The $\beta$-Lipoprotein Doublet in Type 3 Hyperlipoproteinemia

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We used a previously reported agarose-gel electrophoresis system to study the lipoprotein patterns in patients with type 3 hyperlipoproteinemia. All samples from 20 subjects tested revealed the presence of two bands with beta-lipoprotein mobility, irrespective of quantitative changes in plasma lipids induced by diet or by the administration of drugs. If these observations are confirmed, the method will permit the diagnosis of type 3 hyperlipoproteinemia without resort to preparative ultracentrifugation and chemical measurement of the very-low-density lipoproteins.

Although familial type 3 hyperlipoproteinemia (HLP) is a relatively rare disorder, even among subjects with combined increases in plasma cholesterol and triglycerides, its diagnosis is important because of the inordinate incidence of premature cardiovascular disease associated with this disorder and the relative ease with which it is managed.

The diagnosis of type 3 HLP or "broad-$\beta$" disease originally rested on the demonstration of very-low-density lipoproteins (VLDL) of $\beta$ mobility rather than pre-$\beta$ mobility on zonal electrophoresis (3, 4). Hazzard et al. (5) suggested that cholesterol enrichment of the VLDL more accurately reflected the lipoprotein abnormality in type 3 HLP, and Fredrickson et al. (6) have recently concluded that abnormalities of lipoprotein composition rather than electrophoretic mobility permit better segregation of type 3 HLP. Both of these diagnostic approaches involve preparative ultracentrifugation, an expensive and time-consuming technique.

Masket et al. (7) suggested a test for which a preparative ultracentrifuge is not needed. The method involves the dual electrophoresis of plasma in different supporting media. The diagnosis of type 3 HLP depends on the absence of $\beta$-migrating lipoproteins on polyacrylamide gel electrophoretic separations and their presence on paper, agarose, or cellulose acetate electrophoresis patterns. The method has two disadvantages: the polyacrylamide gel system is complex and not suited to routine application, and "false positives" may occur in other forms of hypertriglyceridemia associated with subnormal concentrations of low-density lipoproteins (LDL) in plasma.

We report here a lipoprotein electrophoresis pattern not previously recognized in type 3 HLP. It may permit the diagnosis without reliance on preparative ultracentrifugation.

Materials and Methods

Plasma and Serum Samples

Blood was sampled (disodium ethylenediaminetetraacetate, 1 mg/ml, anticoagulant) after 12- to 14-h fast, and the lipoprotein pattern was classified by analytical techniques previously described (4). Serum and plasma samples were stored at 4 °C until analyzed. All patients with type 3 HLP had a VLDL-cholesterol/plasma triglyceride ratio of 0.3 or greater (6).

Electrophoresis on Agarose-Gel

The agarose-gel electrophoresis system we used is described elsewhere (8, 9). It incorporates a number of unique features: (a) a low concentration of pure agarose (5 g/liter), which permits relatively free migration and clear separation of the major classes of serum lipoproteins; (b) use of a buffer of low ionic strength and cooling with petroleum ether allow high voltage to be applied and decrease electrophoresis time to 12 min; and (c) 3 μl of serum is loaded into a narrow slit, which results in sharp lipoprotein bands being produced. The usual lipoprotein fractions are clearly resolved and subfractions are better separated.

Results

We studied plasma from 20 subjects with type 3 HLP on one or more occasions. The clinical, biochemical, and genetic features of these patients and others whose cases
have been followed in the Molecular Disease Branch clinic are detailed elsewhere (10). The unique feature demonstrated in the serum electrophoretic patterns of all of them was that two distinct, although closely migrating, bands of β-lipoprotein mobility were present (Figure 1). The two patterns frequently encountered in controls with normal plasma cholesterol and triglyceride concentrations are presented for comparison. One of these (cholesterol, 1.95 g/liter; triglyceride, 1.10 g/liter) has a β band, a single, rapidly migrating pre-β band, and two lipoprotein bands of α-mobility (Figure 1A). The other (cholesterol, 2.52 g/liter; triglyceride, 1.05 g/liter) demonstrates a β band, both slow and rapidly migrating pre-β bands, and two α bands. The patient with type 3 HLP was on therapy with clofibrate and restricted diet and had a cholesterol concentration of 2.22 g/liter and a triglyceride of 0.92 g/liter. On electrophoresis her serum showed two β, two pre-β, and two α bands, although the second α band was relatively weak. The more rapidly migrating of the two β bands was clearly less mobile than the slower pre-β band.

To determine whether one or both of the β bands seen on agarose electrophoresis were the LDL (1.006 < d < 1.063 g/ml), we centrifuged plasma from another subject with type 3 HLP at the relative density of plasma and separated the VLDL by tube slicing. The whole plasma (Figure 2A) contained two β, one pre-β, and two α bands. The VLDL fraction (d < 1.006 g/ml) contained one of the two β bands (the one with the greater mobility) and one pre-β band (Figure 2C). The 1.006 g/ml infranatant fraction contained the α bands and a single band of β mobility (Figure 2B). Therefore, the β band of greater mobility corresponds by definition to a VLDL subfraction and the slower migrating β band to an LDL.

