Effects of heparin infusion on plasma lipoproteins in subjects with lipoprotein lipase deficiency

Evidence for a role of hepatic endothelial lipase in the metabolism of high-density lipoprotein subfractions in man

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The role of heparin-releasable hepatic endothelial lipase (HL) in human plasma lipoprotein metabolism was investigated by examining the effects of intravenous infusion of heparin (180 units/kg over 2 h) in 8 subjects with primary extrahepatic lipoprotein lipase deficiency. In addition to reducing the triglyceride concentration in very low-density lipoproteins, heparin-induced release of HL reduced the phopholipid and protein concentrations in the HDL_2 subclass of high-density lipoprotein (by 28% and 36% respectively, mean values) and simultaneously increased the HDL_3 phospholipid concentration (by 23%), providing the first in vivo evidence for a function of HL in the interconversion of the major HDL subfractions in man.

Lipoproteins *Hepatic lipase* *Heparin* *Hyperlipidaemia* *Lipoprotein lipase* *High-density lipoprotein*

1. INTRODUCTION

Chylomicrons and very low-density lipoproteins (VLDL) are catabolised through a cascade from large to small particles by mechanisms involving the heparin-releasable enzyme, lipoprotein lipase (LPL), present largely in adipose tissue and skeletal muscle [1]. The enzyme requires a specific co-factor, apoprotein (apo) C-II [2], and is inhibited by protamine sulphate and 1.0 M NaCl. The fractional rate of catabolism of VLDL in man is closely correlated with the activity of LPL in adipose tissue [3], and familial deficiency of the enzyme [4], or of apoC-II [5] produces severe hypertriglyceridaemia on an uncontrolled diet. In vitro, incubation of VLDL with LPL in the presence of the HDL_3 subfraction of high-density lipoprotein (HDL) results in the appearance of particles of similar density and size to the HDL_2 subclass [6], due apparently to the transfer of surface cholesterol, phospholipid and C apoproteins to HDL_3. Evidence from studies in vivo also suggests that the lipolysis of VLDL and chylomicrons results in the transfer of surface material to HDL [5].

Less is known about the functions of hepatic endothelial lipase (HL). It is also released into the circulation by intravenous (i.v.) heparin, but does not require apoC-II and is not inhibited by 1.0 M NaCl [1]. Two functions have been suggested for the enzyme:

(1) It has been implicated in the catabolism of the 'remnants' of chylomicrons and VLDL. This is supported by the accumulation of remnants in rats after hepatectomy [7] and after the injection of an antibody to HL [8]. Furthermore, the triglyceride of VLDL remnants has been shown to be a better substrate for the enzyme in vitro than the triglyceride of chylomicrons or VLDL [9]. On the other hand, Reardon et al. [10] found that the fractional catabolic rate of VLDL remnants in man was unrelated to the activity of HL in post-heparin plasma.

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(2) The catabolism of HDL_2. Oxandrolone, an anabolic steroid, has been shown to elevate post-heparin plasma HL activity and to simultaneously decrease the concentration of HDL_2 in man [11]. Females have lower HL activity and higher HDL_2 concentrations than men [12], and negative correlations between HL activity in post-heparin plasma and the concentrations of HDL_2 lipid and protein components have been reported in healthy men [13]. In addition, there have been recent reports that injection of antibody to HL into rats increases the HDL_2 phospholipid concentration [14–16].

In order to clarify the role of HL in man, we have studied the changes in plasma lipoproteins which are associated with the release of the enzyme into plasma during a 2 h intravenous infusion of heparin in subjects with documented primary LPL deficiency. The results have provided evidence for a role of HL both in the catabolism of VLDL and in the interconversion of HDL_2 to HDL_3.

