Infusion of atherogenic lipoprotein particles increases hepatic lipase activity in the rabbit

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Abstract Hepatic lipase plays a key role in the turnover of potentially atherogenic lipoprotein remnants and in determining the relative distribution of high density lipoprotein (HDL) particle size subclasses. Rabbits fed a cholesterol-enriched diet have been found to accumulate potentially atherogenic chylomicron remnants and β-very low density lipoprotein (β-VLDL) and show a rapid increase in liver and postheparin plasma hepatic lipase activity. To determine whether the particles that accumulate during cholesterol feeding are a stimulus for this increase in hepatic lipase activity, we infused normal chow-fed rabbits with a chylomicron remnant plus β-VLDL-enriched plasma fraction isolated from rabbits fed 0.5% cholesterol-supplemented chow. The infusion of this plasma fraction for 4 h increased hepatic lipase activity up to 2.9-fold over control rabbits and resulted in a loss of larger sized HDL particles consistent with the action of hepatic lipase. The increase in activity was significantly correlated with the concentration of infusate phospholipid, unesterified cholesterol, and esterified cholesterol, but not with the infusate triglyceride concentration. The change in the plasma cholesterol concentration of recipient rabbits, which reflects the degree of lipoprotein accumulation in these rabbits, was also significantly correlated with the change in hepatic lipase activity. However, a chylomicron remnant and β-VLDL-depleted fraction of plasma from cholesterol-fed rabbits did not increase hepatic lipase activity. Furthermore, triglyceride presented as an artificial lipid emulsion (Intralipid®) was not able to stimulate hepatic lipase activity, although triglyceride is a substrate for hepatic lipase. Consistent with previous observations in the cholesterol-fed rabbit, the increase in hepatic lipase activity is not dependent on an increase in specific mRNA levels. We propose that the fraction of plasma that accumulates in the cholesterol-fed rabbit is a positive regulator of hepatic lipase activity and, therefore, may act to enhance its own clearance.—**Ebert, D. L., R. J. Warren, P. J. Barter, and A. Mitchell.** Infusion of atherogenic lipoprotein particles increases hepatic lipase activity in the rabbit. *J. Lipid Res.* 1993. **34:** 89–94.

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The cholesterol-fed rabbit rapidly produces large vessel atherosclerosis and a marked increase in plasma cholesterol. This cholesterol is primarily in the d<1.006 g/ml fraction of plasma (1) in the form of chylomicron remnants and β-migrating very low density lipoprotein (β-VLDL), which are enriched with apoE (2) and potentially atherogenic (3). Accompanying this rise in plasma cholesterol is a reduction in the level of high density lipoprotein (HDL) protein (1).

In addition to its effects on lipoproteins, cholesterol feeding also produces an increase in the activity of hepatic lipase (HL), which rises nearly threefold (4) above the low level normally found in the rabbit (5). This increase is not dependent on increased mRNA levels (4), leading to the speculation that the enzyme is activated or stabilized.

HL plays an important role in lipoprotein homeostasis. Its preferred substrates are the triglycerides and phospholipids of chylomicron remnants, VLDL remnants, and HDL. Hydrolysis of the triglyceride and phospholipid of the remnant particles facilitates their clearance from plasma (6–8), while HDL lipid hydrolysis results in a reduction of HDL_2 levels with their conversion to smaller HDL_3 particles (9). It is our hypothesis that the observed increase in HL activity in the cholesterol-fed rabbit is mediated by a factor that is associated with the potentially atherogenic lipoproteins that accumulate in the plasma. We have measured changes in HL activity that occurred upon the direct, intravenous infusion of these lipoproteins into normal, chow-fed rabbits. In this way we were able to assess the influence of these lipoproteins on HL activity independent of the effects of dietary cholesterol absorption. We report that this lipoprotein fraction produced a significant and rapid rise in HL activity in the recipient rabbits. Accompanying the rise in activity was an appreciable reduction in HDL size.

Abbreviations: HL, hepatic lipase; VLDL, very low density lipoprotein; HDL, high density lipoprotein; apo, apolipoprotein.

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MATERIALS AND METHODS

Materials

All reagents were from Sigma Chemical Co. (St. Louis, MO) or BDH Chemicals (Victoria, Australia) unless otherwise stated. Intralipid® was manufactured by KabiVitrum AB (Stockholm, Sweden) and contained 20% (w/v) triglyceride (soya oil) and 1.2% (w/v) phospholipid (egg phospholipids).

