipoproteins, and that this effect can be corrected by administration by exogenous apoE (89). This may occur by displacement of apoE from the lipoprotein surface by apoC-III (39–42) by interference with the interaction of apoE and the receptors mediating the clearance of these particles, or by impairing the interaction of apoE and HL, since apoE may modulate the activity of this enzyme (HL) (90, 91). However, from studies in apoE knockout mice it has been concluded that apoC-III mediated hypertriglyceridemia is not due to effects on apoE (92). A role for apoC-III in impairing triglyceride rich lipoprotein catabolism in humans was suggested by a recent study in normolipidemic women in which there was reduced FCR of VLDL and IDL particles that were enriched in apoE and apoC-III (93).

As described above, factors contributing to increased plasma triglyceride levels can promote triglyceride enrichment of larger LDL particles that may give rise to smaller, denser products by lipolysis. There is growing evidence that HL has a critical role in this process (Fig. 1). While VLDL lipids are hydrolyzed more effectively by LpL than HL, IDL, and LDL lipids are significantly better substrates for HL than LpL (94). HL has a higher affinity for LDL than LpL and has the capability to act as a phospholipase as well, hence removing both core and surface components from the particle (90, 95). Buoyant, triglyceride-rich LDL particles accumulate in patients with HL deficiency (96, 97) after acute inhibition of HL activity in the cynomolgus monkey (82) and in hepatic knockout mice (98). A significant inverse relation of HL activity with levels of large buoyant LDL has been reported (99). We have found that in normolipidemic subjects, HL is significantly positively correlated with plasma triglyceride, apoB, and mass of large VLDL and small dense LDL (LDL-III), but not correlated with mass of large LDL (LDL-I) (80). Based on these observations, it may be suggested that HL is a critical step in the formation of small dense LDL-III, although there is as yet no direct evidence as to the lipoprotein substrate for this activity. In vitro incubations of

plasma VLDL with both LpL and HL have failed to generate smaller LDL products (81). This approach is of course limited since it provides neither nascent precursor particles nor remodeling steps that may exist in vivo. Diet induced changes in HL activity have been found to correlate inversely with changes in levels of small IDL-2/LDL-I (80), implicating a possible role for HL in the clearance or catabolism of these particles. While it is evident that there must be sufficient triglyceride in VLDL and IDL precursor particles to enable full lipolytic transformation to smaller species, there may be substantial variation in core lipid content of each species. Hence, if output of hepatic VLDL triglyceride were to significantly exceed that of VLDL cholesterol (VLDL-C), IDL and LDL products of VLDL catabolism could remain triglyceride enriched. It has been hypothesized that this may be responsible for the small, triglyceride-enriched LDL found in the plasma of high-expressing human apoB transgenic mice (100) and rabbits (101). It is also possible that the appearance of triglyceride-enriched particles in the LDL density range is due to incomplete lipidation of nascent VLDL particles in the liver. With progressive increase in hepatic VLDL cholesteryl ester secretion, as can occur in cholesterol fed animal models including apoB transgenic mice (102, 103), there is replacement of VLDL triglyceride by cholesterol, and a progression in accumulation of cholesterol enriched LDL, IDL, and ultimately β-VLDL (remnant) subspecies.

Role of cholesteryl ester transfer protein in the metabolism of LDL subclasses

By mediating triglyceride enrichment of IDL and large LDL, CETP may promote lipolytic conversion to smaller denser LDL (Fig. 1) (77). Although CETP activity has not been related to LDL subfraction distribution in normolipidemic subjects (78, 99), it has been reported that CETP levels in moderately hypertriglyceridemic subjects are strongly inversely correlated with HDL-C (104, 105). Similar observations have been made in hypertriglyceridemic mice expressing high levels of human apoC-III and CETP (106). In contrast, in normolipidemic mice, CETP overexpression appears to result in increased LDL particle size (107). These observations are consistent with the hypothesis from in vitro studies that CETP activity may be rate limiting for the transfer of triglyceride from VLDL to HDL in hypertriglyceridemia, but not normotriglyceridemia (108). This is in accordance with a very recent study in FCHL subjects where CETP (and HL) activity only correlated with LDL size after correction for plasma triglycerides, suggesting that CETP activity is only rate limiting in hypertriglyceridemic FCHL subjects (109). Interestingly, transgenic mice expressing both human apoB and CETP have triglyceride profiles similar to those seen in mice expressing human apoB alone (110), but a higher percentage of total serum cholesterol in the combined LDL and VLDL fraction than in transgenic mice expressing human apoB alone. High CETP also has been observed in a distinct subgroup of subjects with isolated low HDL and normal postheparin lipase activities (111). In view of the strong correlation of both increased triglyceride and reduced HDL with reduced LDL particle size (69, 112, 113), it is possible that CETP may contribute to reduced LDL particle size in subjects with moderate increases in plasma triglyceride and reduced HDL such as are found in subjects with small dense LDL phenotype discussed below. In type 2 diabetes patients it has been demonstrated that CETP contributes significantly to the increased levels of small dense LDL by preferential CE transfer from HDL to small dense LDL, as well as through an indirect mechanism involving enhanced CE transfer from HDL to VLDL-1 (114).

Notably, studies in patients with homozygous familial CETP deficiency have indicated that CETP is not required for the formation of small LDL (115). In these subjects, multiple plasma LDL subpopulations have been identified, including a small, triglyceride-rich LDL subpopulation that overlaps in density distribution with the predominant larger LDL species. Recent evidence has indicated that a primary acceptor for CETP-mediated HDL cholesteryl ester transfer in normolipidemic subjects is a large, buoyant, triglyceride-enriched LDL subclass (116). Thus, it is possible that retention of such triglyceride-rich LDL and subsequent lipolytic processing contributes to the heterogeneous LDL subclass profile found in patients with CETP deficiency. In heavy alcohol drinkers, multiple LDL species are observed on GGE with normalization upon abstention from alcohol and may be associated with secondary, partial CETP deficiency in these patients (117). Consistent with this notion is the observation that incubation of plasma containing "polydisperse" LDL with CETP can shift the LDL to a monodisperse pattern (118). We have shown that human-like LDL subclasses with normal composition are found in fat-fed transgenic mice expressing high levels of human apoB, but with low cholesteryl ester transfer activity (119). Thus, while increased CETP can promote triglyceride enrichment of lipolytic precursors of small dense LDL, results in humans and mice indicate that CETP activity is not critical for the production of LDL size heterogeneity per se.

Other metabolic influences on LDL particle size distribution

With cholesterol feeding on monkeys, hepatic acyl-CoaA acyltransferase (ACAT) activity was found to be positively correlated with hepatic cholesteryl ester secretion and with plasma LDL cholesteryl ester content, which in turn was proportional to LDL particle size (120). Earlier evidence (55) had indicated that secretion rate of nascent lipoproteins of $d < 1.063$ g/ml by perfused livers of cholesterol fed monkeys was proportional to average particle size of plasma LDL from the same animals, and that cholesteryl ester in nascent apoB-containing particles was not LCAT derived, again suggesting a key role for ACAT in process in this species. These studies also indicated that receptor-mediated uptake of LDL was not a factor influencing the distribution of perfusate lipoproteins, although activity of other receptors (e.g., LRP) was not ruled out. It also has been shown that subsets of VLDL particles secreted by perfused monkey livers are rapidly converted to plasma LDL when infused in vivo (56). Therefore, with a high choles-

terol diet, ACAT may promote cholesteryl ester enrichment and secretion of VLDL precursors of large LDL particles that do not arise via progressive intravascular catabolism of a series of intermediate-sized remnants. As described further below, such large LDL, enriched in cholesteryl ester and apoE, have been associated with atherosclerosis severity (121). We hypothesize that secretion of such particles corresponds to the IDL-2/LDL-I pathway shown in the Fig. 1.

It has been reported that activity of a bile-salt dependent pancreatic carboxyl ester lipase (CEL) found in human serum is inversely correlated with serum cholesterol and LDL levels, and that the enzyme can hydrolyze cholesteryl ester in LDL, as well as in HDL-3 in vitro (122). Evidence has also been presented that the enzyme can mediate the production of small LDL from larger LDL, and may contribute to the reduced cholesterol content of the smaller particles (122). However, while CEL knockout mice have been found to have reduced absorption of dietary cholesteryl ester, there were no significant changes in plasma cholesterol levels (123). This finding, together with a lack of evidence for plasma cholesteryl ester hydrolysis in vivo, raises the question as to whether this enzyme has a significant role in plasma lipoprotein metabolism.

It is not known to what extent differences in receptor mediated clearance of LDL subclasses contribute to variation in plasma LDL particle distribution. As noted above, reduced LDL receptor binding has been reported for more buoyant and more dense LDL in comparison with intermediate density LDL subspecies (124, 125), which is in concordance of the finding of reduced LDL fractional catabolic rate in pattern B subjects (67). The reduction in receptor binding affinity of the smaller denser LDL found in hypertriglyceridemic subjects has been shown to be independent of triglyceride content (126). Differences in non-receptor mediated LDL clearance among LDL subpopulations also may contribute to variations in LDL particle distribution. Smaller LDLs bind more avidly to arterial wall proteoglycans, possibly in relation to their reduced content of sialic acid (127–129). Sialic acid, perhaps because of its exposure at the LDL surface, plays a determinant role in the in vitro association of LDL with the polyanionic proteoglycans (128). ApoC-III which has been found to be increased in small LDL particles (La Belle et al., unpublished observations) can also increase proteoglycan binding of apoB containing lipoproteins (130). The retention of small dense LDL in arterial tissue may contribute to its plasma clearance.

SMALL DENSE LDL PHENOTYPE

Genetic and environmental influences on the small dense LDL phenotype

A distinct LDL subclass pattern characterized by a predominance of small dense LDL particles (principally LDL-III) has been identified using both nondenaturing gradient gel analysis (131) and ultracentrifugation (12, 132). The prevalence of this trait, which has been designated LDL subclass pattern B, is 30–35% in adult men, but is much lower (5–10%) in males <20 years and in premenopausal women (131, 133), and is intermediate (15–25%) in postmenopausal women (134, 135). Compared with individuals with larger LDL (pattern A), pattern B is associated with approximately a 2-fold increase in plasma triglycerides, higher plasma apoB and IDL levels, and reduced HDL-C and apoA-I concentrations (136). Evidence from several studies for major gene determinants of this phenotype has been summarized previously (137). Two additional studies using complex segregation analysis have confirmed major gene effects on LDL diameter as a quantitative trait (138, 139). The data have been most consistent with an autosomal dominant or codominant model for inheritance of the pattern B phenotype with varying additive and polygenic effects. Linkage of the pattern B phenotype to the region of the LDL receptor gene locus on chromosome 19p (140) has been confirmed using quantitative sibpair linkage analysis of LDL particle size in 25 kindreds ascertained on the basis of two affected members with coronary artery disease (141). In these families, evidence was also obtained for linkage to regions near three other genetic loci: the apoA-I, C-III, A-IV cluster on chromosome 11; the CETP locus on chromosome 16; and the Mn-SOD gene on chromosome 6. Quantitative sibpair linkage-analysis in 126 dizygotic women twin pairs indicated linkage of the apoB gene to peak LDL size and plasma triglycerides, HDL-C, and of apoB levels (142). These results suggest that multiple genes may contribute to determination of particle size of the major LDL species in plasma, and that the responsible genetic mechanism may differ among affected families. In a recent study, markers in the vicinity of the HL gene locus also were found to contribute to LDL size in a series of Dutch families with familial combined hyperlipidemia using nonparametric sibpair linkage and association analyses (143). In addition, in a genome-wide scan there was evidence for linkage of LDL particle size, HDL-C, and triglyceride levels to the vicinity of the HL gene locus (143). However, there was no evidence in this study for linkage of LDL size to a polymorphism in the hepatic lipase gene promoter that has been associated with HDL-C (143). Recently linkage of peak LDL size to the CETP gene has been confirmed (141, 144). No study to date has identified a DNA sequence causally related to LDL size (145). The linkage between LDL size and apoE protein polymorphism is uncertain: no relationship could be demonstrated between LDL particle size and apoE phenotype in healthy 35-year-old males (146) and a recent study by Austin et al. using sibpair linkage analysis demonstrated no linkage of LDL size to the apoE gene (142). However, Haffner et al. reported an association between LDL size and the apoE polymorphism in 337 nondiabetic subjects from the San Antonio Heart Study (147).

