Roles of Lipoprotein Lipase and Hepatic Triglyceride Lipase in the Catabolism In Vivo of Triglyceride-Rich Lipoproteins

Michael F. Reardon, Hideyo Sakai, and George Steiner

To define the roles, in vivo, of hepatic triglyceride lipase and lipoprotein lipase in the catabolism of triglyceride-rich lipoproteins, we investigated the relationship between the activities of the above enzymes in postheparin plasma and the fractional removal rates of very low density lipoproteins (VLDL) and VLDL remnant particles. In 22 patients, the fractional removal rates of VLDL and VLDL-remnant particles were determined from analyses of the disappearance of radiolabeled Sf 60-400 and Sf 12-60 lipoprotein B apoprotein. The maximal activities of hepatic triglyceride lipase and lipoprotein lipase were determined in plasma samples drawn 2-60 minutes after heparin injection (60 U/kg). A positive correlation was observed between the fractional removal rate of VLDL and postheparin plasma lipoprotein lipase activity (r = 0.65). When all 22 patients were considered together, no relationship was demonstrable between remnant fractional removal and postheparin plasma lipoprotein lipase activity. However, humans may be subdivided with respect to the way in which they catabolize remnants. In some, all remnant may be catabolized to form LDL. In others, some of the remnant may also be directly removed from the circulation. Those subjects in whom previous studies indicate that all remnant is converted to LDL demonstrated a positive correlation between remnant fractional removal rate and postheparin plasma lipoprotein lipase activity (n = 8, r = 0.83). No correlations between postheparin plasma hepatic triglyceride lipase activity and any of the fractional removal rates were found. These data are consistent with the following: 1) Lipoprotein lipase plays a key regulatory role in the catabolism of triglyceride-rich lipoproteins; 2) this role applies only to those catabolic processes involving the formation of particles of higher density VLDL remnants and low density lipoprotein; and 3) hepatic triglyceride lipase plays no rate-limiting role in the catabolism of VLDL or VLDL-remnant particles. (Arteriosclerosis 2: 396-402, September/October 1982)

Lipoproteins of the VLDL class transport triglyceride of endogenous (i.e., nondietary) origin into the circulation.¹,² The catabolism of VLDL is essentially a delipidation process, facilitated by lipases. It involves primarily the removal of triglyceride, but the other lipid components are also lost from the lipoprotein particle.³-⁴ This results in the formation of smaller particles of higher hydrated density that initially fall into the remnant range and are ultimately catabolized to form LDL.

The role of the triglyceride-rich lipoproteins in atherosclerosis is currently the subject of considerable interest both in the general population⁵ and in certain high risk populations such as patients with diabetes⁶ and others with chronic renal failure.⁷ There is mounting evidence that the subfraction of the triglyceride-rich lipoproteins which constitute the VLDL-remnant, or intermediate density lipoproteins may be particularly atherogenic.⁷-⁸,¹⁰ Hence, it becomes important to understand those factors that regulate the production and removal of these lipoproteins. For this reason, we have examined the relationship between the turnover of both VLDL (Sf 60–400) and its intermediate density remnant (Sf 12–60) to the activities of both postheparin plasma lipoprotein lipase and hepatic triglyceride lipase.¹¹-¹⁵

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tein lipase and of postheparin plasma hepatic triglyceride lipase.

A number of tissues contain lipases that may play a role in the catabolism of VLDL to low density lipoprotein (LDL). These tissues are primarily adipose tissue, cardiac and skeletal muscle, and liver, but may also include the kidney, spleen, aorta, and lung. The administration of intravenous heparin results in a release of lipases into the circulation. Plasma postheparin lipolytic activity may be subdivided into two classes, i.e., activity of hepatic and of extrahepatic origin. Lipases of extrahepatic origin, collectively referred to as lipoprotein lipase (LPL), require the presence of apolipoprotein C-II for activity. LPL may also be inhibited, in vitro, by protamine sulphate or 1M NaCl. Postheparin lipolytic activity of hepatic origin, referred to as hepatic triglyceride lipase (HTGL) does not require the presence of apolipoprotein (LDL). These tissues are primarily adipose tissue, cardiac and skeletal muscle, and liver, but may also include the kidney, spleen, aorta, and lung. The administration of intravenous heparin results in a release of lipases into the circulation. Plasma postheparin lipolytic activity may be subdivided into two classes, i.e., activity of hepatic and of extrahepatic origin. Lipases of extrahepatic origin, collectively referred to as lipoprotein lipase (LPL), require the presence of apolipoprotein C-II for activity. LPL may also be inhibited, in vitro, by protamine sulphate or 1M NaCl. Postheparin lipolytic activity of hepatic origin, referred to as hepatic triglyceride lipase (HTGL) does not require the presence of apolipoprotein cofactors for activity and is not subject to inhibition by protamine sulphate or 1M NaCl. The conversion of VLDL to its remnant and then finally to LDL is dependent upon lipolytic activity. The administration, in vivo, of heparin into the circulation results in the rapid disappearance of circulating VLDL with a resultant increase in particles of higher density. Similarly, in vitro studies have illustrated that "LDL-like" lipoproteins may be produced upon exposing VLDL to lipolytic activity. We have conducted a series of studies relating the fractional removal rates of the B apolipoprotein of VLDL and of VLDL remnant to the activities of lipases of hepatic or extrahepatic origin. These studies have given some insight into the processes involved in the catabolism of VLDL and VLDL remnants. They also indicate the rate-limiting role of LPL, but not of HTGL, in the catabolism of VLDL to its remnant and then to LDL.