Animals

A multicolored strain of rabbits maintained at the Baker Medical Research Institute was used in these studies. All rabbits were individually housed females, weighing from 2.0–2.5 kg, and were fed either commercial rabbit chow (4% fat w/w, Clark King, Australia) or the same chow supplemented with 0.5% (w/w) cholesterol. The cholesterol-supplemented chow was prepared by first dissolving 50 g of solid cholesterol in 1 liter of diethyl ether, then mixing with 10 kg of chow and allowing the ether to evaporate. The animals were housed individually, had free access to water, and were weighed before use.

Preparation of infusate

Each infusate was prepared on the day of the infusion. Blood from animals fed cholesterol-supplemented chow for more than 28 days was collected in the absence of heparin into tubes containing ethylenediamine tetraacetic acid, final concentration of 0.1% (w/v). The cholesterol concentration in the plasma of the cholesterol-fed rabbits varied between 1350 mg/dl and 2000 mg/dl. Plasma was separated by centrifugation at 4°C, diluted threefold with 0.9% (w/v) saline (d 1.006 g/ml) and spun in a Ti 55.2 rotor (Beckman, Palo Alto, CA) at 35,000 rpm (64,000 g_{min}), 90 min, 4°C. The tube was sliced and the top third was collected. This fraction was highly enriched for chylomicron remnants and β-migrating VLDL as shown by agarose gel electrophoresis. This fraction was diluted with 0.9% saline, when necessary, to give an appropriate range of infusate lipid concentrations. Two experiments were done in which the bottom third was collected after the removal of the top third. This fraction was depleted in chylomicron remnants and β-VLDL. The conditions for ultracentrifugation were chosen to cause minimal disruption to the lipoprotein particle and to minimize the time delay before infusing.

Infusion

A catheter was inserted into the central ear vein under local anesthetic. A bolus of 10 ml of prepared infusate was administered over 10 min, followed by 40 ml over 4 h. Age- and weight-matched rabbits received an identical infusion of 0.9% saline to control for any influence that insertion of the catheter and infusion might have had on HL activity.

Hepatic lipase activity

HL activity was measured in eluates from small samples (45–55 mg) of liver tissue as described previously (4). A linear relationship between enzyme activity and liver tissue mass was found in the range of 20 to 100 mg (4, 10). Changes in liver HL activity are known to closely reflect changes in postheparin plasma HL activity (4, 11). As several batches of triolein/gum arabic emulsions were used as HL substrates over the course of these experiments, all activities are expressed relative to the activity obtained from the paired control animals that were assayed at the same time.

Lipid determinations

Total cholesterol, unesterified cholesterol, phospholipid, and triglyceride were determined on a Cobas-Bio centrifugal analyzer (Roche Diagnostics, Zurich, Switzerland) using commercially available kits (Boehringer-Mannheim, Germany). The triglyceride determination kit excluded free glycerol. The concentration of cholesteryl ester was calculated as the difference between total cholesterol and unesterified cholesterol concentrations assuming the molecular weight for cholesteryl ester to be that of cholesteryl oleate.

mRNA quantification

Liver RNA was prepared by the method of Chomczynski and Sacchi (12), enriched for poly $(A)^+$ RNA by oligo (dT) selection (13), and applied to a nylon membrane (Zetaprobe, Bio-Rad, Richmond, CA) using a slot-blot apparatus (Bio-Rad) according to the manufacturer's recommendations. The membrane was probed with the rabbit HL cDNA (14) labeled by random priming with [^{32}P]α-dATP to a specific activity of 10^9 cpm/μg using a kit (Boehringer Mannheim, Germany). Final wash conditions were 3 mM sodium citrate, pH 7, 30 mM sodium chloride, 0.1% sodium dodecyl sulfate, 55°C, 30 min. The resultant autoradiograph was scanned by laser densitometry (Ultrascan XL, LKB, Bromma, Sweden) to quantify specific mRNA levels and corrected for total mRNA content by reprobing with oligo dT_{30}. Data were in the linear range of the signal response curve. Relative values are presented as arbitrary absorbance units.

Gradient gels

HDL particle size was determined by gradient gel electrophoresis (4–30% polyacrylamide gels, Pharmacia-LKB, Uppsala, Sweden) as previously described (15, 16). Equal volumes of the $d < 1.25$ g/ml plasma fraction from recipient animals were loaded onto the gel. After electrophoresis the gels were stained with the protein-specific dye, Coomassie G-250, and scanned with a laser densitometer (Ultrascan XL, LKB, Bromma, Sweden).