Estimates of heritability of LDL particle size have ranged from 35–45% (148), indicating the importance of nongenetic and environmental influences. In addition to age and gender, effects on LDL particle size and density distribution have been shown for abdominal adiposity (149), estrogen (150), and oral contraceptive use (151). Dietary intervention studies have shown that variation in dietary fat

and carbohydrate can strongly influence expression of the small LDL phenotype (152, 153), and contribute to variations in LDL particle size distribution that are observed among individuals and population groups (154). It has been demonstrated in offspring genetically predisposed to phenotype B that a very low fat, high carbohydrate diet can induce expression of this phenotype (155, 156). Recently, both genetic and dietary factors have been reported to affect LDL size phenotypes in baboons (157). Thus, LDL subclass phenotypes may result from interaction of multiple genetic and environmental determinants, and the trait can be viewed as a marker for the mechanism underlying these effects. In view of the close relationship of change in plasma triglyceride levels with change in LDL particle size (75, 139), the clustering of metabolic changes associated with pattern B, including increased VLDL and IDL, reduced HDL, and insulin resistance (135, 158), and the metabolic relationships described above, it is likely that both genetic and non-genetic determinants of pattern B involve coordinate effects on metabolism of plasma triglyceride-rich lipoproteins and LDL subclasses. On the basis of the strength of the relationship between insulin resistance and the pattern B, small dense LDL has been added to the list of abnormalities that characterize the "metabolic syndrome" (158).

It is noteworthy that a predominance of small dense LDL is commonly found in conjunction with familial disorders of lipoprotein metabolism that are associated with increased risk of premature coronary artery disease. These include familial combined hyperlipidemia (159), as well as hyperapobetalipoproteinemia (160) and hypoalphalipoproteinemia (161). There is evidence that the inheritance of familial combined hyperlipidemia involves at least two major gene loci responsible for increased plasma apoB levels and the second for LDL subclass pattern B (162, 163). Such interactions of the genes underlying pattern B with other genes or environmental factors may contribute to familial dyslipidemic syndromes that are commonly found in patients with coronary artery disease (161).

Metabolic influences on small dense LDL phenotype

Consistent with the metabolic influences described above (Fig. 1), we have found in preliminary stable isotope kinetic studies that subjects with small, dense LDL phenotype have an increased rate of production and reduced rate of catabolism of large VLDL subspecies (S_F 60–400) (50). This finding is consistent with a recent report of Packard et al., who demonstrated lower VLDL-1 and VLDL-2 apoB fractional transfer rates and a lower LDL apoB fractional catabolic rate in subjects with predominantly small dense LDL compared with those with large LDL (67).

Reduced activity of LpL (80, 164) as well as an increase in apoC-III may be among the factors that contribute to impairment in VLDL clearance in pattern B. Moreover, patients with heterozygous LpL deficiency have a lipoprotein phenotype that appears to be similar to that in subjects with pattern B (165). Coupled with the kinetic studies described above, and evidence for reduced exogenous triglyceride clearance in pattern B subjects, independent of fasting triglyceride level (166), this suggests that one or more factors resulting in retardation of triglyceride-rich lipoprotein metabolism may have an etiologic or contributory role in a high proportion of subjects with the small dense LDL phenotype.

Based on the relationship of HL activity to levels of small dense LDL described above, it has been hypothesized that increases in HL contribute to the pattern B phenotype (60, 80, 163, 164). This hypothesis carries with it the notion that, with low HL activity, the metabolic antecedent of small dense LDL profile may be present, as manifest by increased production of VLDL remnants, but the pattern B "fingerprint" of this metabolic abnormality may only be fully manifest with permissive HL activity. Thus, it may be that factors influencing HL activity (e.g., adiposity and sex steroid hormones) can modulate levels of small dense LDL and the expression of the pattern B trait to a greater extent in susceptible individuals who generate increased levels of precursor lipoproteins.

Insulin resistance and small dense LDL

Hypertriglyceridemia, low HDL and small dense LDL particles are common lipid abnormalities in individuals with insulin resistance and non-insulin dependent diabetes mellitus (135, 166, 167). Thus, the cardiovascular disease (CVD) risk factor profile of persons with small dense LDL consists of essentially the same factors as those associated with an increased risk for the insulin resistance syndrome. Moreover, in a nested case control study of 204 elderly men and women from Finland, it has been demonstrated that subjects with predominance of small dense LDL had a greater than 2-fold increased risk for developing diabetes type 2 over a 3.5-year follow up period, independent from age, sex, glucose tolerance, and body mass index. Importantly, an increase of 5 A in LDL diameter was associated with a 16% decrease in risk of type 2 diabetes (168).

The link between the atherogenic lipoprotein profile, insulin resistance and diabetes mellitus may be explained by the effects of insulin and triglycerides on VLDL production and secretion, hepatic lipase activity, and the resulting remodeling of triglyceride enriched LDL particles to denser more atherogenic species. It is known that insulin is an important regulator of VLDL plasma concentrations (169, 170). Insulin regulates the influx of substrates for triglyceride synthesis in the liver by suppression the release of free fatty acids from adipose tissue. It has been suggested that de novo VLDL fatty acid synthesis represents a minor pathway (171), and about 70% of the secreted VLDL triglycerides are produced by reesterification of intracellular free fatty acids (172). Elevation of plasma free fatty acids during insulin and intralipid infusions attenuated the suppressive effect of insulin on the production of VLDL triglycerides, while VLDL apoB production remained unchanged (173), suggesting that insulin has direct inhibitory effects on VLDL production in the liver. In healthy normolipidemic subjects, it has been reported using stable isotopes that insulin acutely suppressed VLDL-1 apoB production, but had no effect on de novo VLDL-2 apoB production, suggesting that VLDL-1

and VLDL-2 apoB production are regulated independently (174). In obese subjects, it has been demonstrated that acute hyperinsulinemia reduced large triacylglycerol rich VLDL concentrations in insulin sensitive but not in insulin resistant subjects and modified the LDL subfraction profile toward a greater prevalence of small dense LDL (175). The reduction of triacylglycerol-rich VLDL may be the result of decreased secretion from the liver (176). However, it is not established that insulin resistance or hyperinsulinemia are directly responsible for hypertriglyceridemia; it is also possible that factors leading to hypertriglyceridemia such as altered plasma free fatty acid transport also contribute to insulin resistance (177). In normoglycemic men, LDL size was significantly positively correlated with the rates of whole body glucose uptake only when not adjusted for plasma triglycerides (178). This is consistent with evidence reviewed above that triglyceride metabolism is of critical importance in production of small dense particles and that triglyceride enrichment of precursors of small dense LDL may be favored by postprandial hypertriglyceridemia and hyperinsulinemia, with subsequent lipolysis by hepatic lipase (33, 179).

LIPOPROTEIN HETEROGENEITY AND RISK OF CORONARY ARTERY DISEASE

Interrelated coronary disease risk factors

The plasma lipoprotein profile accompanying a predominance of small dense LDL particles (specifically LDL-III) is associated with approximately a 3-fold increased risk of coronary artery disease. This has been demonstrated in case control studies of myocardial infarction (133, 136, 180) and of angiographically documented coronary disease (181). In most, but not all (180) of these studies, the disease risk associated with small dense LDL was no longer significant after adjusting for covariates, including triglycerides (136, 181) or other risk factors (133). Prospective studies of the relation of peak LDL particle size to the development of coronary artery disease have been carried out using nested case-control analysis in the Physician's Health Study (182), the population based Stanford Five Cities Project (183), and the Quebec Cardiovascular Study (184–186). In these studies, reduced LDL particle diameter was a significant univariate predictor of coronary disease. In the Physician's Health Study, this relationship was no longer significant after adjusting for non-fasting triglyceride level, while in the Five Cities study, the relationship, and the case-control difference in LDL diameter were independent of triglyceride, but not independent of the total/HDL-C ratio. In the Quebec Cardiovascular Study, both small LDL particle size and plasma apoB level were predictive of coronary disease risk independent of other risk factors, including plasma triglyceride (185). Moreover, there was a strong interaction of small LDL size ($<$256 A) with other risk factors, resulting in an odds ratio 4.9 for coronary heart disease in combination with total/HDL-C ratio $>$ 6 and an odds ratio of 6.2 in combination with an apoB level $>$120mg/dl.

These findings have been confirmed and extended in further studies in this population (184, 186). These studies support the concept that the clustered metabolic changes associated with the production of small dense LDL may jointly contribute to coronary disease risk, and that increased numbers of atherogenic particles must be present for disease risk to be manifest. Mykkänen et al. found that LDL size was not a predictor of CHD events in elderly men and women after controlling for diabetes status. This may be explained in part by a survival bias that is supported by the low prevalence of phenotype B subjects in this study. Further, the follow-up period of 3.5 years in this study was relatively short. Interestingly, a recent study analyzing data from the CARE trial in a prospective nested case control study found that larger LDL size after adjustment for other variables was an independent predictor of recurrent coronary events in a population with coronary artery disease (187). In this study, however, cases and controls (CAD patients without recurrent events) were closely matched for baseline lipid levels, including triglycerides, and also for prevalence of LDL subclass pattern B (approximately 40%). Thus, the population was one in which the atherogenic lipoprotein phenotype did not discriminate risk for recurrent events, and in this context a strong risk associated with larger LDL was detected. As described below, this is associated with other lines of evidence that particles at both extremes of the LDL size range and density spectrum have atherogenic properties compared with LDL particles of intermediate size and density. Further, as suggested in the Fig. 1, the production of large and small LDL may be metabolically linked in pathways regulated by triglyceride availability.

Potential for enhanced atherogenicity of small dense LDL

It has been reported that smaller, denser LDL have a greater propensity for uptake by arterial tissue than larger LDL (188), suggesting greater transendothelial transport of smaller particles. In addition, as described above, smaller LDL particles may also have decreased receptor mediated uptake and increased proteoglycan binding (127–129).

