Plasma Triglyceride Determines Structure-Composition in Low and High Density Lipoproteins

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Because the association of hypertriglyceridemia and premature atherosclerosis is not due to the direct effects of the triglyceride molecule itself, we studied the effects of increased plasma triglyceride-rich lipoproteins on the composition and structure of low density lipoprotein (LDL) and high density lipoprotein (HDL). We found profound changes in the core and surface domains of both lipoproteins with increasing triglyceridemia. Core cholesterol esters were progressively depleted and replaced by triglyceride molecules. Highly significant negative correlations were found between cholesterol ester/protein ratios ($r = -0.64$ for LDL and -0.58 for HDL ($p < 0.001$); positive correlations were found for triglyceride/protein ratios ($r = 0.62$ for LDL and 0.58 for HDL) and for triglyceride/cholesterol ester ratios ($r = 0.70$ for LDL and 0.83 for HDL) when these variables were assayed as a function of plasma triglyceride concentrations. With severe hypertriglyceridemia, triglyceride/cholesterol ester ratios of more than 1.0 were consistently observed (normal, <0.02). This leads to an underestimation of LDL and HDL levels when cholesterol alone is measured. At the surface, LDL and HDL were depleted of phospholipid and free cholesterol, with a relative enrichment of protein. These changes can be explained on the basis of high levels of plasma triglyceride-rich lipoproteins serving as acceptors for cholesterol esters and other constituents from LDL and HDL. Concomitantly, triglycerides are transferred to LDL and HDL. These transfer processes are likely to be mediated by the activity of lipid transfer proteins present in human plasma.
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Increased levels of plasma low density lipoprotein (LDL) cholesterol[1] and very low density lipoprotein (VLDL) cholesterol and triglyceride[2] are risk factors for premature atherosclerosis. Conversely, high levels of high density lipoprotein (HDL) cholesterol have been correlated with longevity and freedom from early coronary disease.[3,4] For example, high levels of LDL and normal levels of HDL are considered a cause of accelerated coronary artery disease in familial hypercholesterolemia.[5] The increased risk for coronary artery disease observed in hypertriglyceridemia, however, is poorly understood and probably does not reflect the direct effects of the triglyceride molecule itself. Recently, Hulley and associates[6] have suggested that the low HDL cholesterol levels found in hypertriglyceridemic patients contribute significantly to this phenomen. Such patients also have low LDL cholesterol levels and high VLDL cholesterol and triglyceride levels. The metabolic pathways responsible for these observations have not been previously elucidated.

In this paper we suggest that these abnormalities may merely reflect the state of hypertriglyceridemia. Specifically, we propose that the replacement of cholesterol ester in HDL and LDL by triglycerides contributes to the low levels of cholesterol in these lipoproteins. In recent in vitro experiments,[7–9] we showed that cholesterol ester-triglyceride bidirectional transfer processes, originally observed by Nichols and Smith,[10] play a major role in determining the structure and composition of LDL and HDL. We

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now provide evidence that these processes also mediate structure-composition relationships of LDL and HDL in vivo. The metabolic consequences of these pathways help in understanding the increased atherogenicity associated with hypertriglyceridemia.

Methods

LDL and HDL were isolated from the plasma of 43 male and female subjects with plasma triglyceride levels between 31 mg/dl and 2820 mg/dl and plasma cholesterol levels between 28 mg/dl and 494 mg/dl. These subjects were not selected specifically for this study but, rather, provided plasma for a variety of lipoprotein-related studies being carried out in our laboratories. The subject with the lowest plasma lipid levels (cholesterol 28 mg/dl and triglyceride 31 mg/dl) had abetalipoproteinemia. One subject with plasma triglyceride levels over 1000 mg/dl had Type IV hyperlipidemia, one had apoprotein C-II deficiency, and the others were children with Type I primary hypertriglyceridemias associated with variable patterns of lipase deficiencies. All other blood donors were either normolipemic or phenotypically Type IV hyperlipidemic adults. All patients with hypertriglyceridemia had normal or low LDL levels. We did not differentiate between familial combined hyperlipoproteinemia and familial hypertriglyceridemia because of lack of data on family members.