Statistics

All statistical tests were performed using the commercial software package, StatWorks™ (Cricket Software). All average values are presented as the mean ± standard deviation.

RESULTS

To test the influence of different concentrations of chylomicron remnant plus β-VLDL lipid on HL activity, nine different infusates were prepared as described in Materials and Methods from the plasma of cholesterol-fed donor rabbits. These plasma fractions had the following average composition expressed as a percentage of total lipid mass: unesterified cholesterol, 12.0 ± 1.1; cholesteryl ester, 66.8 ± 3.7; triglyceride, 6.2 ± 4.0; phospholipid, 15.0 ± 1.8.

Different concentrations of these preparations were infused for 4 h into each of nine different recipient rabbits. HL activity is expressed relative to that in a paired control animal infused with 0.9% saline. Infusion of the chylomicron remnant plus β-VLDL-enriched plasma fraction resulted in an increase in HL activity that correlated with the concentrations in the infusate of phospholipid (R^2 = 0.94, $P < 0.001$), unesterified cholesterol (R^2 = 0.93, $P < 0.001$), and cholesteryl ester (R^2 = 0.86, $P < 0.001$) (**Fig. 1**). The increase in HL activity did not correlate significantly with the concentration of infusate triglyceride (R^2 = 0.37, P = 0.08), possibly reflecting the variation in triglyceride content of the different infusate preparations. Not only did the increase in HL activity correlate with the amount of chylomicron remnant plus β-VLDL lipid infused, but it also correlated positively and significantly with the increase in plasma cholesterol concentration in the recipient animals (R^2 = 0.73, P = 0.007) (**Fig. 2**).

In all of the experiments described above, age- and weight-matched animals infused with 0.9% saline served as controls for the infusion of the chylomicron remnant plus β-VLDL plasma fraction. This fraction was prepared by diluting plasma threefold with 0.9% saline prior to brief ultracentrifugation, then collecting the contents of the top third of the ultracentrifuge tube. As an additional