Several studies have documented that LDL subfractions differ in susceptibility to in vitro oxidative stress, a factor of significance in atherogenesis (151, 189–193). Specifically, large buoyant LDL are more resistant and small dense LDL are more susceptible to oxidation, as assessed by the length of the lag time before the propagation phase of free radical generation upon incubation with copper. A number of factors have been proposed to contribute to this differential oxidative susceptibility, including altered properties of the surface lipid layer associated with reduced content of free cholesterol (189), diminished antioxidant content (194), and increased content of polyunsaturated fatty acids (193).

Atherogenic potential of other apoB-containing lipoprotein subspecies

Elevated levels of remnant lipoproteins and IDL appear to be of particular importance with regard to coronary disease risk. In the National Heart, Lung, and Blood Institute Type II

Coronary Disease Intervention Trial, lipoprotein subfractions measured by analytical ultracentrifugation were correlated with angiographic progression of coronary disease in patients with primary hypercholesterolemia treated with cholestyramine and/or diet (2). Progression status was most closely related to changes in mass of small IDL/large LDL particles of flotation rate (S_f) 10–14 ($P < 0.03$), while there was a weaker association with mass of small dense LDL of S_f 0–7. In the St. Thomas Atherosclerosis Regression Study, change in coronary segment lumen diameter was assessed by quantitative coronary angiography in 74 men treated with cholestyramine and/or diet (195). Among a number of lipid and lipoprotein subfraction measures, IDL, small LDL, and HDL all were correlated significantly with both of the measures of change in segment lumen diameter that were employed in this study. Coronary atherosclerosis progression in a more recent study (196) was most strongly correlated with change in cholesterol levels in VLDL remnants and IDL, with much weaker relationship for cholesterol and apoB in LDL. Finally, in patients with coronary artery disease participating in the Monitored Atherosclerosis Regression Study (MARS), mass concentrations of lipoproteins across the VLDL and IDL particle spectrum as assessed by analytic ultracentrifugation were related to coronary angiographic progression (197), while only on-trial IDL mass concentrations predicted change in carotid artery intimal medial thickness assessed by B-mode ultrasound (198).

Several studies have examined the relationship of apolipoprotein specific subpopulations to risk of coronary artery disease. Increased levels of both apoC-III:B and apoE:B particles have been found in myocardial infarction survivors vs. controls, independent of other standard lipid and lipoprotein measures (199). Moreover, increased levels of apoB-bound apoC-III were strong predictors of angiography progression of coronary artery disease in two intervention trials (200, 201). In a recently published prospective, nested case-control study of the CARE trial it was demonstrated that VLDL apoB and apoC-III concentrations in VLDL and LDL were independent predictors of recurrent coronary events (202). Finally, it has been reported that apoC-III distribution among lipoprotein species discriminated differences in coronary disease risk between two populations better than did other lipid and lipoprotein variables (203). These findings suggest that within the spectrum of triglyceride-rich lipoproteins and their lipolytic remnants, those containing apoC-III, as well as apoE, may be of particular importance of the pathogenesis of coronary artery disease.

There is also considerable evidence that certain forms of large LDL particles may be atherogenic, possibly because of alterations in cholesteryl fatty acid composition (204). A direct pathologic role of small IDL/large LDL particles in atherogenesis is evident from studies in cholesterol fed animal models (2, 204). Notably, as described above in hypercholesterolemic monkeys, both increases in LDL particle size and apoE content (121) have been related to extent of coronary atherosclerosis, and it is likely that the atherogenic apoE-containing large LDL in this species have properties similar to IDL-2/LDL-I in humans (4). Among lipoproteins that accumulated in fat-fed LDL receptor knockout mice, a subpopulation of IDL/large LDL particles that is preferentially depleted by overexpression of LpL has been strongly and specifically implicated in the development of atherosclerosis (205). Increased particle size of LDL-I has been associated with coronary artery risk in a population of subjects selected for normolipidemia, in whom selection criteria effectively excluded subjects with a predominance of small dense LDL (206). Generally, in normolipidemic individuals with a predominance of larger LDL, the potentially atherogenicity of these particles can be attenuated since these subjects generally have relatively higher HDL-C and lower triglyceride levels. As discussed above in a recent report from the CARE study (187), increased LDL peak particle size was significantly related to CAD risk after adjustment for triglycerides, HDL, and other variables.

Thus, in various reports both particle with the characteristics of IDL/large LDL and small dense LDL have been associated with clinical and angiographic indices of coronary artery disease. Given the relationship of these lipoprotein subclasses with each other, and potentially with other unmeasured pathological factors, these studies do not allow assessment of causality. Nevertheless, it is reasonable to suppose that one or both of these subclasses contribute directly to risk of coronary artery disease, particularly in subjects with LDL subclass pattern B in whom levels of both are elevated. It is possible that IDL and dense LDL particles promote different pathologic events in the development of atherosclerotic cardiovascular disease, or, as discussed above, that they share common features or metabolic properties that result in additive or overlapping effects on this process.

LDL subclass profiles as predictors of coronary artery disease in response to lipid-altering therapies

Information regarding LDL subclasses and response of coronary disease progression to lipid altering treatment was first reported in the Stanford Coronary Risk Intervention Project (SCRIP) (132). SCRIP was a multiple risk factor intervention trial in patients with angiographically documented coronary disease in which the most commonly used regimens included bile acid binding resins and nicotinic acid. Despite similar levels of total LDL-C at entry and similar reduction with therapy, only subjects with predominantly small dense LDL (approximately 40% of the total group), and not those with larger more buoyant LDL, demonstrated reduced angiographic progression compared with the control (usual care) groups (132). In conjunction with the clustering of other metabolic features associated with the dense LDL trait, levels of triglyceride, VLDL, and IDL above the median and levels of HDL-2 below the median, were also predictive of greater therapeutic benefit. It is noteworthy that a post hoc analysis of the results of the Helsinki Heart Trial (207) indicated that the major benefit of diet plus gemfibrozil on clinical events was confined to 10% of the subjects with triglyceride levels greater than 204 mg/dl and LDL/HDL-C

ratios greater than five, a subgroup that would be expected to consist primarily if not exclusively of subjects with predominantly smaller, denser LDL particles. Moreover, a recent post hoc analysis of the results of the Cholesterol Lowering Atherosclerosis Study (CLAS) has revealed that the benefit of intervention with diet, colestipol, and nicotinic on coronary disease progression was confined to subjects in the top tertile of the triglyceride distribution (>190 mg/dl), a group expected to be highly enriched in LDL subclass pattern B subjects (208). Reductions in LDL and increases in HDL in subjects in this group were of similar magnitude to those in the other triglyceride tertiles.

Intensive lipid-lowering therapy (colestipol plus lovastatin or niacin plus colestipol) in the Familial Atherosclerosis Treatment Study demonstrated that increases in LDL buoyancy associated with reduced hepatic lipase activity were correlated with reduced coronary artery stenosis (209). In a multiple regression analysis, the increased LDL buoyancy was the risk factor most strongly associated with CAD regression, accounting for 37% of the variance of change in coronary stenosis. This result suggests that therapeutic modulation of the small dense LDL phenotype can be of benefit in reducing atherosclerosis risk. These analyses, while all post hoc, indicate that at least certain lipid altering drug regimens achieve selective benefit on angiographic progression in individuals with the dyslipidemic phenotype associated with a predominance of small dense LDL. It appears likely that these interventions act on metabolic pathways that are particularly important for atherosclerosis risk in subjects with this phenotype. That this may not be a universal effect of all lipid altering therapies is indicated in recent observations from MARS in which no significant benefit of the HMG-CoA reductase inhibitor lovastatin on coronary artery narrowing were observed in subjects with small dense LDL phenotype, whereas substantial benefit was found in subjects with larger more buoyant LDL (210). In the prospective, nested case control study from the CARE trial, discussed above, pravastatin therapy eliminated the excess in risk for recurrent events in patients with larger LDL particles (187). However, the overall risk reduction in CARE of approximately 25% leaves considerable opportunity for further risk reduction by treatment of other atherogenic abnormalities, including those associated with high triglycerides, low HDL and small dense LDL.

In summary, these results suggest that metabolic factors underlying differing LDL subclass phenotypes may influence not only risk of coronary artery disease, but also the likelihood of benefit of specific lipid altering therapies and diets. Improved understanding of these factors and their genetic determinants and modifying influences should lead to more effective identification and management of individuals at high risk for coronary artery disease.

This work was supported by the National Institutes of Health Program Project Grant HL 18574 from the National Heart, Lung and Blood Institute, a grant from the National Dairy Promotion and Research Board and administered in cooperation with the National Dairy Council and was conducted at the Ernest Orlando Lawrence Berkeley National Laboratory through the US Department of Energy under Contract No. DE-AC03-76SF00098. K.K.B. was supported by a research award from the Swiss National Foundation

REFERENCES

1. Krauss, R. M. 1987. Physical heterogeneity of apolipoprotein B-containing lipoproteins. *In* Proceedings of the Workshop on Lipoprotein Heterogeneity. K. Lippel, editor. US Govt. Printing Office, Washington, D.C. 15–21.
2. Krauss, R. M. 1987. Relationship of intermediate and low-density lipoprotein subspecies to risk of coronary artery disease. *Am. Heart J.* **113:** 578–582.
3. Alaupovic, P. 1992. The lipoprotein family concept and its clinical significance. *Nutr. Metab. Cardiovasc. Dis.* **2:** 52–59.
4. Musliner, T. A., C. Giotas, and R. M. Krauss. 1986. Presence of multiple subpopulations of lipoproteins of intermediate density in normal subjects. *Arteriosclerosis.* **6:** 79–87.
5. Meyer, B. J., M. J. Caslake, M. M. McConnell, and C. J. Packard. 2000. Two subpopulations of intermediate density lipoprotein and their relationship to plasma triglyceride and cholesterol levels. *Atherosclerosis.* **153:** 355–362.
6. Packard, C. J., J. Shepherd, S. Joerns, A. M. Gotto, Jr., and O. D. Taunton. 1979. Very low density and low density lipoprotein subfractions in type III and type IV hyperlipoproteinemia. Chemical and physical properties. *Biochim. Biophys. Acta.* **572:** 269–282.
7. Sata, T., R. J. Havel, and A. L. Jones. 1972. Characterization of subfractions of triglyceride-rich lipoproteins separated by gel chromatography from blood plasma of normolipemic and hyperlipemic humans. *J. Lipid Res.* **13:** 757–768.
8. Krauss, R., and D. Burke. 1982. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J. Lipid Res.* **23:** 97–104.
9. McNamara, J. R., H. Campos, J. M. Ordovas, J. Peterson, P. W. Wilson, and E. J. Schaefer. 1987. Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution. Results from the Framingham Offspring Study. *Arteriosclerosis.* **7:** 483–490.
10. Segrest, J. P., M. K. Jones, H. De Loof, and N. Dashti. 2001. Structure of apolipoprotein B-100 in low density lipoproteins. *J. Lipid Res.* **42:** 1346–1367.
11. Swinkels, D. W., H. L. Hak-Lemmers, and P. N. Demacker. 1987. Single spin density gradient ultracentrifugation method for the detection and isolation of light and heavy low density lipoprotein subfractions. *J. Lipid Res.* **28:** 1233–1239.
12. Krauss, R. M., and P. J. Blanche. 1992. Detection and quantification of LDL subfractions. *Curr. Opin. Lipidol.* **3:** 377–383.
13. Otvos, J. D., E. J. Jeyarajah, D. W. Bennett, and R. M. Krauss. 1992. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. *Clin. Chem.* **38:** 1632–1638.
14. Alaupovic, P. 1991. Apolipoprotein composition as the basis for classifying plasma lipoproteins. Characterization of ApoA- and ApoB-containing lipoprotein families. *Prog. Lipid Res.* **30:** 105–138.
15. Kandoussi, A., C. Cachera, D. Parsy, J. M. Bard, and J. C. Fruchart. 1991. Quantitative determination of different apolipoprotein B containing lipoproteins by an enzyme linked immunosorbent assay: apoB with apoC-III and apoB with apoE. *J. Immunoassay.* **12:** 305–323.
16. Lee, D. M., and P. Alaupovic. 1974. Composition and concentration of apolipoproteins in very-low-and low-density lipoproteins of normal human plasma. *Atherosclerosis.* **19:** 501–520.
17. Campos, E., S. Jackle, G. Chi Chen, and R. J. Havel. 1996. Isolation and characterization of two distinct species of human very low density lipoproteins lacking apolipoprotein E. *J. Lipid Res.* **37:** 1897–1906.
18. Fielding, P. E., and C. J. Fielding. 1986. An apo-E-free very low density lipoprotein enriched in phosphatidylethanolamine in human plasma. *J. Biol. Chem.* **261:** 5233–5236.
19. Campos, H., D. Perlov, C. Khoo, and F. M. Sacks. 2001. Distinct patterns of lipoproteins with apoB defined by presence of apoE