Venous blood samples in disodium EDTA (1 mg/ml) were obtained after a 12- to 14-hour fast, and LDL and HDL were separated using either sequential salt density[11] or rate zonal ultracentrifugation.[12] With the first method, LDL was recovered between the densities of 1.019 and 1.063 g/ml and HDL, between 1.063 and 1.21 g/ml. With the second method, LDL and HDL were obtained directly from the zonal rotor effluent at the end of 140 minutes or 22 hours of centrifugation runs, respectively, in a 14Ti rotor, as previously detailed.[13] The LDL and HDL were dialyzed against several changes of normal saline (0.15 M NaCl–1 mM EDTA, pH 8.5, 100:1 (vol/vol). Lipids were extracted with chloroform/methanol 2:1 (vol/vol).[14] The total phospholipid content was determined using the method of Bartlett.[15] The total cholesterol was determined using the method of Chiamori and Henry,[16] and thin-layer chromatography was used to separate free and esterified cholesterol.[17] The triglyceride content was determined by the AutoAnalyzer method.[18] The protein content was measured by the method of Lowry et al.[19]

Pearson product-moment correlation and partial correlation analyses were performed to determine the associations between individual lipoprotein constituents and/or combinations of constituents and plasma triglyceride and plasma cholesterol levels. To correct the marked skew in plasma triglyceride levels at higher concentrations, plasma triglyceride levels were transformed to logarithmic scales (log 10). Linear regression analysis was performed by the method of least squares. Analysis of variance for the regression was performed to obtain the significance of F ratios.[20]

All 43 subjects had LDL analyzed; 40 subjects had HDL analyzed. LDL and HDL were separated from the plasma of 20 subjects by salt density ultracentrifugation; in the remaining subjects rate zonal ultracentrifugation was used. A separate analysis of variables recorded with each centrifugation method showed no significant differences; therefore, data from both groups, i.e., salt density and zonal ultracentrifugation groups, were pooled.

Results

The composition of LDL and HDL in subjects were divided over three ranges of plasma triglyceride levels (<250, 250–1000, >1000 mg/dl) and demonstrated several consistent changes (Table 1). With increased triglyceridemia, the relative contribution of protein and triglyceride increases, and that of phospholipid, free cholesterol, and cholesterol ester de-

Table 1. Composition Analysis of LDL and HDL over Three Ranges of Plasma Triglyceride Levels

Plasma *triglyceride* concentration (mg/dl)		Protein	Phospholipid	Cholesterol, free	Cholesterol ester	Triglyceride
Low density lipoprotein (LDL)						
<250	(18)	22.9±3.4	22.4±2.9	9.3±1.1	39.0±5.6	6.5±2.8
251–1000	(18)	25.7±4.0	23.9±7.6	7.8±1.6	34.3±6.1	8.3±3.3
>1000	(5)	28.4±6.1	15.3±4.8	5.6±1.7	23.9±3.5	26.7±7.0
High density lipoprotein (HDL)						
<250	(19)	51.5±7.1	24.3±5.0	2.3±1.2	17.2±4.5	4.7±2.1
251–1000	(17)	51.8±6.0	25.3±7.5	1.8±0.3	13.8±2.6	7.3±2.0
>1000	(4)	55.9±5.1	17.2±4.8	2.2±0.6	12.8±3.2	11.9±1.7

Numbers in parentheses refer to the number of subjects studied in each group. Since full composition analysis was available in only 41 of the 43 subjects analyzed for LDL, the total number in the three groups is 41 for LDL. Composition is expressed as the relative percentage of weight contribution to total lipoprotein mass, mean ± SD.

