Two Methods Compared for Measuring Lipase Activity in Plasma after Heparin Administration

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We compared two methods for the direct selective measurement of hepatic lipase and lipoprotein lipase activities in human plasma after intravenous administration of heparin. Except for the emulsifier (gum arabic vs lecithin), the two assay media for hepatic lipase are essentially similar. Results for hepatic lipase by these two assays correlate well (r = 0.99). The assays for lipoprotein lipase in the two procedures differ in the way that hepatic lipase activity is eliminated (immunological inhibition vs a specific substrate emulsion), and also with regard to the emulsifier. The substrate emulsion stabilized by gum arabic (immunological assay) consistently yielded about three times higher enzymic activity than the specific substrate stabilized by lecithin. Experiments in which purified enzymes were used demonstrated that this systematic difference can be accounted for by the different emulsifiers. The satisfactory correlation (r = 0.92) between the two lipoprotein lipase assays, however, demonstrates that they measure the same enzymic activity.

Additional Keyphrases: lipoprotein lipase • hepatic lipase

"postheparin" plasma

Intravenous injection of heparin rapidly releases into the blood stream several enzymes with catalytic activity on tri-, di-, and monoacylglycerols as well as phospholipids. Two different such "postheparin" lipase activities have been identified and purified: lipoprotein lipase (LPL, EC 3.1.1.34), which is characterized by its sensitivity to 1 mol/L NaCl and its need for apolipoprotein CII for optimal catalytic activity (1, 2), and hepatic lipase (HL), which is not inhibited by high salt concentrations and requires no serum cofactors for optimal activity (2, 3). LPL catalyzes the initial rate-limiting step in the degradation of triacylglycerol-rich lipoproteins (4), but the role of HL is under debate. Numerous experimental and clinical studies have been conducted to delineate a function of HL in lipoprotein metabolism and to evaluate the role of the two enzyme activities in relation to hyperlipoproteinemia, atherosclerosis, and metabolic disturbances such as obesity and diabetes. Evaluation and comparison of results of such studies is often difficult, because different assay systems are used (5–9).

Assays of lipoprotein lipase and hepatic lipase activity in plasma present problems because of the overlapping substrate specificity of the two enzymes. Triacylglycerol emulsions are generally used as substrate in both assays. Krauss et al. (5) exploited the inhibition of LPL by protamine to obtain a specific assay system for the HL component of postheparin lipolytic activity; the LPL component was calculated by subtracting HL activity from total postheparin lipolytic activity. Another assay (6) is based on chromatographic separation of the enzyme activities before assay. We have described two rapid, simple assay systems for direct selective individual measurement of the two postheparin lipase activities. In one, which will be referred to here as the "Helsinki method" (7), an antisera is used for immunological inhibition of the HL component of postheparin lipolytic activity. The other, referred to here as the "Lund method" (8), relies upon a substrate preparation that, due to the specific sonication procedure, is readily attacked by LPL but not by HL. Here, we demonstrate a satisfactory correlation between values for HL and LPL obtained by the two different assay systems.

Materials and Methods

Subjects. Healthy men and women laboratory workers, all with normal plasma cholesterol and triacylglycerol concentrations (less than 5.5 and 1.5 mmol/L, respectively) served as donors of postheparin plasma. Samples also were obtained from 10 patients from the outpatient clinic of the Department of Internal Medicine, five of whom had slightly supranormal plasma triacylglycerol concentrations (2.1–4.0 mmol/L) but normal plasma cholesterol concentrations. All subjects had fasted for at least 12 h before the blood was sampled.

Postheparin plasma. "Postheparin plasma" was plasma separated from blood drawn 15 min after intravenous administration of 100 USP units of heparin (AB Vitrum, Stockholm, Sweden) per kilogram of body weight. Duplicate samples were drawn in evacuated blood-collection tubes containing either disodium EDTA or heparin as anticoagulant. The final heparin concentration in the heparin-containing tubes was 20–30 kilounits per liter of blood. The tubes were immediately placed on ice. Plasma, separated by centrifugation at 4 °C, was stored at −20 °C.

Assays for lipase activities. In each method LPL activity and HL activity are measured separately.

In the Helsinki method (7) sonicated tri[3H]acylglycerol emulsions, stabilized with gum arabic, are used. HL activity is separately measured by incubating postheparin plasma in 1 mol/L sodium chloride at pH 8.4 in the absence of serum, conditions under which LPL is inhibited. LPL activity is selectively assayed in 0.1 mol/L sodium chloride at pH 8.4, in the presence of normal human serum and an antiserum to hepatic lipase. In both the LPL and HL assay, bovine serum albumin is present to bind fatty acid released during the incubations.

In the Lund method (8) tri[3H]acylglycerol emulsions stabilized with lecithin are used. HL activity is assayed at pH 9.0, in 1 mol/L sodium chloride and in the absence of serum. In this assay, albumin is added before the emulsion is sonicated. In the LPL assay, albumin is added after sonication of the lipids; this procedure gives a substrate that cannot be attacked by HL, and therefore provides a specific assay system for LPL. This assay is performed in 0.15 mol/L sodium chloride, in the presence of serum and at pH 8.4.