or apoC-III in hypercholesterolemia and hypertriglyceridemia. *J. Lipid Res.* **42:** 1239–1249.
20. Packard, C. J., A. Gaw, T. Demant, and J. Shepherd. 1995. Development and application of a multicompartmental model to study very low density lipoprotein subfraction metabolism. *J. Lipid Res.* **36:** 172–187.
21. Ehnholm, C., R. W. Mahley, D. A. Chappell, K. H. Weisgraber, E. Ludwig, and J. L. Witztum. 1984. Role of apolipoprotein E in the lipolytic conversion of beta-very low density lipoproteins to low density lipoproteins in type III hyperlipoproteinemia. *Proc. Natl. Acad. Sci. USA.* **81:** 5566–5570.
22. Gaw, A., C. J. Packard, E. F. Murray, G. M. Lindsay, B. A. Griffin, M. J. Caslake, B. D. Vallance, A. R. Lorimer, and J. Shepherd. 1993. Effects of simvastatin on apoB metabolism and LDL subfraction distribution. *Arterioscler. Thromb.* **13:** 170–189.
23. Caslake, M. J., C. J. Packard, A. Gaw, E. Murray, B. A. Griffin, B. D. Vallance, and J. Shepherd. 1993. Fenofibrate and LDL metabolic heterogeneity in hypercholesterolemia. *Arterioscler. Thromb.* **13:** 702–711.
24. Gaw, A., C. J. Packard, M. J. Caslake, B. A. Griffin, G. M. Lindsay, J. Thomson, B. D. Vallance, D. Wosornu, and J. Shepherd. 1994. Effects of ciprofibrate on LDL metabolism in man. *Atherosclerosis.* **108:** 137–148.
25. Packard, C. J., A. Munro, A. R. Lorimer, A. M. Gotto, and J. Shepherd. 1984. Metabolism of apolipoprotein B in large triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2178–2192.
26. Stalenhoef, A. F., M. J. Malloy, J. P. Kane, and R. J. Havel. 1984. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in normal and lipoprotein lipase-deficient humans. *Proc. Natl. Acad. Sci. USA.* **81:** 1839–1843.
27. Gofman, J. W., O. Delalla, F. Glazier, N. K. Freeman, D. Lindgren, A. V. Nichols, B. Strisower, and A. R. Tramplin. 1954. The serum lipoprotein transport system in health, metabolic disorders, atherosclerosis and coronary artery disease. *Plasma.* **2:** 413–484.
28. Demant, T., L. A. Carlson, L. Holmquist, F. Karpe, P. Nilsson-Ehle, C. J. Packard, and J. Shepherd. 1988. Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. *J. Lipid Res.* **29:** 1603–1611.
29. James, R. W., B. Martin, D. Pometta, J. C. Fruchart, P. Duriez, P. Puchois, J. P. Farriaux, A. Tacquet, T. Demant, R. J. Clegg, A. Munro, M. F. Oliver, C. J. Packard, and J. Shepherd. 1989. Apolipoprotein B metabolism in homozygous familial hypercholesterolemia. *J. Lipid Res.* **30:** 159–169.
30. Demant, T., D. Bedford, C. J. Packard, and J. Shepherd. 1991. Influence of apolipoprotein E polymorphism on apolipoprotein B-100 metabolism in normolipemic subjects. *J. Clin. Invest.* **88:** 1490–1501.
31. Shepherd, J., C. J. Packard, J. M. Stewart, R. F. Atmeh, R. S. Clark, D. E. Boag, K. Carr, A. R. Lorimer, D. Ballantyne, H. G. Morgan, and T. D. Veitch Lawrie. 1984. Apolipoprotein A and B (Sf 100–400) metabolism during bezafibrate therapy in hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2164–2177.
32. Dachet, C., E. Cavallero, C. Martin, G. Girardot, and B. Jacotot. 1995. Effect of gemfibrozil on the concentration and composition of very low density and low density lipoprotein subfractions in hypertriglyceridemic patients. *Atherosclerosis.* **113:** 1–9.
33. Tan, C. E., L. Foster, M. J. Caslake, D. Bedford, T. D. Watson, M. McConnell, C. J. Packard, and J. Shepherd. 1995. Relations between plasma lipids and postheparin plasma lipases and VLDL and LDL subfraction patterns in normolipemic men and women. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1839–1848.
34. Pease, R. J., and J. M. Leiper. 1996. Regulation of hepatic apolipoprotein-B-containing lipoprotein secretion. *Curr. Opin. Lipidol.* **7:** 132–138.
35. Lin, M. C., D. Gordon, and J. R. Wetterau. 1995. Microsomal triglyceride transfer protein (MTP) regulation in HepG2 cells: insulin negatively regulates MTP gene expression. *J. Lipid Res.* **36:** 1073–1081.
36. Chappell, D. A., G. L. Fry, M. A. Waknitz, L. E. Muhonen, and M. W. Pladet. 1993. Low density lipoprotein receptors bind and mediate cellular catabolism of normal very low density lipoproteins in vitro. *J. Biol. Chem.* **268:** 25487–25493.
37. Tacken, P. J., B. Teusink, M. C. Jong, D. Harats, L. M. Havekes, K. W. van Dijk, and M. H. Hofker. 2000. LDL receptor deficiency unmasks altered VLDL triglyceride metabolism in VLDL receptor transgenic and knockout mice. *J. Lipid Res.* **41:** 2055–2062.
38. Gianturco, S. H., A. M. Gotto, Jr., S. L. Hwang, J. B. Karlin, A. H. Lin, S. C. Prasad, and W. A. Bradley. 1983. Apolipoprotein E mediates uptake of Sf 100–400 hypertriglyceridemic very low density lipoproteins by the low density lipoprotein receptor pathway in normal human fibroblasts. *J. Biol. Chem.* **258:** 4526–4533.
39. Aalto-Setala, K., E. A. Fisher, X. Chen, T. Chajek-Shaul, T. Hayek, R. Zechner, A. Walsh, R. Ramakrishnan, H. N. Ginsberg, and J. L. Breslow. 1992. Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apoCIII and reduced apoE on the particles. *J. Clin. Invest.* **90:** 1889–1900.
40. Windler, E., Y. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. Opposing effects of homologous apolipoprotein E and individual C apolipoproteins. *J. Biol. Chem.* **255:** 8303–8307.
41. Windler, E., Y. Chao, and R. J. Havel. 1980. Determinants of hepatic uptake of triglyceride-rich lipoproteins and their remnants in the rat. *J. Biol. Chem.* **255:** 5475–5480.
42. Windler, E., and R. J. Havel. 1985. Inhibitory effects of C apolipoproteins from rats and humans on the uptake of triglyceride-rich lipoproteins and their remnants by the perfused rat liver. *J. Lipid Res.* **26:** 556–565.
43. Beisiegel, U., W. Weber, and G. Bengtsson-Olivecrona. 1991. Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. *Proc. Natl. Acad. Sci. USA.* **88:** 8342–8346.
44. Hussain, M. M., F. R. Maxfield, J. Mas-Oliva, I. Tabas, Z. S. Ji, T. L. Innerarity, and R. W. Mahley. 1991. Clearance of chylomicron remnants by the low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor. *J. Biol. Chem.* **266:** 13936–13940.
45. Beisiegel, U., A. Krapp, W. Weber, and G. Olivecrona. 1994. The role of alpha 2M receptor/LRP in chylomicron remnant metabolism. *Ann. NY Acad. Sci.* **737:** 53–69.
46. Mann, W. A., N. Meyer, W. Weber, S. Meyer, H. Greten, and U. Beisiegel. 1995. Apolipoprotein E isoforms and rare mutations: parallel reduction in binding to cells and to heparin reflects severity of associated type III hyperlipoproteinemia. *J. Lipid Res.* **36:** 517–525.
47. Ji, Z. S., S. Fazio, and R. W. Mahley. 1994. Variable heparan sulfate proteoglycan binding of apolipoprotein E variants may modulate the expression of type III hyperlipoproteinemia. *J. Biol. Chem.* **269:** 13421–13428.
48. Gianturco, S. H., M. P. Ramprasad, R. Song, R. Li, M. L. Brown, and W. A. Bradley. 1998. Apolipoprotein B-48 or its apolipoprotein B-100 equivalent mediates the binding of triglyceride-rich lipoproteins to their unique human monocyte-macrophage receptor. *Arterioscler. Thromb. Vasc. Biol.* **18:** 968–976.
49. Yamada, N., D. M. Shames, K. Takahashi, and R. J. Havel. 1988. Metabolism of apolipoprotein B-100 in large very low density lipoproteins in blood plasma. *J. Clin. Invest.* **82:** 2106–2113.
50. Krauss, R. M., M. K. Hellerstein, R. A. Neese, P. J. Blanche, M. La Belle, and D. M. Shames. 1995. Altered metabolism of large low density lipoproteins in subjects with predominance of small low density lipoproteins. *Circulation.* **92:** I–102.
51. Packard, C. J., and J. Shepherd. 1997. Lipoprotein heterogeneity and apolipoprotein B metabolism. *Arterioscler. Thromb. Vasc. Biol.* **17:** 3542–3556.
52. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1982. The metabolism of very low density and intermediate density lipoproteins in patients with familial hypercholesterolaemia. *Atherosclerosis.* **43:** 217–231.
53. Fidge, N. H., and P. Poulis. 1975. Metabolic heterogeneity in the formation of low density lipoprotein in the rat: evidence for the independent production of a low density lipoprotein subfraction. *J. Lipid Res.* **19:** 342–349.
54. Murthy, V. N., C. A. Marzetta, L. L. Rudel, L. A. Zech, and D. M. Foster. 1990. Hepatic apoB-100 lipoproteins and plasma LDL heterogeneity in African green monkeys. *Am. J. Physiol.* **258:** E1041–E1057.
55. Johnson, F. L., R. W. St Clair, and L. L. Rudel. 1983. Studies on the production of low density lipoproteins by perfused livers from nonhuman primates. Effect of dietary cholesterol. *J. Clin. Invest.* **72:** 221–236.
56. Marzetta, C. A., F. L. Johnson, L. A. Zech, D. M. Foster, and L. L. Rudel. 1989. Metabolic behavior of hepatic VLDL and plasma LDL apoB-100 in African green monkeys. *J. Lipid Res.* **30:** 357–370.