Table 2. Relative Contribution of Individual Constituents to Total LDL and HDL Mass Correlated with Plasma Triglyceride Concentrations

	Correlation coefficients (r) Plasma triglyceride concentrations (mg/dl)		
	<250	<1000	All subjects
Low density lipoprotein (LDL)			
Protein	−0.06 (N.S.)	+0.39 (0.02)	+0.45 (0.003)
Phospholipid	−0.16 (N.S.)	−0.06 (N.S.)	−0.32 (0.040)
Free cholesterol	+0.12 (N.S.)	−0.44 (0.007)	−0.63 (0.001)
Cholesterol ester	−0.15 (N.S.)	−0.27 (N.S.)	−0.57 (0.001)
Triglyceride	+0.49 (0.035)	+0.38 (0.02)	+0.72 (0.001)
No.	18	36	41
High density lipoprotein (HDL)			
Protein	+0.44 (0.060)	+0.16 (N.S.)	+0.36 (0.020)
Phospholipid	−0.30 (N.S.)	−0.02 (N.S.)	−0.39 (0.060)
Free cholesterol	−0.66 (0.002)	−0.50 (0.002)	−0.39 (0.013)
Cholesterol ester	−0.52 (0.023)	−0.55 (0.001)	−0.60 (0.001)
Triglyceride	+0.68 (0.001)	+0.71 (0.001)	+0.64 (0.001)
No.	19	36	40

r refers to the coefficient of correlation between the relative weight contribution (percentage of total lipoprotein mass) of an individual compositional component and $\log_{10}$ plasma triglyceride concentration for each group of subjects. Numbers in parentheses give the p value for the coefficient of correlation. NS = not significant, with $p > 0.10$.

creases. The correlation of individual components to the relative lipoprotein mass over the entire range of plasma triglyceride levels demonstrates similar significant trends (Table 2). In both LDL and HDL, the decrease of cholesterol ester and the increase in triglyceride are highly significant.

In lipoproteins, phospholipid, protein, and free cholesterol make up the surface shell of the particle emulsifying the core neutral lipids, cholesterol ester and triglyceride. To better understand the events that affect the structure, composition, and metabolism of the particles, we correlated the ratios of different components contained in the two domains of the lipoprotein with continually increasing plasma triglyceride (Table 3, Figure 1).

In the core, the amount of triglyceride relative to cholesterol ester was highly correlated to plasma triglyceride levels, and this ratio increased very substantially with triglyceridemia (Figure 1, Table 3). Considering the entire range of plasma triglycerides, we noted some differences between LDL and HDL (Figure 1). In LDL, the increase of triglyceride/cholesterol ester ratio was moderate at plasma triglyceride levels below 1000 mg/dl, but increased steeply with more severe hypertriglyceridemia. This was reflected in the correlation coefficients of LDL triglyceride/cholesterol ester ratios vs (log) plasma triglyceride concentrations: 0.37 ($p < 0.020$) at plasma triglyceride levels less than 1000 mg/dl and 0.70 ($p < 0.001$) for the entire group. By contrast, in HDL the increase of the triglyceride/cholesterol ester ratio was linear over the entire range of plasma triglyceride levels with a correlation coefficient of 0.77 ($p < 0.001$) for plasma triglyceride levels less than 1000 mg/dl and 0.83 ($p < 0.001$) for all subjects.

Table 3. Mass Ratios of Different Constitutents of LDL and HDL Correlated with Plasma Triglyceride Concentrations

Mass ratio	LDL (r)	HDL (r)
Triglyceride/ cholesterol ester	+0.70 (0.001)	+0.83 (0.001)
Triglyceride/ phospholipid	+0.64 (0.001)	+0.78 (0.001)
Triglyceride/protein	+0.62 (0.001)	+0.58 (0.001)
Triglyceride/ (phospholipid + protein + free cholesterol)	+0.68 (0.001)	+0.62 (0.001)
Cholesterol ester/ phospholipid	−0.10 (NS)	−0.15 (NS)
Cholesterol ester/ protein	−0.64 (0.001)	−0.58 (0.001)
Cholesterol ester/ (phospholipid + protein + free cholesterol)	−0.44 (0.004)	−0.54 (0.001)
Phospholipid/protein	−0.37 (0.017)	−0.29 (0.070)
(Cholesterol ester + triglyceride)/ (phospholipid + protein + free cholesterol)	−0.25 (NS)	−0.16 (NS)

Mass ratios were calculated from the relative concentrations of individual lipoprotein constituents on a weight/weight basis. r refers to coefficient of correlation of the given ratio and to $\log_{10}$ plasma triglyceride concentration over the entire range of plasma triglycerides in all subjects. Numbers in parentheses are the p values for each correlation coefficient. NS = not significant ($p > 0.10$).