2. MATERIALS AND METHODS

2.1. *Subjects*

Eight adults with primary LPL deficiency were studied. In each subject absence of the enzyme from needle biopsies of gluteal adipose tissue (heparin-elutable) and/or from post-heparin plasma (60 units heparin/kg) had been demonstrated as in [5]. At the time of referal to the Lipid Clinic of St. Thomas' Hospital, plasma triglyceride concentrations were 7.3–37.2 mmol/l. Five normolipidaemic healthy subjects, all with normal LPL activity in post-heparin plasma, were also studied.

2.2. *Clinical procedures*

Heparin infusions were carried out in a metabolic ward, the subjects having fasted for 14 h overnight. Each LPL-deficient subject had consumed a low-fat diet (< 5 g fat/day) for 2–4 days before admission to the ward, and all had been following a diet containing < 20 g fat/day for several weeks prior to admission. At the time of the study 5 patients (no. 1–5) were without fasting chylomicronaemia; in the remaining 3 patients (no. 6–8) chylomicrons were still present in plasma at the start of the heparin infusion.

After obtaining a baseline venous blood sample (20 ml; anticoagulated with 1 mg Na_2 EDTA/ml), a bolus i.v. injection of heparin, 60 units/kg, was given. A constant i.v. infusion of heparin was then commenced at the rate of 60 units $.kg^{-1}.h^{-1}$ [5]. Blood samples were collected into ice-cold glass tubes 10, 30, 60, 90 and 120 min after the bolus injection. They were promptly centrifuged at 0–4°C, and the plasma was stored at the same temperature until analysis (commenced the same day).

2.3. *Laboratory procedures*

After the removal of any chylomicrons from the plasma samples by ultracentrifugation [17], VLDL ($d \times 1.006$ g/ml), intermediate density lipoprotein (IDL; $d = 1.006–1.019$ g/ml), low-density lipoprotein (LDL; $d = 1.019–1.063$ g/ml) and HDL subclasses 2 and 3 (HDL_2, $d = 1.063–1.125$ g/ml; HDL_3, $d = 1.125–1.210$ g/ml) were isolated by further sequential preparative ultracentrifugation at 40000 rev./min and 4°C in an MSE Prepspin 50 ultracentrifuge, using a rotor with geometry similar to the Beckman 40.3 rotor [17]. Centrifugation times were 16 h, 20 h, 24 h and 40 h for flotation of VLDL, IDL, LDL, HDL_2 and HDL_3, respectively.

Total cholesterol, glyceride and phospholipid in each lipoprotein fraction were measured manually by enzymatic procedures (Boehringer Mannheim, cat. no. 187313 and 16448; Wako Phospholipid kit, Wako Chemistry, Osaka). Total protein in each lipoprotein (except HDL_3) was measured as in [18], using bovine serum albumin as standard. All analyses for each subject were carried out as one batch. Results were expressed as the mean values of triplicate determinations.

Activities of LPL in post-heparin plasma were measured as in [19], using as substrate Intralipid into which glycerol tri-(3)oleate (Radiochemical Centre, Amersham) had been incorporated by the manufacturers (Vitrum, Sweden).

2.4. *Statistical analyses*

As the lipoprotein responses to heparin in the 5 LPL-deficient subjects who were without chylomicronaemia at the start of the infusion appeared to differ in some respects from those observed in the 3 patients in whom chylomicronaemia was still present, the changes observed in these two sub-groups were considered separately.

Statistical analyses of significance were by the paired Student *t*-test and the Wilcoxon matched-pairs signed rank test [20].

3. RESULTS

As anticipated, the LPL-deficient subjects had negligible post-heparin plasma LPL activity during the infusions (not shown).

As expected, VLDL triglyceride concentration decreased in the normal subjects during the infusion. VLDL triglyceride also decreased in the LPL-deficient subjects ($P<0.005$ at 120 min), although the percentage reduction was less than that in the normal subjects (table 1). The VLDL phospholipid and protein concentrations showed no consistent changes in any group.