57. Checovich, W. J., R. J. Aiello, and A. D. Attie. 1991. Overproduction of a buoyant low density lipoprotein subspecies in spontaneously hypercholesterolemic mutant pigs. *Arterioscler. Thromb.* **11:** 351–361.
58. Cooper, S. T., R. J. Aiello, W. J. Checovich, and A. D. Attie. 1992. Low density lipoprotein heterogeneity in spontaneously hypercholesterolemic pigs. *Mol. Cell. Biochem.* **113:** 133–140.
59. Knipping, G., A. Birchbauer, E. Steyrer, and G. M. Kostner. 1987. Action of lecithin-cholesterol acyltransferase on low-density lipoproteins in native pig plasma. *Biochemistry.* **26:** 7945–7953.
60. Gaw, A., C. J. Packard, G. M. Lindsay, B. A. Griffin, M. J. Caslake, A. R. Lorimer, and J. Shepherd. 1995. Overproduction of small very low density lipoproteins (Sf 20–60) in moderate hypercholesterolemia: relationships between apolipoprotein B kinetics and plasma lipoproteins. *J. Lipid Res.* **36:** 158–171.
61. Fisher, W. R., L. A. Zech, L. L. Kilgore, and P. W. Stacpoole. 1991. Metabolic pathways of apolipoprotein B in heterozygous familial hypercholesterolemia: studies with a [^{3}H]leucine tracer. *J. Lipid Res.* **32:** 1823–1836.
62. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1977. Simultaneous measurement of apolipoprotein B turnover in very-low- and low-density lipoproteins in familial hypercholesterolaemia. *Atherosclerosis.* **28:** 247–256.
63. Nakaya, N., B. H. Chung, J. R. Patsch, and O. D. Taunton. 1977. Synthesis and release of low density lipoproteins by the isolated perfused pig liver. *J. Biol. Chem.* **252:** 7530–7533.
64. Noel, S. P., L. Wong, P. J. Dolphin, L. Dory, and D. Rubenstein. 1979. Secretion of cholesterol-rich lipoproteins by perfused livers of hypercholesterolemic rats. *J. Clin. Invest.* **64:** 674–683.
65. Ardern, H. A., G. M. Benson, K. E. Suckling, M. J. Caslake, J. Shepherd, and C. J. Packard. 1999. Apolipoprotein B overproduction by the perfused liver of the St. Thomas' mixed hyperlipidemic (SMHL) rabbit. *J. Lipid Res.* **40:** 2234–2243.
66. Boren, J., M. M. Veniant, and S. G. Young. 1998. Apo B100-containing lipoproteins are secreted by the heart. *J. Clin. Invest.* **101:** 1197–1202.
67. Packard, C. J., T. Demant, J. P. Stewart, D. Bedford, M. J. Caslake, G. Schwertfeger, A. Bedynek, J. Shepherd, and D. Seidel. 2000. Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions. *J. Lipid Res.* **41:** 305–318.
68. Musliner, T. A., K. M. McVicker, J. F. Iosefa, and R. M. Krauss. 1987. Metabolism of human intermediate and very low density lipoprotein subfractions from normal and dysbetalipoproteinemic plasma. In vivo studies in rat. *Arteriosclerosis.* **7:** 408–420.
69. Krauss, R. M., P. T. Williams, F. T. Lindgren, and P. D. Wood. 1988. Coordinate changes in levels of human serum low and high density lipoprotein subclasses in healthy men. *Arteriosclerosis.* **8:** 155–162.
70. Shelness, G. S., and J. A. Sellers. 2001. Very-low-density lipoprotein assembly and secretion. *Curr. Opin. Lipidol.* **12:** 151–157.
71. Foster, D. M., A. Chait, J. J. Albers, R. A. Failor, C. Harris, and J. D. Brunzell. 1986. Evidence for kinetic heterogeneity among human low density lipoproteins. *Metabolism.* **35:** 685–696.
72. Shepherd, J., M. J. Caslake, A. R. Lorimer, B. D. Vallance, and C. J. Packard. 1985. Fenofibrate reduces low density lipoprotein catabolism in hypertriglyceridemic subjects. *Arteriosclerosis.* **5:** 162–168.
73. Galeano, N. F., M. Al-Haideri, F. Keyserman, S. C. Rumsey, and R. J. Deckelbaum. 1998. Small dense low density lipoprotein has increased affinity for LDL receptor-independent cell surface binding sites: a potential mechanism for increased atherogenicity. *J. Lipid Res.* **39:** 1263–1273.
74. Lund-Katz, S., P. M. Laplaud, M. C. Phillips, and M. J. Chapman. 1998. Apolipoprotein B-100 conformation and particle surface charge in human LDL subspecies: implication for LDL receptor interaction. *Biochemistry.* **37:** 12867–12874.
75. McNamara, J. R., J. L. Jenner, Z. Li, P. W. Wilson, and E. J. Schaefer. 1992. Change in LDL particle size is associated with change in plasma triglyceride concentration. *Arterioscler. Thromb.* **12:** 1284–1290.
76. Krauss, R. M., F. T. Lindgren, and R. M. Ray. 1980. Interrelationships among subgroups of serum lipoproteins in normal human subjects. *Clin. Chim. Acta.* **104:** 275–290.
77. Deckelbaum, R. J., E. Granot, Y. Oschry, L. Rose, and S. Eisenberg. 1984. Plasma triglyceride determines structure-composition in low and high density lipoproteins. *Arteriosclerosis.* **4:** 225–231.
78. Karpe, F., P. Tornvall, T. Olivecrona, G. Steiner, L. A. Carlson, and A. Hamsten. 1993. Composition of human low density lipoprotein: effects of postprandial triglyceride-rich lipoproteins, lipoprotein lipase, hepatic lipase and cholesteryl ester transfer protein. *Atherosclerosis.* **98:** 33–49.
79. Johansson, J., P. Nilsson-Ehle, L. A. Carlson, and A. Hamsten. 1991. The association of lipoprotein and hepatic lipase activities with high density lipoprotein subclass levels in men with myocardial infarction at a young age. *Atherosclerosis.* **86:** 111–122.
80. Campos, H., D. M. Dreon, and R. M. Krauss. 1995. Associations of hepatic and lipoprotein lipase activities with changes in dietary composition and low density lipoprotein subclasses. *J. Lipid Res.* **36:** 462–472.
81. Murdoch, S. J., and W. C. Breckenridge. 1995. Influence of lipoprotein lipase and hepatic lipase on the transformation of VLDL and HDL during lipolysis of VLDL. *Atherosclerosis.* **118:** 193–212.
82. Goldberg, I. J., N. A. Le, H. N. Ginsberg, R. M. Krauss, and F. T. Lindgren. 1988. Lipoprotein metabolism during acute inhibition of lipoprotein lipase in the cynomolgus monkey. *J. Clin. Invest.* **81:** 561–568.
83. Le, N. A., J. C. Gibson, and H. N. Ginsberg. 1988. Independent regulation of plasma apolipoprotein C–II and C–III concentrations in very low density and high density lipoproteins: implications for the regulation of the catabolism of these lipoproteins. *J. Lipid Res.* **29:** 669–677.
84. Ginsberg, H. N., N. A. Le, I. J. Goldberg, J. C. Gibson, A. Rubinstein, P. Wang-Iverson, R. Norum, and W. V. Brown. 1986. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. *J. Clin. Invest.* **78:** 1287–1295.
85. Maeda, N., H. Li, D. Lee, P. Oliver, S. H. Quarfordt, and J. Osada. 1994. Targeted disruption of the apolipoprotein C–III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. *J. Biol. Chem.* **269:** 23610–23616.
86. Brown, W. V., and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by an apolipoprotein of human very low density lipoprotein. *Biochem. Biophys. Res. Commun.* **46:** 375–382.
87. Havel, R. J., C. J. Fielding, T. Olivecrona, V. G. Shore, P. E. Fielding, and T. Egelrud. 1973. Cofactor activity of protein components of human very low density lipoproteins in the hydrolysis of triglycerides by lipoproteins lipase from different sources. *Biochemistry.* **12:** 1828–1833.
88. Shachter, N. S., T. Hayek, T. Leff, J. D. Smith, D. W. Rosenberg, A. Walsh, R. Ramakrishnan, I. J. Goldberg, H. N. Ginsberg, and J. L. Breslow. 1994. Overexpression of apolipoprotein CII causes hypertriglyceridemia in transgenic mice. *J. Clin. Invest.* **93:** 1683–1690.
89. de Silva, H. V., S. J. Lauer, J. Wang, W. S. Simonet, K. H. Weisgraber, R. W. Mahley, and J. M. Taylor. 1994. Overexpression of human apolipoprotein C–III in transgenic mice results in an accumulation of apolipoprotein B48 remnants that is corrected by excess apolipoprotein E. *J. Biol. Chem.* **269:** 2324–2335.
90. Thuren, T., P. Sisson, and M. Waite. 1991. Activation of hepatic lipase catalyzed phosphatidylcholine hydrolysis by apolipoprotein E. *Biochim. Biophys. Acta.* **1083:** 217–220.
91. Landis, B. A., F. S. Rotolo, W. C. Meyers, A. B. Clark, and S. H. Quarfordt. 1987. Influence of apolipoprotein E on soluble and heparin-immobilized hepatic lipase. *Am. J. Physiol.* **252:** G805–G810.
92. Ebara, T., R. Ramakrishnan, G. Steiner, and N. S. Shachter. 1997. Chylomicronemia due to apolipoprotein CIII overexpression in apolipoprotein E-null mice. Apolipoprotein CIII-induced hypertriglyceridemia is not mediated by effects on apolipoprotein E. *J. Clin. Invest.* **99:** 2672–2681.
93. Tomiyasu, K., B. W. Walsh, K. Ikewaki, H. Judge, and F. M. Sacks. 2001. Differential metabolism of human VLDL according to content of ApoE and ApoC-III. *Arterioscler. Thromb. Vasc. Biol.* **21:** 1494–1500.
94. Musliner, T. A., P. N. Herbert, and M. J. Kingston. 1979. Lipoprotein substrates of lipoprotein lipase and hepatic triacylglycerol lipase from human post-heparin plasma. *Biochim. Biophys. Acta.* **575:** 277–288.
95. Nicoll, A., and B. Lewis. 1980. Evaluation of the roles of lipoprotein lipase and hepatic lipase in lipoprotein metabolism: in vivo and in vitro studies in man. *Eur. J. Clin. Invest.* **10:** 487–495.