The assay systems compare well with regard to reproducibility, sensitivity, and simplicity, and except for the anti-HL serum used in the Helsinki method, all reagents cur-

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Received June 9, 1980; accepted May 22, 1984.
rently are commercially available. Substrate emulsions must be sonicated daily. The assay procedures include incubation of diluted postheparin plasma and substrate after addition of either sodium chloride (in one procedure) or anti-HL (in the other). Both assays involve a rapid one-step liquid–liquid partition system (9) for isolating the radiolabeled fatty acid released. An assay takes less than 1 h, and 100 incubations can easily be performed in 2 to 3 h. Both assay systems have an interassay imprecision (CV) of 5 to 8% and a sensitivity that allows adequate quantitation of enzymic activity in 2 to 5 uL of postheparin plasma.

Results

Effects of heparin in vitro. Postheparin plasma from blood samples drawn in tubes containing heparin as anticoagulant gave distinctly lower values for LPL activity when measured with the Lund method. The LPL activity in tubes containing heparin was only about 40% (range 21–73%) of that measured in samples drawn in EDTA-containing tubes. Inhibition of this assay system by heparin could also be demonstrated by adding heparin (30 or 60 kilounits/L) to postheparin plasma samples drawn in EDTA-containing tubes, which resulted in an inhibition of about 60% of the enzymic activity.

In contrast, LPL activity as measured by the Helsinki method was not significantly different for samples drawn in tubes containing EDTA or heparin.

The activity of HL as measured by either assay system was not affected by the presence of heparin.

Comparison of assay methods. HL activity as measured by the two methods correlated well (Figure 1). For the 20 samples the correlation coefficient was 0.99, the slope of the regression line was close to 1, and the intercept negligible, demonstrating a close identity of the two activities measured.

The two assay systems for LPL activity (Figure 2) also showed a satisfactory correlation (correlation coefficient, 0.92), but the variability was distinctly greater than for assays of hepatic lipase, and activities as measured by the Helsinki method were about threefold those recorded with the Lund method.

LPL and HL activities as determined with either assay system were not correlated.

Exchange of assay components. To elucidate the reason for the systematic difference between the two assays for LPL activity, we interchanged the emulsifiers (gum arabic and lecithin). The modified substrates were incubated with postheparin plasma as well as with semipurified enzymes separated from postheparin plasma by affinity chromatography on heparin–Sepharose (10).

Substitution of lecithin for gum arabic in the Lund assay resulted in a loss of specificity. About 50–60% of HL activity (measured under optimal conditions) was then expressed in the LPL assay. Under these conditions, the enzyme activity measured in the semipurified LPL preparation was two- to 2.5-fold that obtained with the standard Lund method, indicating that the different emulsifiers account for the systematic difference between the two methods for LPL measurement.

Discussion

Postheparin plasma contains two separate enzymes with catalytic activity against long-chain triacylglycerols, LPL and HL. Their individual determination relies on selective inhibition of one of the lipases.

Selective assay of HL activity is easy, because LPL activity can be eliminated by omitting the serum cofactor (apolipoprotein CII) for the enzyme system and by performing the assay in 1 mol/L sodium chloride or in the presence of protamine. High salt concentrations seem to be the more effective means of excluding LPL activity, because protamine may activate HL to varying extents (10). The two methods compared in this study both involve inhibition of LPL by NaCl. Despite the differences in substrate concentration and the choice of emulsifier, results of the two methods correlated very well.

Direct selective measurement of LPL activity in postheparin plasma is more complicated, because no chemical inhibitors of hepatic lipase have been reported. In earlier assay systems LPL activity either was measured indirectly, as the difference between total postheparin lipolytic activity and HL activity (5)—unsatisfactory because of the larger methodological error introduced—or the two enzyme activities were assayed after chromatographic separation (6), which is laborious. One of the two methods compared in the present study, the Helsinki method, involves an antiserum against HL, whereas the Lund method takes advantage of the fact that LPL, but not HL, readily attacks a triacylgly-
cerol-phospholipid emulsion sonicated in the absence of albumin. The lack of activity of HL against such substrates is not understood, but may be related to the surface properties of the substrate emulsion particles (8). This interpretation is consistent with the results we obtained after we exchanged emulsifiers, demonstrating that the specificity of the Lund assay for LPL depends on the lecithin component of the substrate. Despite the different principles used in eliminating HL activity, LPL activity as measured by the two techniques correlated satisfactorily. However, the Helsinki method consistently yielded higher enzymic activities, on the average about threefold. As demonstrated by exchange of emulsifier between the assay systems, this difference is also probably due to the different emulsifiers used (lecithin and gum arabic). The different characteristics of the two substrate preparations are also illustrated by the different sensitivity to inhibition by heparin.

An emulsifier must be present in the substrates to provide a reasonably stable substrate emulsion, and various ones have been used. Phospholipid is one of the surface components of very-low-density lipoproteins and chylomicrons, and a rationale for using lecithin-stabilized emulsions is that they most closely resemble these physiological substrates. However, both of the enzymic activities assayed have catalytic activity against phospholipid, and this emulsifier thus may compete with the triacylglycerol substrate. This problem is eliminated in assay systems in which gum arabic is used as emulsifier. On the other hand, such substrates are more clearly artificial.

From the practical point of view, the systematic difference between the LPL activities as measured by the two techniques seems to be of little importance. The sensitivity of both assays is sufficient to quantify enzymic activity in 2-5 μL of postheparin plasma. When one considers the complexity of lipase assays, the correlation obtained in the present study seems sufficient to warrant the conclusion that the two methods measure the same enzymic activity.

Ms. Gerd Nilsson gave excellent technical assistance. Financial support was obtained from the Swedish Medical Research Council (04966), the Medical Faculty, University of Lund, and the Sigrid Juselius Foundation.

References