In the normal subjects heparin also decreased the plasma concentrations of IDL phospholipid and protein ($P<0.05$ at 60 min) (table 2). The LPL-deficient subjects without chylomicronaemia showed similar trends, whereas those with chylomicronaemia showed increases in IDL phospholipid and IDL protein levels during the infusion. In neither group of LPL-deficient subjects, however, were these changes statistically significant. The

Table 1

Effects of intravenous infusion of heparin on plasma VLDL triglyceride concentration (mmol/l) in 5 normal subjects, 5 LPL-deficient patients without chylomicronaemia at the time of study (no. 1–5) and 3 LPL-deficient patients with chylomicronaemia (no. 6–8)

Time (min)	0	30	60	90	120[a]
Normal subjects	0.83 ± 0.2	0.31 ± 0.1	0.37 ± 0.1	0.32 ± 0.1	0.24 ± 0.1 (−71%)
LPL-deficiency no. 1–5	5.35 ± 0.8	4.45 ± 0.7	4.95 ± 1.3	4.16 ± 0.8	3.17 ± 0.7 (−41%)
LPL-deficiency no. 6–8	9.75 ± 1.5	8.29 ± 1.3	7.83 ± 1.50	6.73 ± 2.2	6.94 ± 2.5 (−29%)

[a] Results at 120 min were significantly different ($P < 0.005$) from those at time zero in all 3 groups (Wilcoxon test)

Mean ± SEM; Mean % changes relative to time zero are given in parentheses

Table 2

Effects of intravenous infusion of heparin on plasma IDL phospholipid and protein concentrations in 5 normal subjects, 5 LPL-deficient subjects without chylomicronaemia at the time of study (no. 1–5) and 3 LPL-deficient subjects with chylomicronaemia (no. 6–8)

Time	0	30	60	90	120
Phospholipid (mg/dl)					
Normal subjects	3.4 ± 1.0	3.8 ± 1	1.0 ± 1[a] (−71%)	1.2 ± 1	1.0 ± 0.5
LPL-deficiency no. 1–5	21 ± 6	18 ± 6	19 ± 7	18 ± 7	18 ± 7
LPL-deficiency no. 6–8	16 ± 8	26 ± 18	42 ± 36	34 ± 28	32 ± 25
Protein (μg/ml)					
Normal subjects	95.6 ± 12	35.4 ± 8	22.6 ± 6[a] (−76%)	31.2 ± 4	52.4 ± 15
LPL-deficiency no. 1–5	114.8 ± 29	82.4 ± 23	99.0 ± 32	111.6 ± 30	95.2 ± 32
LPL-deficiency no. 6–8	84.7 ± 35	109.7 ± 28	131.0 ± 44	130.3 ± 45	124.0 ± 72

[a] $P < 0.05$ relative to time zero (Wilcoxon test)

Mean ± SEM mean % changes relative to time zero are given in parentheses

Table 3

Effects of intravenous infusion of heparin on plasma HDL_2 phospholipid, HDL_2 protein and HDL_3 phospholipid in 5 normal subjects, 5 LPL-deficient subjects without chylomicronaemia at the time of study (no. 1–5) and 3 LPL-deficient subjects with chylomicronaemia (no. 6–8)