96. Breckenridge, W. C., J. A. Little, P. Alaupovic, C. S. Wang, A. Kuksis, G. Kakis, F. Lindgren, and G. Gardiner. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* **45:** 161–179.
97. Auwerx, J. H., C. A. Marzetta, J. E. Hokanson, and J. D. Brunzell. 1989. Large buoyant LDL-like particles in hepatic lipase deficiency. *Arteriosclerosis.* **9:** 319–325.
98. Blanche, P., L.G. Holl, M.I. Shoukry, and R.M. Krauss. 1999. Absence of hepatic lipase results in reduction of human-like small dense LDL in Fat-Fed human apoB Transgenic mice. *Circulation.* **100:** A-13.
99. Watson, T. D., M. J. Caslake, D. J. Freeman, B. A. Griffin, J. Hinnie, C. J. Packard, and J. Shepherd. 1994. Determinants of LDL subfraction distribution and concentrations in young normolipidemic subjects. *Arterioscler. Thromb.* **14:** 902–910.
100. Linton, M. F., R. V. Farese, Jr., G. Chiesa, D. S. Grass, P. Chin, R. E. Hammer, H. H. Hobbs, and S. G. Young. 1993. Transgenic mice expressing high plasma concentrations of human apolipoprotein B100 and lipoprotein(a). *J. Clin. Invest.* **92:** 3029–3037.
101. Fan, J., S. P. McCormick, R. M. Krauss, S. Taylor, R. Quan, J. M. Taylor, and S. G. Young. 1995. Overexpression of human apolipoprotein B-100 in transgenic rabbits results in increased levels of LDL and decreased levels of HDL. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1889–1899.
102. Callow, M. J., J. Verstuyft, R. Tangirala, W. Palinski, and E. M. Rubin. 1995. Atherogenesis in transgenic mice with human apolipoprotein B and lipoprotein (a). *J. Clin. Invest.* **96:** 1639–1646.
103. Purcell-Huynh, D., R. J. Farese, D. Johnson, F. Flynn, V. Pierotti, D. Newland, M. Linton, D. Sanan, and S. Young. 1995. Transgenic mice expressing high levels of human apolipoprotein B develop severe atherosclerotic lesions in response to a high fat diet. *J. Clin. Invest.* **95:** 2246–2257.
104. Tato, F., G. L. Vega, A. R. Tall, and S. M. Grundy. 1995. Relation between cholesterol ester transfer protein activities and lipoprotein cholesterol in patients with hypercholesterolemia and combined hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **15:** 112–120.
105. Foger, B., A. Konigsrainer, G. Palos, A. Ritsch, G. Trobinger, H. J. Menzel, M. Lechleitner, A. Doblinger, P. Konig, G. Utermann, R. Margreiter, and J. R. Patsch. 1996. Effects of pancreas transplantation on distribution and composition of plasma lipoproteins. *Metabolism.* **45:** 856–861.
106. Hayek, T., N. Azrolan, R. B. Verdery, A. Walsh, T. Chajek-Shaul, L. B. Agellon, A. R. Tall, and J. L. Breslow. 1993. Hypertriglyceridemia and cholesteryl ester transfer protein interact to dramatically alter high density lipoprotein levels, particle sizes, and metabolism. Studies in transgenic mice. *J. Clin. Invest.* **92:** 1143–1152.
107. Dinchuk, J., J. Hart, G. Gonzalez, G. Karmann, D. Schmidt, and D. O. Wirak. 1995. Remodelling of lipoproteins in transgenic mice expressing human cholesteryl ester transfer protein. *Biochim. Biophys. Acta.* **1255:** 301–310.
108. Mann, C. J., F. T. Yen, A. M. Grant, and B. E. Bihain. 1991. Mechanism of plasma cholesteryl ester transfer in hypertriglyceridemia. *J. Clin. Invest.* **88:** 2059–2066.
109. Vakkilainen, J., M. Jauhiainen, K. Ylitalo, I. O. Nuotio, J. S. Viikari, C. Ehnholm, and M. R. Taskinen. 2002. LDL particle size in familial combined hyperlipidemia. Effects of serum lipids, lipoprotein-modifying enzymes, and lipid transfer proteins. *J. Lipid Res.* **43:** 598–603.
110. Grass, D. S., U. Saini, R. H. Felkner, R. E. Wallace, W. J. Lago, S. G. Young, and M. E. Swanson. 1995. Transgenic mice expressing both human apolipoprotein B and human CETP have a lipoprotein cholesterol distribution similar to that of normolipidemic humans. *J. Lipid Res.* **36:** 1082–1091.
111. Tato, F., G. L. Vega, and S. M. Grundy. 1995. Bimodal distribution of cholesteryl ester transfer protein activities in normotriglyceridemic men with low HDL cholesterol concentrations. *Arterioscler. Thromb. Vasc. Biol.* **15:** 446–451.
112. McNamara, J. R., D. M. Small, Z. Li, and E. J. Schaefer. 1996. Differences in LDL subspecies involve alterations in lipid composition and conformational changes in apolipoprotein B. *J. Lipid Res.* **37:** 1924–1935.
113. McNamara, D. J., R. Kolb, T. S. Parker, H. Batwin, P. Samuel, C. D. Brown, and E. H. Ahrens, Jr. 1987. Heterogeneity of cholesterol homeostasis in man. Response to changes in dietary fat quality and cholesterol quantity. *J. Clin. Invest.* **79:** 1729–1739.
114. Guerin, M., W. Le Goff, T. S. Lassel, A. Van Tol, G. Steiner, and M. J. Chapman. 2001. Atherogenic role of elevated CE transfer from HDL to VLDL(1) and dense LDL in type 2 diabetes: impact of the degree of triglyceridemia. *Arterioscler. Thromb. Vasc. Biol.* **21:** 282–288.
115. Sakai, N., Y. Matsuzawa, K. Hirano, S. Yamashita, S. Nozaki, Y. Ueyama, M. Kubo, and S. Tarui. 1991. Detection of two species of low density lipoprotein particles in cholesteryl ester transfer protein deficiency. *Arterioscler. Thromb.* **11:** 71–79.
116. Guerin, M., P. J. Dolphin, and M. J. Chapman. 1994. A new in vitro method for the simultaneous evaluation of cholesteryl ester exchange and mass transfer between HDL and apoB-containing lipoprotein subspecies. Identification of preferential cholesteryl ester acceptors in human plasma. *Arterioscler. Thromb.* **14:** 199–206.
117. Hirano, K., Y. Matsuzawa, N. Sakai, H. Hiraoka, S. Nozaki, T. Funahashi, S. Yamashita, M. Kubo, and S. Tarui. 1992. Polydisperse low-density lipoproteins in hyperalphalipoproteinemic chronic alcohol drinkers in association with marked reduction of cholesteryl ester transfer protein activity. *Metabolism.* **41:** 1313–1318.
118. Lagrost, L., H. Gandjini, A. Athias, V. Guyard-Dangremont, C. Lallemant, and P. Gambert. 1993. Influence of plasma cholesteryl ester transfer activity on the LDL and HDL distribution profiles in normolipidemic subjects. *Arterioscler. Thromb.* **13:** 815–825.
119. Blanche, P. J., M. J. Callow, L. G. Holl, E. M. Rubin, and R. M. Krauss. 1995. Similar low density lipoprotein subclasses in fat-fed human-apoB transgenic mice and humans. *Circulation.* **92 (Suppl.):** I-104.
120. Carr, T. P., J. S. Parks, and L. L. Rudel. 1992. Hepatic ACAT activity in African green monkeys is highly correlated to plasma LDL cholesteryl ester enrichment and coronary artery atherosclerosis. *Arterioscler. Thromb.* **12:** 1274–1283.
121. Stevenson, S. C., J. K. Sawyer, and L. L. Rudel. 1992. Role of apolipoprotein E on cholesteryl ester-enriched low density lipoprotein particles in coronary artery atherosclerosis of hypercholesterolemic nonhuman primates. *Arterioscler. Thromb.* **12:** 28–40.
122. Brodt-Eppley, J., P. White, S. Jenkins, and D. Y. Hui. 1995. Plasma cholesterol esterase level is a determinant for an atherogenic lipoprotein profile in normolipidemic human subjects. *Biochim. Biophys. Acta.* **1272:** 69–72.
123. Howles, P. N., C. P. Carter, and D. Y. Hui. 1996. Dietary free and esterified cholesterol absorption in cholesterol esterase (bile salt-stimulated lipase) gene-targeted mice. *J. Biol. Chem.* **271:** 7196–7202.
124. Nigon, F., P. Lesnik, M. Rouis, and M. J. Chapman. 1991. Discrete subspecies of human low density lipoproteins are heterogeneous in their interaction with the cellular LDL receptor. *J. Lipid Res.* **32:** 1741–1753.
125. Campos, H., K. S. Arnold, M. E. Balestra, T. L. Innerarity, and R. M. Krauss. 1996. Differences in receptor binding of LDL subfractions. *Arterioscler. Thromb. Vasc. Biol.* **16:** 794–801.
126. Galeano, N. F., R. Milne, Y. L. Marcel, M. T. Walsh, E. Levy, T. D. Ngu'yen, A. Gleeson, Y. Arad, L. Witte, M. al-Haideri. 1994. Apolipoprotein B structure and receptor recognition of triglyceride-rich low density lipoprotein (LDL) is modified in small LDL but not in triglyceride-rich LDL of normal size. *J. Biol. Chem.* **269:** 511–519.
127. La Belle, M., and R. M. Krauss. 1990. Differences in carbohydrate content of low density lipoproteins associated with low density lipoprotein subclass patterns. *J. Lipid Res.* **31:** 1577–1588.
128. Camejo, G., A. Lopez, F. Lopez, and J. Quinones. 1985. Interaction of low density lipoproteins with arterial proteoglycans. The role of charge and sialic acid content. *Atherosclerosis.* **55:** 93–105.
129. Jaakkola, O., T. Solakivi, V. V. Tertov, A. N. Orekhov, T. A. Miettinen, and T. Nikkari. 1993. Characteristics of low-density lipoprotein subfractions from patients with coronary artery disease. *Coron. Artery Dis.* **4:** 379–385.
130. Olin, K., R. M. Krauss, M. La Belle, N. W. Hokanson, and A. Chait. 1999. Apo CIII modulates lipoprotein binding to Vascular Proteoglycans. *Circulation.* **100 (Suppl. I693–694):** 3658.
131. Austin, M. A., M. C. King, K. M. Vranizan, and R. M. Krauss. 1990. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. *Circulation.* **82:** 495–506.
132. Miller, B. D., E. L. Alderman, W. L. Haskell, J. M. Fair, and R. M. Krauss. 1996. Predominance of dense low-density lipoprotein