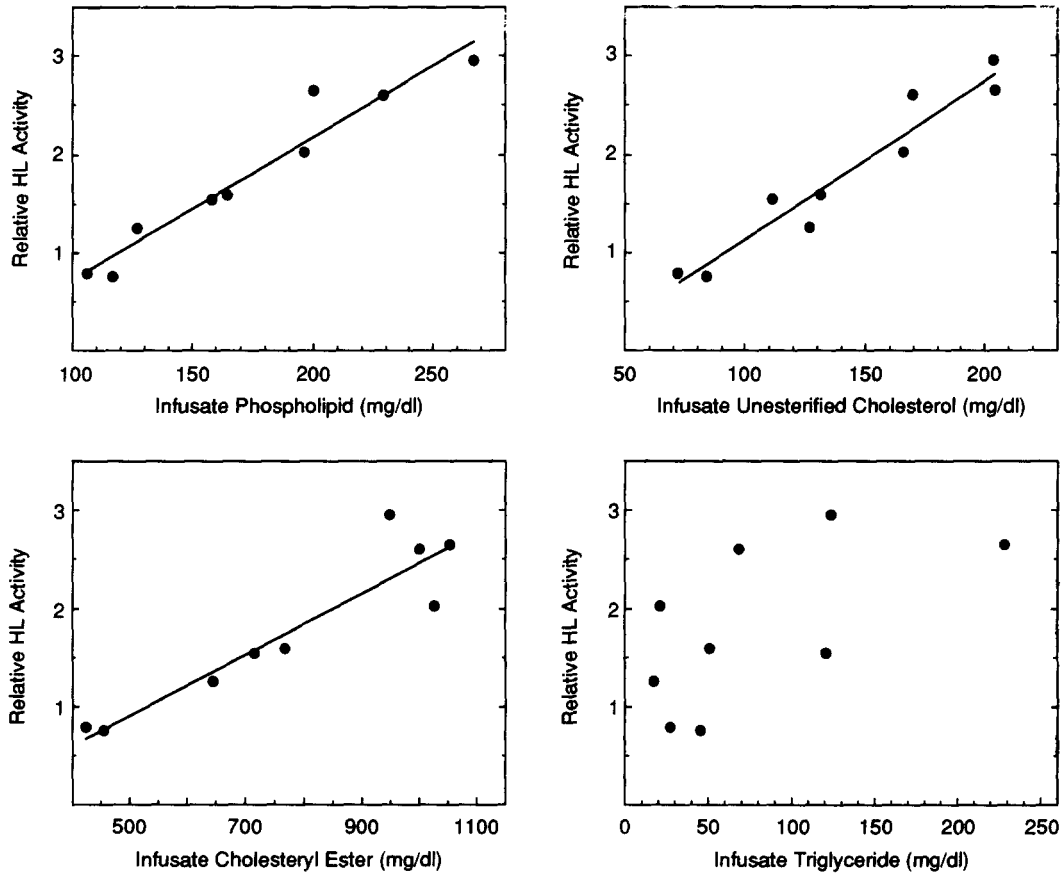

Fig. 1. The relationship between infusate lipid concentration and relative hepatic lipase activity. Infusates were enriched with chylomicron remnants and β-VLDL as described in Materials and Methods. This was infused as a 10-ml bolus, then at a rate of 10 ml/h for 4 h. Activity is expressed relative to the activity of control animals infused with physiological saline, and is plotted against the concentration of the various lipid components.

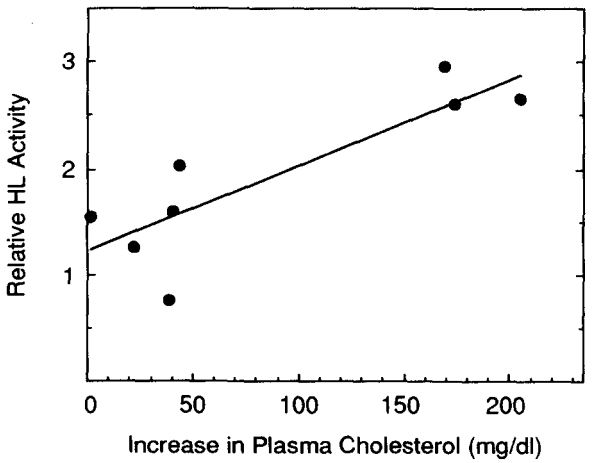

Fig. 2. The relationship between the change in recipient plasma cholesterol and relative hepatic lipase activity. In the experiments shown in Fig. 1, plasma cholesterol was measured before and after infusion of the chylomicron remnant plus β-VLDL-enriched plasma fraction. The change in plasma cholesterol is plotted against the relative hepatic lipase activity.

control for plasma components that would remain distributed throughout the tube and might influence HL activity, we performed two additional experiments in which plasma samples from different cholesterol-fed donor rabbits were each divided into two fractions that were either depleted (bottom third) of or enriched (top third) for chylomicron remnants and β-VLDL. These fractions were then infused into separate chow-fed rabbits. The two enriched fractions had cholesteryl ester concentrations of 1000 mg/dl and 995 mg/dl and compositions that were within one standard deviation of those summarized above, and would therefore be expected to be associated with a significant rise in HL activity. The lipid concentrations of the two bottom fractions would not be expected to have a significant influence on HL activity (cholesteryl ester concentrations of 468 mg/dl and 353 mg/dl). Infusion of the enriched fractions gave rises of 2.2- and 2.6-fold relative to saline infused controls, while infusion of the corresponding depleted fractions yielded rises of only 1.3- and 1.2-fold.

To control for factors that may be present in a chylomicron and VLDL-enriched fraction of plasma independent of cholesterol feeding, three further infusions were performed in which the infusate was prepared from normal, chow-fed rabbits. These control infusates were prepared in a manner identical to that described for the preparation of infusates with the highest cholesterol concentrations from cholesterol-fed animals. The infusion of this control fraction had little influence on HL activity (1.1 ± 0.4) relative to HL activity in saline-infused animals.

While the results strongly suggested that triglyceride was not the component causing the rise in HL activity, we examined this question further by testing whether a triglyceride-rich, artificial lipid emulsion that is metabolized in a manner similar to chylomicrons would produce a change in HL activity. Three rabbits were infused with Intralipid® at a concentration of total lipid equivalent to that in the chylomicron remnant plus β-VLDL-enriched infusates that had the greatest stimulatory effect on HL activity. The triglyceride concentration of 2% (w/v) was nine times that in the infusate with the highest triglyceride concentration. There was no increase in HL activity in these recipient rabbits with values being, on average, 0.95 ± 0.29 that of animals infused with saline. The phospholipid concentration in the Intralipid® infusates was 120 mg/dl. In no experiments was this concentration of phospholipid associated with a rise in HL activity.