Time (min)	0	30	60	90	120
HDL_2 Phospholipid (mg/dl)					
Normal subjects	25.7 ± 4	25.6 ± 3	25.7 ± 3	24.7 ± 3	23.4 ± 3
LPL-deficiency no. 1–5	42.2 ± 11	36.4 ± 12	35.8 ± 9[a] (−15%)	32.4 ± 10	33.0 ± 10[a] (−22%)
LPL-deficiency no. 6–8	47.3 ± 14.3	37.0 ± 13	41.8 ± 13[a] (−12%)	38.8 ± 14	29.7 ± 8[a] (−37%)
HDL_2 Protein (μg/ml)					
Normal subjects	294 ± 33	271 ± 29	295 ± 41	262 ± 26	269 ± 31
LPL-deficiency no. 1–5	374 ± 35	269 ± 28	273 ± 42[a] (−27%)	260 ± 17	235 ± 8[b] (−37%)
LPL-deficiency no. 6–8	215 ± 47	188 ± 39	174 ± 43[a] (−19%)	169 ± 43	145 ± 16[b] (−33%)
HDL_3 Phospholipid (mg/dl)					
Normal subjects	47.1 ± 5	46.5 ± 4	47.3 ± 4	45.3 ± 3	46.3 ± 3
LPL-deficiency no. 1–5	39.1 ± 4	44.5 ± 7	45.4 ± 6	42.3 ± 8	48.0 ± 7[a] (+23%)
LPL-deficiency no. 6–8	40.0 ± 8	42.0 ± 10	43.3 ± 10	47.7 ± 8	49.3 ± 11[a] (+23%)

[a] $P < 0.05$; [b] $P < 0.01$ relative to time zero (Wilcoxon test)

Mean + SEM; Mean % changes relative to values at time zero are given in parentheses

IDL cholesterol and tryglyceride levels did not change consistently in any group; nor were there any significant changes in LDL components.

The most consistent and striking changes were observed in HDL_2 and HDL_3 (table 3). In both sub-groups of LPL-deficient patients, the concentrations of HDL_2 phospholipid and HDL_2 protein, but not of HDL_2 cholesterol, decreased significantly during the infusion ($P < 0.01$ and $P < 0.05$ at 60 min, respectively). In the same subjects significant increases in HDL_3 phospholipid were observed ($P < 0.05$ at 120 min). No such changes were recorded in the HDL subfractions of normal subjects; nor did HDL_2 or HDL_3 cholesterol change significantly in this group.

4. DISCUSSION

This study is based on the assumptions that the lipoprotein changes which occur in subjects with complete deficiency of LPL during an infusion of heparin are due to release of HL into the circulation, and that those which occur in healthy subjects reflect effects of HL and LPL acting simultaneously. The decrease in VLDL triglyceride concentration which was observed during the infusions in the LPL-deficient subjects supported evidence from studies in vitro and in vivo [9] that HL can hydrolyse the triglyceride of VLDL, although less efficiently than LPL. The decreases in the phospholipid and protein concentrations in IDL observed in normal subjects could have reflected the activities of either or both of the enzymes. The similar trend which was observed in the LPL-deficient subjects without chylomicronaemia conforms with the view [7,8] that HL may play a role in the hepatic catabolism of IDL, but as these changes were not statistically significant no confident conclusions about IDL metabolism can be drawn from the present experiments. The increases

in IDL components observed during the first hour of infusion in the LPL-deficient subjects with chylomicronaemia may have reflected the catabolism of the large pool of triglyceride-rich precursors of IDL in this sub-group.

The most consistent findings in LPL-deficient subjects were the changes in HDL subfractions. In all patients, both those with, and those without chylomicronaemia, decreases in HDL_2 phospholipid and HDL_2 protein occurred during the infusion. Over the same period the HDL_3 phospholipid concentration showed a reciprocal increase in these subjects. In contrast, normal subjects showed no significant changes in HDL-subfractions. These results are consistent with a role for hepatic lipase in the catabolism of HDL_2 or in the conversion of HDL_2 to HDL_3 [14–16,21]. Since there is evidence that the hydrolysis of VLDL triglyceride by LPL may contribute to the conversion of HDL_3 to HDL_2 [6], the absence of any change in HDL_2 or HDL_3 in the normal subjects during heparin infusion can be attributed to the opposing effects of the two heparin-releasable enzymes on the interconversion of HDL subclasses. The absence of any significant changes in HDL_2 and HDL_3 cholesterol in the LPL-deficient subjects, in spite of the changes in other HDL components, might have reflected a redistribution of cholesteryl ester between the subfractions and other lipoproteins [22].

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