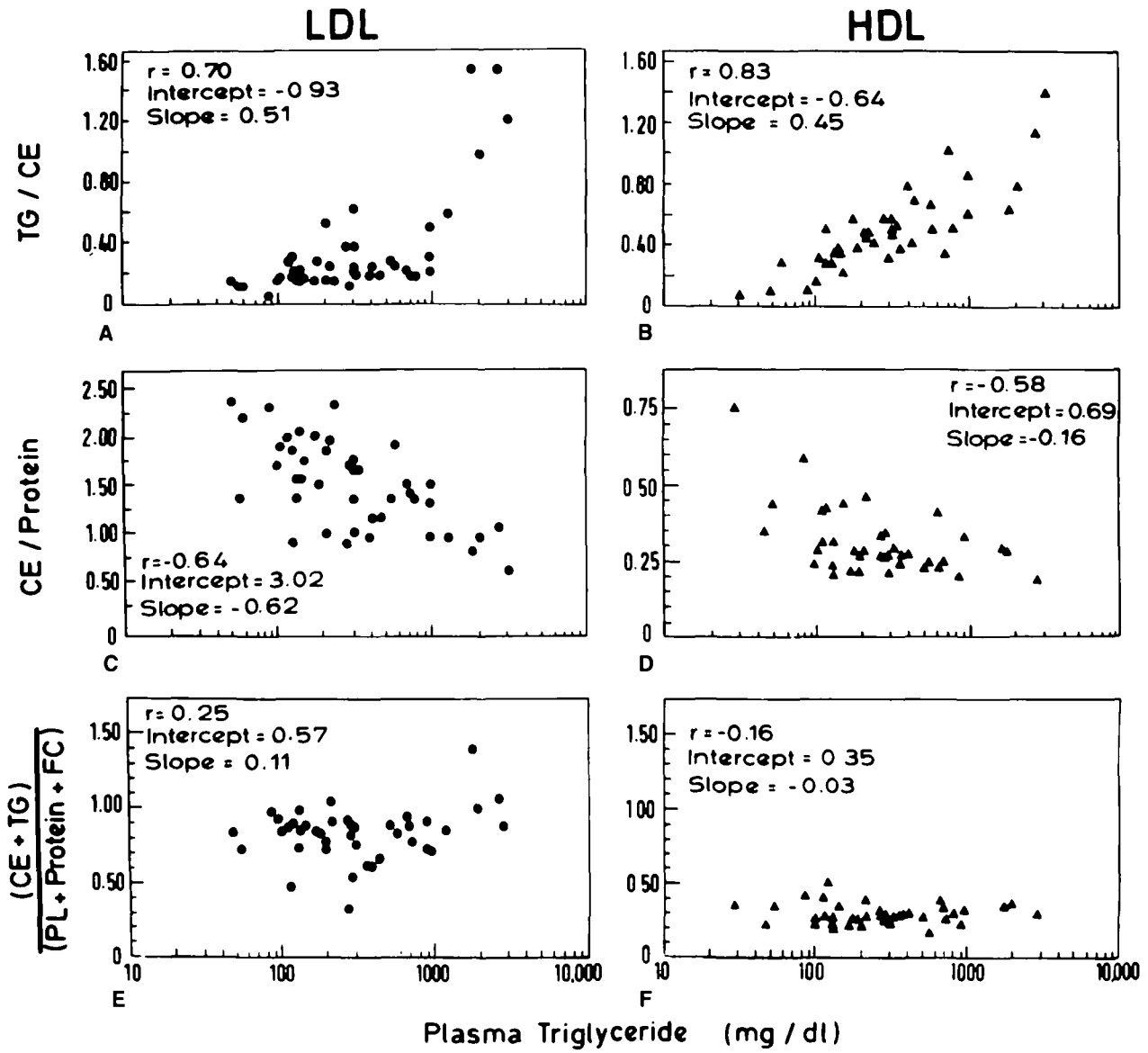

Figure 1. Mass ratios of LDL and HDL constituents compared to plasma triglyceride concentrations. Triglyceride/cholesterol ester ratios in LDL (**A**) and in HDL (**B**). Cholesterol ester/protein ratios in LDL (**C**) and in HDL (**D**). (Triglyceride + cholesterol ester)/(phospholipid + protein + free cholesterol) ratios in LDL (**E**) and in HDL (**F**). Ratios are plotted as a function of plasma triglyceride concentrations.

At the surface, the phospholipid/protein ratio decreased with elevated plasma triglyceride concentrations, but the degree of correlation reached statistical significance ($p < 0.05$) only for LDL. The ratios of core constituents to individual or combined surface constituents changed accordingly, i.e., highly significant positive correlations were observed for triglyceride and negative correlations were seen for cholesterol ester. Since neutral lipid transfers are not significantly associated with the transfer of LDL and HDL major apoproteins,[7,8] the loss of cholesterol ester molecules from both LDL and HDL was substantiated by a decreasing cholesterol ester/protein ratio with increasing triglyceridemia (Figure 1). An exception, the cholesterol ester/phospholipid ratio, did not seem to correlate with plasma triglyceride levels (Table 3).

These findings suggest that, as triglyceride increases in plasma, cholesterol ester leaves the core of LDL and HDL and is replaced by triglyceride. If this assumption is correct, then the ratio of the total core components (triglyceride + cholesterol ester) to the total surface components (protein + phospholipid + free cholesterol) should change little over the wide range of plasma triglyceride levels studied. The data in Table 3 and Figure 1 demonstrate that this is the case.