There is considerable variation among patients and even in individuals with type 3 HLP when they are repeatedly tested. Typical electrophoretograms from four additional subjects illustrate the range of findings (Figure 3). A subject with type IV HLP had a plasma triglyceride concentration of 3.42 g/liter and a cholesterol concentration of 2.94 g/liter (Figure 3A). The β band is single. The intensity of the rapidly migrating pre-β band is much greater than that of the slower pre-β band and both components of the α-doublet are light. One of the patients with type 3 HLP (Figure 3B) had a plasma triglyceride concentration of 1.71 g/liter and a cholesterol concentration of 2.42 g/liter. The two bands of β mobility are of about equal intensity and the single pre-β band is not intense (Figure 3B). The slower β band in the second patient’s plasma (Figure 3C) is present, but very faint, while the β band of greater mobility is intensely stained. Two heavily stained pre-β bands are also visible in the pattern for this plasma, with triglyceride concentration equal to 7.77 g/liter and cholesterol concentration equal to 3.85 g/liter. The fourth pattern (Figure 3D) is of theoretical interest. The plasma is from a normolipidemic 14-year-old boy (triglyceride, 1.64 g/liter; cholesterol, 1.21 g/liter) whose father suffered a myocardial infarction at age 38. Two bands of β mobility are clearly demonstrated in the child’s plasma in addition to two pre-β and two α bands. This pattern is similar to the type 3 HLP pattern illustrated in Figure 1.

Figure 4 illustrates the effect of dietary perturbations on the serum lipoprotein patterns in a subject with type 3 HLP. Serum was analyzed when the patient was consuming an unrestricted diet (Figure 4A), after seven days on an isocaloric high-carbohydrate diet containing less than 5 g of fat per day (Figure 4B; cholesterol, 4.30...
g/liter; triglyceride, 10 g/liter), after caloric restriction (800 kcal/day) for seven days (Figure 4C; cholesterol, 2.92 g/liter; triglyceride, 2.00 g/liter), and after caloric restriction for 21 days had decreased the plasma triglyceride concentration to normal (Figure 4D; cholesterol, 1.88 g/liter; triglyceride, 1.57 g/liter). These patterns illustrate that the electrophoretic diagnosis of type 3 HLP can be made over a wide range of cholesterol and triglyceride concentrations. However, the relative intensity of all lipoprotein bands can be expected to change with diet or drug effects on lipoprotein metabolism. The larger VLDL are likely precursors of smaller VLDL, including that VLDL of β mobility (11). Moreover, the LDL appear to be catabolic products of VLDL (12, 13), and factors influencing the catabolic sequence from VLDL to LDL predictably will influence the lipoprotein electrophoresis pattern.

Discussion

The technique of lipoprotein electrophoresis we used (8, 9) has made it possible for us to identify two species of lipoproteins of β mobility in the plasma of every patient with type 3 HLP that we have examined, irrespective of diet or drugs prescribed. The relative intensity of the two β bands varies inter- and intra-individually, particularly as plasma lipid concentrations change.

Preparative ultracentrifugation demonstrated that one of the two lipoproteins of β mobility was associated with the VLDL, and undoubtedly this is the “β VLDL” that long has been considered pathognomonic of type 3 HLP. In almost all subjects this was the more intensely stained of the two β bands until plasma lipid concentrations had been decreased to near normal.

The β lipoproteins of slower mobility probably correspond to true LDL. They sediment with the plasma proteins of relative density greater than 1.006 g/ml and their mobility is identical to that of normal β lipoproteins. This electrophoretic band was frequently quite light, reflecting the long-recognized relative or absolute deficiency of Sφ0 0–12 lipoproteins in type 3 HLP (14–16). The paucity of normal LDL was in fact the basis for the electrophoretic test of Masket et al. (7).

Increased plasma concentrations of intermediate density lipoproteins (Sφ0 12–20) are often present in type 3 HLP. These lipoproteins reportedly (17) have mobility intermediate between β and pre-β lipoproteins on agarose-gel electrophoresis. It is not known if they generate discrete bands on electrophoresis of uncentrated whole plasma. We did not investigate this lipoprotein fraction.

A word of caution is appropriate. The technique of lipoprotein electrophoresis used here (8, 9) requires careful attention to the preparation of agarose-coated microscopic slides, buffer concentration, application of sample, and the time and temperature of electrophoresis. Moreover, preliminary results suggest that aging

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**Fig. 3.** Serum lipoprotein electrophoretic patterns of a patient with type 4 hyperlipoproteinemia (A), two patients with type 3 hyperlipoproteinemia (B and C), and normolipidemic child who demonstrates the β-doublet (D).

**Fig. 4.** Effects of experimental diets on plasma lipoprotein electrophoretic patterns in a patient with type 3 hyperlipoproteinemia.
A, unrestricted diet; B, after seven days of a high-carbohydrate diet; C, after seven days on an 800 kcal per day diet; and D, after 21 days of caloric restriction.
of samples may greatly decrease the resolution of the lipoprotein zones. The separation between the two \( \beta \) bands is not great, even under optimal conditions. The production of a simple "broad \( \beta \) band" with ill-defined boundaries may result from sample aging, overloading, or excessive heating during electrophoresis. The number of lipoprotein subfractions demonstrated is clearly greater than that usually observed when agarose electrophoresis is used to diagnose type 3 HLP (17–20). If attention to technical considerations is overlooked, much of the diagnostic power of this technique is forfeited.

Demonstration of the specificity of the \( \beta \)-doublet for type 3 HLP will require analysis of hundreds of normal and hyperlipidemic samples using a single blinded approach. We have already analyzed 22 samples from subjects with hypertriglyceridemia who did not meet the chemical criteria (6) for type 3 HLP. None of these, including several patients with type 5 HLP and "\( \beta \)-VLDL" on paper electrophoresis (6), demonstrated the \( \beta \)-doublet on agarose-gel electrophoresis.

References

2. Papadopoulos, N. M., Type III hyperlipoproteinemia can be diagnosed by electrophoresis. Circulation 54, II-152 (1976).