Methods

Population Group and Dietary Management

We selected for study 22 patients, having a wide range of plasma triglyceride concentrations (ranging from 116 to 583 mg/dl). Their individual characteristics are in table 1. No subjects were receiving medication known to affect lipid metabolism during the study period.

Four weeks before the study, all subjects were instructed to consume a balanced, weight maintaining diet (approximately 40% fat, 40% carbohydrate, and 20% protein). During the actual study period, their dietary intake consisted of the same mass of protein and carbohydrate as before the study but with no fat (less than 1%). This dietary regime has been found to maintain B apoprotein and triglyceride concentrations in steady state conditions over the investigation period in this and in other studies.

To minimize uptake of radioactive iodine by the thyroid, all subjects consumed potassium iodide (300 mg/day) for 3 days before and 14 days after the study period.

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*Body mass index (BMI) = weight (kg) ÷ height (m)².
Procedures for Metabolic Investigations

The fractional removal rates of the B apoprotein of VLDL and of VLDL remnants were determined by analysis of the decline in B apoprotein specific activity following injection of autologous radiolabelled VLDL and remnant lipoprotein preparations.

Five days before the study, blood was drawn from the subject (after a 14-hour fast) into tubes containing EDTA (1 mg/ml). Plasma was separated (4 \times 10^4 \text{ g • min}, 4°C). VLDL (Sf 60–400) and VLDL remnant (Sf 12–60) fractions were isolated and washed once as previously described. The suitability of the above flotation ranges for the isolation of VLDL and remnant lipoproteins has been discussed and verified previously. The VLDL fraction was radioiodinated with $^{125}$I, and the remnant fraction with $^{131}$I (Atomic Energy of Canada Limited, Ottawa, Ontario, Canada) using a modification of the iodine-monochloride procedure.

On the first day of study, subjects were injected with 2-30 µCi of each of the radiolabelled lipoprotein preparations. Blood samples (20 ml) were subsequently collected at timed intervals up to 46 hours after injection. The plasma was separated immediately (4 \times 10^4 \text{ g • min}, 4°C). VLDL and remnant lipoprotein fractions were isolated and washed once using conditions identical to those used to isolate the injectates. The B apoprotein of these lipoprotein samples was then isolated, and its specific activity ($^{125}$I in the VLDL fractions and $^{131}$I in the remnant fractions) was determined.

In most patients, the decline in both the VLDL and the remnant B apoprotein specific activity with time conformed, over the 46-hour study period, to a biexponential curve. These curves were analyzed using the 2-pool model described by Gurpide et al. The fractional removal rate ($k_e$) was an expression of irreversible removal from the primary metabolic compartment. The application of the Gurpide model to describe B apoprotein kinetics has been discussed previously. In those cases where the disappearance was monoeXponential, the fractional removal rate was calculated as the slope of the single exponential.

Following completion of the 46-hour period to examine the decline in VLDL and remnant B apoprotein specific activity, the patient was injected with 60 IU/kg porcine heparin (as the sodium salt obtained from Harris Laboratories, Brantford, Ontario, Canada). Blood samples were then collected at 2, 5, 10, 20, 40, 45, 60, and 120 minutes after heparin injection into chilled tubes containing EDTA (1 mg/ml). The plasma was separated immediately and then frozen (−20°C) until it was assayed for LPL and HTGL activity. Both activities were determined by the method of Krauss et al. In many cases, these activities were cross-checked using the immunochemical inhibition method of Huttunen et al. For the latter procedure, two antiheparic lipase preparations were used (one raised in our laboratory and another kindly supplied by Dr. Esko Nikkila, Helsinki). Identical values were obtained using either antibody preparation. Values obtained with immunochemical inhibition closely

Figure 1. Example of the pattern of release of hepatic triglyceride lipase and lipoprotein lipase into the circulation following the administration of heparin (60 U/kg). In this patient, the maximal observed activity of hepatic lipase (8.5 µmol • ml$^{-1}$ • hr$^{-1}$) occurred at 10 minutes whereas the lipoprotein lipase activity peaked (11.9 µmol • ml$^{-1}$ • hr$^{-1}$) at 20 minutes.