particles predicts angiographic benefit of therapy in the Stanford Coronary Risk Intervention Project. *Circulation.* **94:** 2146–2153.
133. Campos, H., J. J. Genest, Jr., E. Blijlevens, J. R. McNamara, J. L. Jenner, J. M. Ordovas, P. W. Wilson, and E. J. Schaefer. 1992. Low density lipoprotein particle size and coronary artery disease. *Arterioscler. Thromb.* **12:** 187–195.
134. Campos, H., E. Blijlevens, J. R. McNamara, J. M. Ordovas, B. M. Posner, P. W. Wilson, W. P. Castelli, and E. J. Schaefer. 1992. LDL particle size distribution. Results from the Framingham Offspring Study. *Arterioscler. Thromb.* **12:** 1410–1419.
135. Selby, J. V., M. A. Austin, B. Newman, D. Zhang, C. P. Quesenberry, Jr., E. J. Mayer, and R. M. Krauss. 1993. LDL subclass phenotypes and the insulin resistance syndrome in women. *Circulation.* **88:** 381–387.
136. Austin, M. A., J. L. Breslow, C. H. Hennekens, J. E. Buring, W. C. Willett, and R. M. Krauss. 1988. Low-density lipoprotein subclass patterns and risk of myocardial infarction. *J. Am. Med. Assoc.* **260:** 1917–1921.
137. Austin, M. A., B. Newman, J. V. Selby, K. Edwards, E. J. Mayer, and R. M. Krauss. 1993. Genetics of LDL subclass phenotypes in women twins. Concordance, heritability, and commingling analysis. *Arterioscler. Thromb.* **13:** 687–695.
138. Bu, X., R. M. Krauss, D. Puppione, R. Gray, and J. I. Rotter. 1992. Major gene control of atherogenic lipoprotein phenotype (ALP): a quantitative segregation analysis in 20 coronary artery disease (CAD) pedigrees. *Am. J. Hum. Genet.* **51:** A336.
139. Austin, M. A., G. P. Jarvik, J. E. Hokanson, and K. Edwards. 1993. Complex segregation analysis of LDL peak particle diameter. *Genet. Epidemiol.* **10:** 599–604.
140. Nishina, P. M., J. P. Johnson, J. K. Naggert, and R. M. Krauss. 1992. Linkage of atherogenic lipoprotein phenotype to the low density lipoprotein receptor locus on the short arm of chromosome 19. *Proc. Natl. Acad. Sci. USA.* **89:** 708–712.
141. Rotter, J. I., X. Bu, R. M. Cantor, C. H. Warden, J. Brown, R. J. Gray, P. J. Blanche, R. M. Krauss, and A. J. Lusis. 1996. Multilocus genetic determinants of LDL particle size in coronary artery disease families. *Am. J. Hum. Genet.* **58:** 585–594.
142. Austin, M. A., P. J. Talmud, L. A. Luong, L. Haddad, I. N. Day, B. Newman, K. L. Edwards, R. M. Krauss, and S. E. Humphries. 1998. Candidate-gene studies of the atherogenic lipoprotein phenotype: a sib-pair linkage analysis of DZ women twins. *Am. J. Hum. Genet.* **62:** 406–419.
143. Allayee, H., K. M. Dominguez, B. E. Aouizerat, R. M. Krauss, J. I. Rotter, J. Lu, R. M. Cantor, T. W. de Bruin, and A. J. Lusis. 2000. Contribution of the hepatic lipase gene to the atherogenic lipoprotein phenotype in familial combined hyperlipidemia. *J. Lipid Res.* **41:** 245–252.
144. Talmud, P. J., K. L. Edwards, C. M. Turner, B. Newman, J. M. Palmen, S. E. Humphries, and M. A. Austin. 2000. Linkage of the cholesteryl ester transfer protein (CETP) gene to LDL particle size: use of a novel tetranucleotide repeat within the CETP promoter. *Circulation.* **101:** 2461–2466.
145. Naggert, J. K., A. Recinos 3rd, J. E. Lamerdin, R. M. Krauss, and P. M. Nishina. 1997. The atherogenic lipoprotein phenotype is not caused by a mutation in the coding region of the low density lipoprotein receptor gene. *Clin. Genet.* **51:** 236–240.
146. Zhao, S. P., M. H. Verhoeven, J. Vink, L. Hollaar, A. van der Laarse, P. de Knijff, and F. M. van 't Hooft. 1993. Relationship between apolipoprotein E and low density lipoprotein particle size. *Atherosclerosis.* **102:** 147–154.
147. Haffner, S. M., M. P. Stern, H. Miettinen, D. Robbins, and B. V. Howard. 1996. Apolipoprotein E polymorphism and LDL size in a biethnic population. *Arterioscler. Thromb. Vasc. Biol.* **16:** 1184–1188.
148. Austin, M. A. 1992. Genetic epidemiology of low-density lipoprotein subclass phenotypes. *Ann. Med.* **24:** 477–481.
149. Terry, R. B., P. D. Wood, W. L. Haskell, M. L. Stefanick, and R. M. Krauss. 1989. Regional adiposity patterns in relation to lipids, lipoprotein cholesterol, and lipoprotein subfraction mass in men. *J. Clin. Endocrinol. Metab.* **68:** 191–199.
150. Campos, H., F. M. Sacks, B. W. Walsh, I. Schiff, M. A. O'Hanesian, and R. M. Krauss. 1993. Differential effects of estrogen on low-density lipoprotein subclasses in healthy postmenopausal women. *Metabolism.* **42:** 1153–1158.
151. de Graaf, J., D. W. Swinkels, P. N. Demacker, A. F. de Haan, and A. F. Stalenhoef. 1993. Differences in the low density lipoprotein subfraction profile between oral contraceptive users and controls. *J. Clin. Endocrinol. Metab.* **76:** 197–202.
152. Krauss, R. M., and D. M. Dreon. 1995. Low-density-lipoprotein subclasses and response to a low-fat diet in healthy men. *Am. J. Clin. Nutr.* **62:** 478S–487S.
153. Dreon, D. M., H. A. Fernstrom, B. Miller, and R. M. Krauss. 1994. Low-density lipoprotein subclass patterns and lipoprotein response to a reduced-fat diet in men. *FASEB J.* **8:** 121–126.
154. Campos, H., W. C. Willett, R. M. Peterson, X. Siles, S. M. Bailey, P. W. Wilson, B. M. Posner, J. M. Ordovas, and E. J. Schaefer. 1991. Nutrient intake comparisons between Framingham and rural and Urban Puriscal, Costa Rica. Associations with lipoproteins, apolipoproteins, and low density lipoprotein particle size. *Arterioscler. Thromb.* **11:** 1089–1099.
155. Dreon, D. M., H. A. Fernstrom, P. T. Williams, and R. M. Krauss. 1997. LDL subclass patterns and lipoprotein response to a low-fat, high-carbohydrate diet in women. *Arterioscler. Thromb. Vasc. Biol.* **17:** 707–714.
156. Dreon, D. M., H. A. Fernstrom, P. T. Williams, and R. M. Krauss. 2000. Reduced LDL particle size in children consuming a very-low-fat diet is related to parental LDL-subclass patterns. *Am. J. Clin. Nutr.* **71:** 1611–1616.
157. Singh, A. T., D. L. Rainwater, C. M. Kammerer, R. M. Sharp, M. Poushesh, W. R. Shelledy, and J. L. VandeBerg. 1996. Dietary and genetic effects on LDL size measures in baboons. *Arterioscler. Thromb. Vasc. Biol.* **16:** 1448–1453.
158. Reaven, G. M., Y. D. Chen, J. Jeppesen, P. Maheux, and R. M. Krauss. 1993. Insulin resistance and hyperinsulinemia in individuals with small dense low density lipoprotein particles. *J. Clin. Invest.* **92:** 141–146.
159. Austin, M. A., J. D. Brunzell, W. L. Fitch, and R. M. Krauss. 1990. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipidemia. *Arteriosclerosis.* **10:** 520–530.
160. Teng, B., G. R. Thompson, A. D. Sniderman, T. M. Forte, R. M. Krauss, and P. O. Kwiterovich, Jr. 1983. Composition and distribution of low density lipoprotein fractions in hyperapobetalipoproteinemia, normolipidemia, and familial hypercholesterolemia. *Proc. Natl. Acad. Sci. USA.* **80:** 6662–6666.
161. Genest, J., Jr., J. M. Bard, J. C. Fruchart, J. M. Ordovas, and E. J. Schaefer. 1993. Familial hypoalphalipoproteinemia in premature coronary artery disease. *Arterioscler. Thromb.* **13:** 1728–1737.
162. Austin, M. A., H. Horowitz, E. Wijsman, R. M. Krauss, and J. Brunzell. 1992. Bimodality of plasma apolipoprotein B levels in familial combined hyperlipidemia. *Atherosclerosis.* **92:** 67–77.
163. Jarvik, G. P., J. D. Brunzell, M. A. Austin, R. M. Krauss, A. G. Motulsky, and E. Wijsman. 1994. Genetic predictors of FCHL in four large pedigrees. Influence of ApoB level major locus predicted genotype and LDL subclass phenotype. *Arterioscler. Thromb.* **14:** 1687–1694.
164. Jansen, H., W. Hop, A. van Tol, A. V. Bruschke, and J. C. Birkenhager. 1994. Hepatic lipase and lipoprotein lipase are not major determinants of the low density lipoprotein subclass pattern in human subjects with coronary heart disease. *Atherosclerosis.* **107:** 45–54.
165. Miesenbock, G., B. Holzl, B. Foger, E. Brandstatter, B. Paulweber, F. Sandhofer, and J. R. Patsch. 1993. Heterozygous lipoprotein lipase deficiency due to a missense mutation as the cause of impaired triglyceride tolerance with multiple lipoprotein abnormalities. *J. Clin. Invest.* **91:** 448–455.
166. Feingold, K. R., C. Grunfeld, M. Pang, W. Doerrler, and R. M. Krauss. 1992. LDL subclass phenotypes and triglyceride metabolism in non-insulin-dependent diabetes. *Arterioscler. Thromb.* **12:** 1496–1502.
167. Syvanne, M., and M. R. Taskinen. 1997. Lipids and lipoproteins as coronary risk factors in non-insulin-dependent diabetes mellitus. *Lancet.* **350(Suppl. 1):** SI20–SI23.
168. Austin, M. A., L. Mykkanen, J. Kuusisto, K. L. Edwards, C. Nelson, S. M. Haffner, K. Pyorala, and M. Laakso. 1995. Prospective study of small LDLs as a risk factor for non-insulin dependent diabetes mellitus in elderly men and women. *Circulation.* **92:** 1770–1778.
169. Lewis, G. F., and G. Steiner. 1996. Acute effects of insulin in the control of VLDL production in humans. Implications for the insulin-resistant state. *Diabetes Care.* **19:** 390–393.
170. Gibbons, G. F. 1990. Assembly and secretion of hepatic very-low-density lipoprotein. *Biochem. J.* **268:** 1–13.
171. Hellerstein, M. K., M. Christiansen, S. Kaempfer, C. Kletke, K. Wu, J. S. Reid, K. Mulligan, N. S. Hellerstein, and C. H. Shackle-