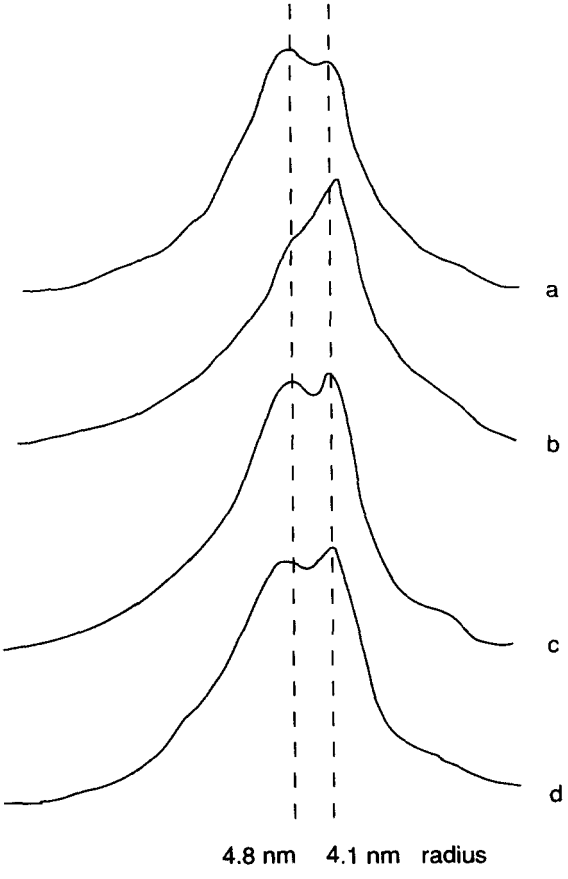

Fig. 3. The influence of infusion on HDL particle size distribution. The plasma fractions of d<1.25 g/ml from control and lipid-infused rabbits, both before and after infusion, were subjected to nondenaturing gradient gel electrophoresis on 4–30% polyacrylamide gels. The tracings represent the absorption at 633 nm obtained by laser densitometry. The numbers at the bottom are the mean particle radii in nm. a) HDL from a recipient rabbit prior to the infusion of the chylomicron remnant plus β-VLDL-enriched plasma fraction. b) HDL from the same rabbit as in (a) after infusion. c) HDL from a recipient rabbit prior to the infusion of physiological saline. d) HDL from the same rabbit as in (c) after infusion.

To test whether the increase in HL activity was associated with an increase in steady-state HL mRNA levels, poly(A)$^+$ mRNA was prepared from the livers of nine saline-infused rabbits and from the livers of four rabbits that had the greatest increase in HL activity after the infusion of the chylomicron remnant plus β-VLDL-enriched fraction. The average amount of HL mRNA present in control samples relative to the amount of poly(A)$^+$ RNA was 1.0 ± 0.3 arbitrary absorbance units. The average value for the test samples was identical (1.0 ± 0.3).

The effect of infusing chylomicron remnants and β-VLDL on HDL particle size was also investigated. The $d < 1.25$ g/ml lipoprotein fraction was isolated from plasma samples taken from recipient rabbits before and after infusion. Equal volumes of this plasma fraction were loaded onto 4–30% nondenaturing gradient gels to determine changes in HDL particle size. The gel scan shown in **Fig. 3** (representative of three experiments) demonstrates that, with the infusion of a chylomicron remnant plus β-VLDL-enriched fraction of high cholesterol concentration, there was a redistribution within the HDL subpopulations. Before infusion, the populations of particles with radii of 4.8 and 4.1 nm were equally represented (Fig. 3a and c). After infusion, there was a predominance of smaller particles with a radius of 4.1 nm (Fig. 3b). There was no change in the particle size of HDL in the saline-infused animals (Fig. 3d).

DISCUSSION

The rationale for this study was provided by our previous finding that cholesterol feeding in the rabbit produces a rise in both plasma cholesterol and HL activity (4). As the rise in plasma cholesterol in the rabbit is primarily in the form of chylomicron remnants and β-VLDL (1), we hypothesized that it is this fraction of plasma that is directly involved in the stimulation of HL activity. The rise in HL activity, therefore, should not be dependent on dietary absorption of cholesterol and should occur quite rapidly upon exposure to the stimulatory agent.