In the subjects studied, plasma cholesterol levels correlated with plasma triglyceride levels ($r = 0.55$, $p < 0.001$). Using Pearson partial correlation coefficient analysis, we found no significant correlation of any of the variables presented in Tables 1–3 with plasma cholesterol levels after an adjustment for the effect of plasma triglyceride on cholesterol levels.

Discussion

Associations between accelerated atherosclerosis, premature coronary heart diseases, and hypertriglyceridemia have been shown in almost every study relating plasma lipids and atherosclerosis. For example, Hulley and colleagues[6] cite no less than 27 studies where significant correlations between these factors were reported using unadjusted plasma triglyceride levels. Whether it is the triglyceride molecule itself, however, that is atherogenic is not known, and it is possible that other abnormalities in plasma lipoproteins or their constituents are responsible for the increased atherogenicity (e.g., low HDL levels). Hence, the importance of defining lipoprotein systems in hypertriglyceridemia.

Abnormal composition of low and high density lipoproteins has been previously shown in patients with unusually severe hypertriglyceridemia.[21-24] In the present study, we demonstrated that specific graded alterations of core and surface constituents in both LDL and HDL are directly and continuously related to plasma triglyceride levels. These changes especially affect the lipoprotein core lipids.

Models for lipoprotein structure predict that the bulk of neutral lipids (cholesterol ester, triglyceride) are localized in the hydrophobic core of the particle surrounded by more hydrophobic surface constituents (phospholipids, free cholesterol, and protein).[25] We show here that, in the frame of this general model, the core of both LDL and HDL undergo considerable alteration in the proportions of cholesterol ester and triglycerides. These changes occur in a predictable fashion dependent on plasma triglyceride or VLDL levels, since VLDL carries most of the triglyceride in plasma.