Figure 2. A second example of the pattern of release of hepatic triglyceride lipase and lipoprotein lipase into the circulation following the administration of heparin (60 U/kg). In this patient, the maximal observed activity of hepatic lipase (7.4 µmol • ml$^{-1}$ • hr$^{-1}$) occurred at 10 minutes whereas lipoprotein lipase activity peaked (13.6 µmol • ml$^{-1}$ • hr$^{-1}$) at 45 minutes.
agreed with those using the procedure of Krauss et al.24 In a comparison of 20 samples, the correlation coefficients between the activities obtained with either procedure was 0.91 for postheparin plasma LPL and 0.92 for postheparin plasma HTGL.

Results

The patterns of HTGL and LPL release into the circulation after heparin administration were determined for each subject. The release patterns of both enzymes into the circulation differed from patient to patient. Examples of some of the patterns are shown for two subjects in figures 1 and 2. For each lipase, the areas under the time vs. lipase activity curves were highly correlated to the peak lipase activities ($r = 0.91$ for LPL and $r = 0.90$ for HTGL). Hence, for simplicity, we chose to select the maximal activity observed in the plasma as indicative of the in situ physiological activity of the enzyme. As there was no consistency in the time at which either enzyme would reach its maximal observable activity in the circulation, it was not possible to select any single time point to sample for enzyme activity. Rather, in each subject, a number of postheparin samples were necessary to enable us to determine the maximal activity releasable into the circulation.

The maximal observed activities of both enzymes were related to the fractional removal rate of VLDL (Sf 60–400) and remnant (Sf 12–60) B apoprotein. As shown in figure 3, we observed a significant correlation between VLDL B apoprotein fractional removal rate and postheparin plasma LPL activity ($r = 0.65$). No significant relationship was demonstrable between the fractional removal of remnant B apoprotein and the activity of postheparin plasma LPL when the group was considered as a whole (figure 4).

No significant correlations were found between postheparin plasma HTGL activity and the fractional removal rates of either VLDL or remnant B apoprotein (figures 5 and 6). Indeed, those insignificant relationships which were found appeared to be negative and certainly were not positive. These observations further point out a lack of any positive association between B apoprotein fractional removal rates and HTGL activity.

Previous studies have established that the catabolism of remnant B apoprotein may proceed by more than one metabolic pathway.5,26–28 Remnant particles are catabolized to particles of higher density (i.e., LDL) or they are directly removed from the circulation (i.e., without conversion to LDL). The nature of this direct removal pathway remains unknown. Recently, Turner, et al. have described the transplanchnic removal of these particles in man,27 thereby indicating the possibility that the liver may play a significant role in this process. As described earlier, when the whole population (n = 22) was considered, no relationship was found between the activity of either lipase and the fractional removal rate of the remnant B apoprotein.

The relationship between the fractional removal rate of the remnant B apoprotein and the activity of each lipase was then examined in the normotriglyceridemic subpopulation of these individuals. Using techniques identical to those employed in this study, previous investigations have found a nearly complete quantitative conversion of remnant B apoprotein.

\[ r = 0.65 \]
\[ p < 0.001 \]

![Figure 3](http://atvb.ahajournals.org/)

**Figure 3.** Relationship between fractional removal rate of VLDL (Sf 60–400) B apoprotein and postheparin lipoprotein lipase activity (n = 22). The equation for the regression line is $Y = 0.014X + 0.086$.

\[ r = 0.30 \]
\[ \text{N.S.} \]

![Figure 4](http://atvb.ahajournals.org/)

**Figure 4.** Relationship between fractional removal rate of VLDL remnant (Sf 12–60) B apoprotein and postheparin lipoprotein lipase activity (n = 22). No significant correlation was found.
tein to LDL B apoprotein in such normotriglyceridemic individuals. Similar observations have been reported by others. By contrast, Turner, et al. suggested that some Sf 12–60 lipoproteins are catabolized, even in normolipidemics, without being converted to LDL. However, their patients were on diets containing 40% fat. Hence, their Sf 12–60 fraction may have had a significant content of chylomicron remnants. In contrast to VLDL remnants, it is generally felt that there is no significant conversion of chylomicron remnants to LDL. In the present study, we did not determine the percentage conversion of remnant B apoprotein to LDL B apoprotein. Nevertheless, when we selected normotriglyceridemic individuals only, a strong positive correlation emerged between the fractional removal rate of remnant B apoprotein and the activity of lipoprotein lipase (figure 7). There was still no correlation observed with postheparin plasma HTGL. We have interpreted the failure to observe any relationship in the whole population as reflecting that, in many of these individuals (i.e., those with hypertriglyceridemia), the fractional removal of remnant B apoprotein was an expression of more than one catabolic process.