Consistent with our hypothesis, we report here that infusion into chow-fed rabbits of a plasma fraction enriched in chylomicron remnants and β-VLDL increased the HL activity in the recipient rabbits within the 4-h period of the infusion. The increase in activity was significantly and positively correlated with the concentration of infused unesterified cholesterol, cholesteryl ester, and phospholipid. The change in the plasma cholesterol concentration of recipient rabbits, which reflects the degree of lipoprotein accumulation in these rabbits, was also significantly correlated with the change in hepatic lipase activity. In contrast, the infusion of a plasma fraction from cholesterol-fed rabbits that was substantially depleted of chylomicron remnants and β-VLDL had minimal influence on HL activity, confirming that the activating factor was associated most strongly with the least dense lipoprotein particles. Furthermore, a fraction of plasma from normal-fed rabbits that was prepared in a manner identical to the fraction of plasma from cholesterol-fed rabbits that produced the greatest rise in HL activity had no effect on HL activity. This establishes that the stimulatory agent had specifically accumulated only in this fraction of cholesterol-fed rabbits. The increase in activity was not reflected by an increase in steady-state HL mRNA levels, indicating that regulation occurred posttranscriptionally, consistent with the regulation of HL in the cholesterol-fed rabbit (4).

Triglyceride and phospholipid in lipoprotein particles are both substrates for HL and, therefore, increased levels of either may potentially regulate HL activity. In testing this possibility with respect to triglyceride, we found no correlation between the increase in HL activity and triglyceride concentration of the infusates. Neither did Intralipid®, a triglyceride-rich emulsion that is metabolized in a manner similar to chylomicrons (17, 18), act to stimulate HL activity. However, phospholipid concentrations were shown to correlate significantly with the increase in HL activity. The fact that the concentrations of unesterified cholesterol and cholesteryl ester also correlated significantly with the increase in HL activity suggests that these lipids as well as phospholipid may simply serve as indicators of the amount of the lipoprotein fraction infused. We cannot rule out the possibility, though, that phospholipid, as a substrate, may stimulate HL activity independently of the other lipoprotein constituents.

Apolipoproteins have been variously reported to enhance, inhibit, or have no effect on HL activity in vitro (19–22). Although we were not able to measure the concentration of the various apolipoproteins present in the infusates, these may also act to influence HL activity in vivo.

Another potential mechanism for the increase in HL activity is a decrease in the rate of HL degradation. An increase in HL protein levels has been observed in a rat hepatoma cell line incubated in the presence of heparin (23). This was found to be due to a decrease in the rate of HL degradation. It is possible that a component of the chylomicron remnant plus β-VLDL-enriched fraction or the lipoprotein particles themselves act to stabilize HL in vivo.

Infusion of the chylomicron remnant plus β-VLDL-enriched plasma fraction also resulted in a change in the relative predominance of HDL populations of differing particle size. In normal rabbits the larger sized population of HDL particles has a triglyceride-rich core that is lost on treatment in vitro with exogenous HL (5). Larger, triglyceride-rich HDL particles are also found in humans postprandially (9), and it has been argued that HL plays a key role in the observed association between HDL cholesterol levels and the ability to clear triglyceride-rich

remnant proteins from plasma (24). Consistent with these observations, in our experiments rabbits showing the greatest increase in HL activity with the infusion of remnant lipoproteins also showed a loss of larger sized HDL particles.

This study has investigated the regulation of HL by lipoproteins that accumulate in the plasma of cholesterol-fed rabbits. We have not tested directly the possibility that other lipoproteins from the cholesterol-fed rabbit may act to stimulate HL activity. However, other lipoproteins, including low density lipoprotein and HDL, are substantially depleted in the cholesterol-fed rabbit (1) and, therefore, would be unlikely to have a stimulatory effect. Clearly, what we have shown is that the chylomicron remnant plus β-VLDL fraction of plasma is associated with positive regulation of HL activity. Considering that increased HL activity is associated with decreased levels of β-VLDL in the estrogen-treated Watanabe heritable hyperlipidemic rabbit (11) and that inhibition of HL activity decreases the clearance of chylomicron remnants (8), our observations would suggest that chylomicron remnants and β-VLDL, through their regulatory influence on HL, may act to enhance their own clearance. Further studies will be necessary to define what component or components of these lipoproteins are responsible for the stimulation of HL activity.

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