These modifications can be explained by the activity of a protein(s) present in human plasma that transfers cholesterol ester from LDL and HDL to VLDL and, in the opposite direction, triglyceride from VLDL to LDL and HDL.[26-28] This suggestion is supported by recent observations that, indeed, cholesterol ester-rich LDL and HDL are substantially modified toward cholesterol ester-poor, triglyceride-rich particles in vitro after incubation with high levels of VLDL and in the presence of lipid transfer proteins.[7, 8] These changes are directly related to the amounts of VLDL relative to LDL or HDL and the time of incubation.[7, 8] It seems, therefore, that the underlying pathways responsible for core lipid alterations in hypertriglyceridemia are similar transfers of core lipids in subjects with high plasma triglyceride levels and prolonged circulating times of their triglyceride-rich lipoproteins.[29-31]

At the lipoprotein surface, we found a fall in free cholesterol and phospholipids, with increased protein correlating to increasing triglyceridemia. Free cholesterol distributes between surface and core domains of lipoproteins[32] and in the presence of high levels of VLDL, the free cholesterol plasma pool must redistribute toward a preponderance in the large VLDL population. Our finding of a constant cholesterol ester/phospholipid ratio independent of plasma triglyceride levels is consistent with coupled transfer of these two molecules in vivo as has been suggested in vitro.[33] Moreover, increases in cholesterol esterification via the lecithin cholesterol acyltransferase reaction, which consumes both free cholesterol and phospholipid, are described in hypertriglyceridemia.[34] Finally, since the major LDL and HDL apoproteins (B, A-I, and A-II) do not redistribute with these transfer processes,[7, 8] they remain on the particles and their relative contribution to the surface increases.

A practical consequence of our observations is that LDL and HDL cholesterol measurements in the presence of increased plasma triglycerides do not accurately reflect plasma LDL and HDL levels. This is in agreement with the conclusions of Phillips et al.[35] who reported that falling HDL cholesterol with increasing plasma VLDL triglyceride was relatively much greater than concomitant slight decreases in plasma's major HDL protein, apo A-I. These authors found increasing absolute amounts of HDL triglycerides correlating with plasma VLDL triglyceride. An earlier study by the same group[36] reported that the ratio of total cholesterol to triglycerides in LDL and HDL decreased as serum triglyceride levels increased. In hypertriglyceridemic diabetics, decreasing HDL cholesterol and increasing HDL triglyceride correlated with increasing total plasma triglyceride, but plasma apoprotein A-I levels did not.[37] Our present study shows that the decrease of cholesterol in LDL and HDL in hypertriglyceridemia is due mainly to a decrease in cholesterol ester in the lipoprotein particle, with a smaller decline in free cholesterol. Therefore, in both LDL and HDL, we suggest that measurements of decreased cholesterol content may not reflect a parallel decrease in the number of circulating particles.

The pathways responsible for these lipoprotein compositional changes also predict that in hypertriglyceridemia, both LDL and HDL would be smaller and denser due to the loss of cholesterol esters for triglyceride, concomitant with LDL and HDL triglyceride hydrolysis. Conversely, in a situation such as abetalipoproteinemia, where plasma is lacking in VLDL to accept cholesterol ester, one could predict that cholesterol ester accumulates in HDL.[9] Figure 2 illustrates that, indeed, this is the case in contrasting zonal ultracentrifugation elution profiles of normal HDL with that of HDL in patients with severe hypertriglyceridemia or abetalipoproteinemia. A similar observation of small, dense HDL in hypertriglyceridemia has been reported by Patsch and Gotto.[38] LDL, as well, has been decribed as smaller and denser in hypertriglyceridemia relative to normolipemic subjects.[39] Inspection of the relative weight composition data presented in Table 1 does not show the obvious decreases in total core/total surface constituent ratios that might be expected with decreasing particle size.