Discussion

The catabolism of VLDL to LDL is a stepwise process involving initially the formation of the remnant. The remnant may then be catabolized by two routes, i.e., it may be catabolized to form LDL or it may be directly removed from the circulation without forming LDL. This latter pathway is significant in hypertriglyceridemic subjects only. Catabolism of VLDL to particles of higher density (i.e., remnants and LDL) is essentially a delipidation process. The only moiety to remain, in toto, with the particle during this catabolic process is the B apoprotein. Thus, the B apoprotein of these lipoproteins may be used as a marker of metabolism of the lipoprotein particle.

Upon examining relationships between fractional removal rates of VLDL and remnant B apoprotein and the activities of postheparin plasma LPL and postheparin plasma HTGL, significant correlations were found between postheparin plasma LPL activity and VLDL B apoprotein fractional removal rate in all subjects; and between postheparin plasma LPL activity and VLDL B apoprotein.

![Figure 5. Relationship between fractional removal rate of VLDL (Sf 60–400) B apoprotein and postheparin hepatic triglyceride lipase activity (n = 22). No significant correlation was found.](image1)

![Figure 6. Relationship between fractional removal rate of VLDL remnant (Sf 12–60) B apoprotein and postheparin hepatic triglyceride lipase activity (n = 22). No significant correlation was found.](image2)

![Figure 7. Relationship between fractional removal rate of VLDL remnant (Sf 12–60) B apoprotein and postheparin plasma lipoprotein lipase in normotriglyceridemic subjects only (n = 8). The VLDL remnant B apoprotein equation for the regression line is Y = 0.033X - 0.069.](image3)
activity and remnant B apoprotein removal, in those whose plasma triglyceride concentrations were less than 185 mg/dl. The reasons for considering this normotriglyceridemic subset of individuals to represent a group in whom the remnant particles are completely or predominantly catabolized to form LDL have been discussed. When all 22 individuals were considered, no relationship was found between postheparin plasma LPL and the fractional catabolic rate of the remnants. We interpret this as resulting from the fact that within the entire population, there were individuals (hypertriglyceridemics) who catabolized a large portion of remnant particles via a direct pathway which did not involve conversion to LDL. These findings suggest that LPL plays a positive regulatory role in the catabolism of triglyceride-rich lipoproteins (both VLDL and remnants), but only in those processes leading to the formation of particles of higher density.

We did not find any positive correlation between the activity of postheparin plasma HTGL and the fractional catabolic rate of either lipoprotein subfraction. This indicates that if HTGL plays any role in the catabolism of the triglyceride-rich lipoproteins, the role is not rate-limiting. However, although some have reported no relationship between postheparin plasma HTGL and plasma triglyceride levels, it has been suggested that HTGL activity may facilitate the uptake of small quantities of triglyceride from VLDL and chylomicron remnants31,32 thereby converting these remnants to LDL.33 However, in vitro studies examining the role of HTGL in these interconversion processes have shown that HTGL does not convert remnants to LDL.33 On the other hand, Murase and Itakura34 and Goldberg et al.35 suggest that blocking HTGL in rats or monkeys may impair the catabolism of intermediate density lipoproteins. Other studies in which hepatic lipase activity was inhibited immunochemically in rats have shown that the major in vivo regulation of lipoprotein metabolism is related to HDL activity and LDL cholesterol levels36,37 thereby allowing its conversion to HDL. Therefore, one possible explanation of the reported correlations between HTGL activity and LDL cholesterol levels36 may be in the interrelationship between HDL levels and the modulation of LDL receptor numbers.39

Our studies clearly indicate that the rates of catabolism of VLDL to its remnant and then to LDL are related to the activity of LPL. A number of in vivo studies using either human postheparin plasma PPL activity, purified postheparin plasma PPL, or perfused isolated rat heart systems have demonstrated an interconversion of VLDL to "LDL-like" particles in the presence of LPL activity.13-18 Studies in which PPL was immunohistochemically inhibited in vivo have shown VLDL catabolism to be blocked with the inactivation of the enzyme. At the same time, LDL formation was stopped, illustrating the requirement, in vivo, for LPL activity in VLDL catabolism to LDL.40

Using a kinetic approach to determine, in vivo, the rates of catabolism of VLDL to its remnant and then to LDL, we have demonstrated that LPL activity plays a regulatory role in the catabolism of triglyceride-rich lipoproteins to particles of higher density. HTGL activity does not appear to play a regulatory role in these catabolic steps, although the requirements for these processes to occur remains uncertain.

Acknowledgments

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