ton. 1991. Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *J. Clin. Invest.* **87:** 1841–1852.
172. Wiggins, D., and G. F. Gibbons. 1992. The lipolysis/esterification cycle of hepatic triacylglycerol. Its role in the secretion of very-low-density lipoprotein and its response to hormones and sulphonylureas. *Biochem. J.* **284:** 457–462.
173. Lewis, G. F., K. D. Uffelman, L. W. Szeto, B. Weller, and G. Steiner. 1995. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J. Clin. Invest.* **95:** 158–166.
174. Malmstrom, R., C. J. Packard, T. D. Watson, S. Rannikko, M. Caslake, D. Bedford, P. Stewart, H. Yki-Jarvinen, J. Shepherd, and M. R. Taskinen. 1997. Metabolic basis of hypotriglyceridemic effects of insulin in normal men. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1454–1464.
175. Bioletto, S., A. Golay, R. Munger, B. Kalix, and R. W. James. 2000. Acute hyperinsulinemia and very-low-density and low-density lipoprotein subfractions in obese subjects. *Am. J. Clin. Nutr.* **71:** 443–449.
176. Malmstrom, R., C. J. Packard, M. Caslake, D. Bedford, P. Stewart, H. Yki-Jarvinen, J. Shepherd, and M. R. Taskinen. 1997. Defective regulation of triglyceride metabolism by insulin in the liver in NIDDM. *Diabetologia.* **40:** 454–462.
177. Steiner, G. 1991. Altering triglyceride concentrations changes insulin-glucose relationships in hypertriglyceridemic patients. Double-blind study with gemfibrozil with implications for atherosclerosis. *Diabetes Care.* **14:** 1077–1081.
178. Mykkanen, L., S. M. Haffner, D. L. Rainwater, P. Karhapaa, H. Miettinen, and M. Laakso. 1997. Relationship of LDL size to insulin sensitivity in normoglycemic men. *Arterioscler. Thromb. Vasc. Biol.* **17:** 1447–1453.
179. Zambon, A., M. A. Austin, B. G. Brown, J. E. Hokanson, and J. D. Brunzell. 1993. Effect of hepatic lipase on LDL in normal men and those with coronary artery disease. *Arterioscler. Thromb.* **13:** 147–153.
180. Griffin, B. A., D. J. Freeman, G. W. Tait, J. Thomson, M. J. Caslake, C. J. Packard, and J. Shepherd. 1994. Role of plasma triglyceride in the regulation of plasma low density lipoprotein (LDL) subfractions: relative contribution of small dense LDL to coronary heart disease risk. *Atherosclerosis.* **106:** 241–253.
181. Coresh, J., P. J. Kwiterovich, H. Smith, and P. Bachorik. 1993. Association of plasma trigglyceride concentration and LDL diameter density, and chemical composition with premature coronary artery disease in men and woman. *J. Lipid Res.* **34:** 1687–1697.
182. Stampfer, M. J., R. M. Krauss, J. Ma, P. J. Blanche, L. G. Holl, F. M. Sacks, and C. H. Hennekens. 1996. A prospective study of triglyceride level, low-density lipoprotein particle diameter, and risk of myocardial infarction. *J. Am. Med. Assoc.* **276:** 882–888.
183. Gardner, C. D., S. P. Fortmann, and R. M. Krauss. 1996. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. *J. Am. Med. Assoc.* **276:** 875–881.
184. Lamarche, B., A. C. St-Pierre, I. L. Ruel, B. Cantin, G. R. Dagenais, and J. P. Despres. 2001. A prospective, population-based study of low density lipoprotein particle size as a risk factor for ischemic heart disease in men. *Can. J. Cardiol.* **17:** 859–865.
185. Lamarche, B., A. Tchernof, S. Moorjani, B. Cantin, G. R. Dagenais, P. J. Lupien, and J. P. Despres. 1997. Small dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. *Circulation.* **95:** 69–75.
186. St-Pierre, A. C., I. L. Ruel, B. Cantin, G. R. Dagenais, P. M. Bernard, J. P. Despres, and B. Lamarche. 2001. Comparison of various electrophoretic characteristics of LDL particles and their relationship to the risk of ischemic heart disease. *Circulation.* **104:** 2295–2299.
187. Campos, H., L. A. Moye, S. P. Glasser, S. P. Stampfer, and F. M. Sacks. 2001. Low-density lipoprotein size, pravastatin treatment, and coronary events. *J. Am. Med. Assoc.* **286:** 1468–1474.
188. Bjornheden, T., A. Babyi, G. Bondjers, and O. Wiklund. 1996. Accumulation of lipoprotein fractions and subfractions in the arterial wall, determined in an in vitro perfusion system. *Atherosclerosis.* **123:** 43–56.
189. Tribble, D. L., L. G. Holl, P. D. Wood, and R. M. Krauss. 1992. Variations in oxidative susceptibility among six low density lipoprotein subfractions of differing density and particle size. *Atherosclerosis.* **93:** 189–199.
190. Chait, A., R. L. Brazg, D. L. Tribble, and R. M. Krauss. 1993. Susceptibility of small dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am. J. Med.* **94:** 350–356.
191. Dejager, S., E. Bruckert, and M. J. Chapman. 1993. Dense low density lipoprotein subspecies with diminished oxidative resistance predominate in combined hyperlipidemia. *J. Lipid Res.* **34:** 295–308.
192. Tribble, D. L., M. Rizzo, A. Chait, D. M. Lewis, P. J. Blanche, and R. M. Krauss. 2001. Enhanced oxidative susceptibility and reduced antioxidant content of metabolic precursors of small dense low-density lipoproteins. *Am. J. Med.* **110:** 103–110.
193. de Graaf, J., H. L. Hak-Lemmers, M. P. Hectors, P. N. Demacker, J. C. Hendriks, and A. F. Stalenhoef. 1991. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. *Arteriosclerosis.* **11:** 298–306.
194. Tribble, D. L., J. J. van den Berg, P. A. Motchnik, B. N. Ames, D. M. Lewis, A. Chait, and R. M. Krauss. 1994. Oxidative susceptibility of low density lipoprotein subfractions is related to their ubiquinol-10 and alpha-tocopherol content. *Proc. Natl. Acad. Sci. USA.* **91:** 1183–1187.
195. Watts, G. F., S. Mandalia, J. N. Brunt, B. M. Slavin, D. J. Coltart, and B. Lewis. 1993. Independent associations between plasma lipoprotein subfraction levels and the course of coronary artery disease in the St. Thomas' Atherosclerosis Regression Study (STARS). *Metabolism.* **42:** 1461–1467.
196. Phillips, N. R., D. Waters, and R. J. Havel. 1993. Plasma lipoproteins and progression of coronary artery disease evaluated by angiography and clinical events. *Circulation.* **88:** 2762–2770.
197. Mack, W. J., R. M. Krauss, and H. N. Hodis. 1996. Lipoprotein subclasses in the Monitored Atherosclerosis Regression Study (MARS). Treatment effects and relation to coronary angiographic progression. *Arterioscler. Thromb. Vasc. Biol.* **16:** 697–704.
198. Hodis, H. N., W. J. Mack, M. Dunn, C. Liu, R. H. Selzer, and R. M. Krauss. 1997. Intermediate-density lipoproteins and progression of carotid arterial wall intima-media thickness. *Circulation.* **95:** 2022–2026.
199. Alaupovic, P., D. H. Blankenhorn, C. Knight-Gibson, M. Tavella, J. M. Bard, D. Shafer, E. T. Lee, and J. Brasuell. 1991. apoB-containing lipoprotein particles as risk factors for coronary artery disease. *Adv. Exp. Med. Biol.* **285:** 299–309.
200. Blankenhorn, D. H., P. Alaupovic, E. Wickham, H. P. Chin, and S. P. Azen. 1990. Prediction of angiographic change in native human coronary arteries and aortocoronary bypass grafts. Lipid and nonlipid factors. *Circulation.* **81:** 470–476.
201. Hodis, H. N., W. J. Mack, S. P. Azen, P. Alaupovic, J. M. Pogoda, L. LaBree, L. C. Hemphill, D. M. Kramsch, and D. H. Blankenhorn. 1994. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin. *Circulation.* **90:** 42–49.
202. Sacks, F. M., P. Alaupovic, L. A. Moye, T. G. Cole, B. Sussex, M. J. Stampfer, M. A. Pfeffer, and E. Braunwald. 2000. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation.* **102:** 1886–1892.
203. Luc, G., C. Fievet, D. Arveiler, A. E. Evans, J. M. Bard, F. Cambien, J. C. Fruchart, and P. Ducimetiere. 1996. Apolipoproteins C–III and E in apoB- and non-apoB-containing lipoproteins in two populations at contrasting risk for myocardial infarction: the ECTIM study. Etude Cas Temoins sur 'Infarctus du Myocarde. *J. Lipid Res.* **37:** 508–517.
204. Rudel, L. L., J. S. Parks, F. L. Johnson, and J. Babiak. 1986. Low density lipoproteins in atherosclerosis. *J. Lipid Res.* **27:** 465–474.
205. Shimada, M., S. Ishibashi, T. Inaba, H. Yagyu, K. Harada, J. I. Osuga, K. Ohashi, Y. Yazaki, and N. Yamada. 1996. Suppression of diet-induced atherosclerosis in low density lipoprotein receptor knockout mice overexpressing lipoprotein lipase. *Proc. Natl. Acad. Sci. USA.* **93:** 7242–7246.
206. Campos, H., G. O. Roederer, S. Lussier-Cacan, J. Davignon, and R. M. Krauss. 1995. Predominance of large LDL and reduced HDL2 cholesterol in normolipidemic men with coro-

nary artery disease. *Arterioscler. Thromb. Vasc. Biol.* **15:** 1043–1048.
207. Manninen, V., L. Tenkanen, P. Koskinen, J. K. Huttunen, M. Manttari, O. P. Heinonen, and M. H. Frick. 1992. Joint effects of serum triglyceride and LDL cholesterol and HDL cholesterol concentrations on coronary heart disease risk in the Helsinki Heart Study. Implications for treatment. *Circulation.* **85:** 37–45.
208. Miller, B., R. M. Krauss, L. Cashin-Hemphill, and D. H. Blankenhorn. 1993. Baseline-triglyceride levels predict angiographic benefit of colestipol plus niacin therapy in the cholesterol-lowering atherosclerosis study (CLAS). *Circulation.* **4(Suppl.):** I-363.
209. Zambon, A., J. E. Hokanson, B. G. Brown, and J. D. Brunzell. 1999. Evidence for a new pathophysiological mechanism for coronary artery disease regression: hepatic lipase-mediated changes in LDL density. *Circulation.* **99:** 1959–1964.
210. Miller, B. D., L. Cashin-Hemphill, W. J. Mack, H. N. Hodis, and R. M. Krauss. 1994. Predominance of mid-density low density lipoproteins predicts angiographic benefit of lovastatin in the monitored atherosclerosis regression study. *Circulation.* **90 (Suppl.):** I460.
211. Pennacchio, L. A., M. Olivier, J. A. Hubacek, J. C. Cohen, D. R. Cox, J. C. Fruchart, R. M. Krauss, and E. M. Rubin. 2001. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science.* **294:** 169–173.