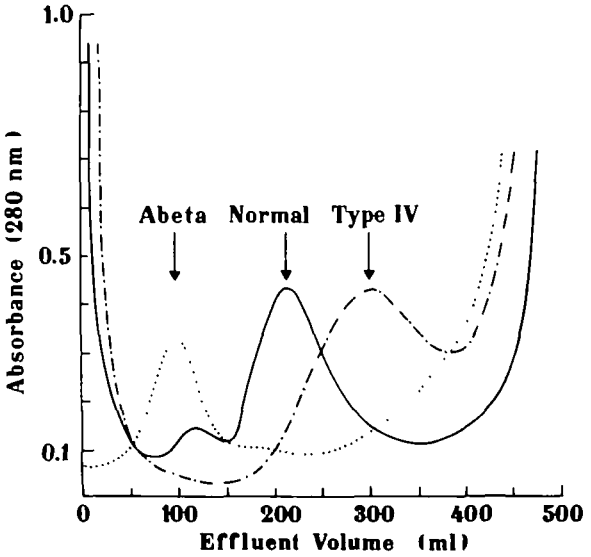

Figure 2. Separation by rate zonal ultracentrifugation of HDL in a normal subject (———), a patient with abetalipoproteinemia (·····), and a patient with Type IV hyperlipidemia (—·—). The plasma triglyceride concentrations were 130, 31, and 927 mg/dl in each subject, respectively. Floatation is from the left to the right, with the lighter and larger particles eluting earlier than the denser and smaller particles. In abetalipoproteinemia, the major HDL peak elutes earlier than in normal HDL. Conversely, in the subject with hypertriglyceridemia, HDL elutes later than in normal HDL.

When we calculated* lipoprotein partial specific volumes,[40] however, both LDL and HDL showed a tendency toward denser particles. In LDL, partial specific volume fell from 0.947 ml/g to 0.937 ml/g, moving from the <250 mg/dl plasma triglyceride group to the 251–1000 mg/dl group. In the severe hypertriglyceridemic group (>1000 mg/dl), LDL partial specific volume was 0.944 ml/g. Similarly in HDL, partial specific volumes decreased from 0.853 to 0.846 ml/g between the lowest and highest plasma triglyceride level groups, respectively. We believe these differences would be greater if a more homogeneous group of hypertriglyceridemic subjects were studied. The group with plasma triglyceride levels over 1000 mg/dl, for example, contained mainly patients with severe Type I-like hyperlipoproteinemia; one patient had a deficiency of lipoprotein lipase activator, apoprotein C-II, while others were children in whom classification of their severe primary hypertriglyceridemia was difficult because of a heterogeneous pattern of lipase deficiencies.[41] Indeed, in an ongoing evaluation of a homogeneous group of normal and hypertriglyceridemic subjects (none with lipase deficiencies) before and after treatment with lipid lowering agents, both LDL and HDL were clearly smaller and denser with increasing triglyceridemia (S. Eisenberg, Y. Oschry, R. Deckelbaum, and D. Gavish, unpublished data). The effects of deficiencies of different lipases concomitant with hypertriglyceridemia, on lipoprotein structure-composition remains to be determined.

Do the lipoprotein compositional changes that occur with increasing plasma triglyceride have metabolic consequences? Most likely, yes. Accelerated catabolism of LDL protein (apoprotein B) has been described in patients with hypertriglyceridemia.[42] This may reflect the low cholesterol content of such LDL with diminished down-regulation of the LDL receptor catabolic pathway[43] due to the delivery of smaller amounts of cholesterol into cells per LDL particle. Indeed, using cultured fibroblasts, recent data[44] show less down-regulation of cellular cholesterol synthesis by triglyceride-enriched LDL modified in vitro. We conclude, therefore, that not only do plasma triglyceride levels determine the structure-composition relationships in LDL and HDL, but probably also affect their biologic behavior.

*LDL and HDL partial specific volumes were calculated by summing the partial specific volume contribution of individual particle constituents where the partial specific volumes were as follows: protein, 0.705 ml/g; phospholipid, 0.970 ml/g; free cholesterol, 0.968 ml/g; cholesterol ester, 1.044 ml/g; and triglyceride, 1.093 ml/g.

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Index Terms: low density lipoprotein • high density lipoprotein • plasma triglyceride • hyperlipidemia