

Kinetics of tissue metabolism of β -very low density lipoproteins: Disparate rates of tissue accumulation during both normal and hypercholesterolemic states

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ABSTRACT

β -Very low density lipoproteins (β -VLDL) can interact with multiple receptors including low density lipoprotein (LDL), abnormal LDL, and LDL-related receptor protein, and thus exhibit complex kinetics and regulation in vivo. The present study defined kinetics of metabolism of β -VLDL by tissues and its modulation by cholesterol feeding in rabbits. β -VLDL was conjugated to a residualizing label, dilactitol-¹²⁵I-tyramine (¹I-DLT), and injected intravenously into rabbits fed normal diet (Gp I), cholesterol-rich diet (Gp II), or cholesterol-rich followed by normal diet (Gp III). Plasma cholesterol concentrations were 49 ± 5 , 813 ± 75 , and 513 ± 80 mg/dl, respectively. Clearance of ¹I-DLT- β -VLDL from plasma was determined and serial autopsy was performed at intervals up to 6 hr for tissue acquisition. ¹I-DLT- β -VLDL was cleared rapidly from plasma of Gp I, (fractional catabolic rates, 0.942 hr^{-1}), but reduced in Gp II (0.093 hr^{-1}) and Gp III (0.199 hr^{-1}). Hepatic accumulation of ¹I-DLT- β -VLDL was very rapid in Gp I with $46 \pm 8\%$ of injected dose (ID) present 5 min after injection. No further accumulation was detected after 5 min. Hepatic accumulation of ¹I-DLT- β -VLDL was also rapid in Gp II although only $10 \pm 2\%$ was present at 5 min. As in Gp I, no further increases were observed after 5 min, although there was an incremental accumulation of radioactivity in Gp III. Adrenal accumulation of ¹I-DLT- β -VLDL was slower than hepatic in Gp I, with a maximum ($0.60 \pm 0.08\%$ ID/g) reached at 2 hr.

Adrenal accretion of β -VLDL was nominally reduced in both Gp II and Gp III (0.16 ± 0.04 and 0.24% ID/g, respectively). (1) β -VLDL is recognized at different rates by liver and adrenals. (2) Hepatic recognition is rapid in both normal and hypercholesterolemic states.

INTRODUCTION

β -Very low density lipoproteins (β -VLDL) are present in the plasma of many animal species during the feeding of high-fat, high-cholesterol diets (Daugherty et al., 1988) and in type III hyperlipidemic subjects (Fainaru et al. 1982). β -VLDL is considered to be a highly atherogenic lipoprotein fraction based the ability to avidly interact with macrophages and augment cholesterol esterification (Mahley et al., 1980). Also, there is a high incidence of accelerated atherosclerotic diseases in human subjects with detectable mass of β -VLDL in plasma.

β -VLDL is a highly heterogeneous lipoprotein fraction consisting of particle of both intestinal and dietary origin. Apo-B100 and apo-E are present in this cholesterol-enriched fraction conveying the ability to interact with both low density lipoprotein (LDL) receptors and the putative chylomicron remnant (apo-E) receptor that may be synonymous with the LDL-receptor-related protein (LRP; Herz et al., 1990). In addition, there is a mutant form of the LDL receptor on macrophages that recognizes β -VLDL but not LDL. Thus, the metabolism of β -VLDL in vivo is a complex interaction of several receptor systems.

It has been well characterized that β -VLDL is cleared rapidly from plasma under normolipidemic conditions and hypercholesterolemia produces

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marked retardation of clearance (Daugherty et al., 1986). The availability of residualizing labels such as dilactitol-¹²⁵I-tyramine (¹-DLT; Strobel et al., 1985) have permitted the determination of the major tissue sites of catabolism of β -VLDL (Daugherty et al., 1985). However, the kinetics of the tissue accumulation of ¹-DLT- β -VLDL have not been defined, and whether these kinetics are equivalent in all tissues. Thus, the purpose of the present study was to determine the rates at which ¹-DLT- β -VLDL accumulated in its major catabolic tissues under conditions of normolipidemic, during diet-induced hypercholesterolemia, and during the feeding of standard laboratory diet following a period of cholesterol feeding.

MATERIAL AND METHODS

Animals

New Zealand rabbits of either sex weighing 1.5 to 2 kg were purchased from a local supplier (Boswell's, Pacific, MO). Diets were either normal laboratory food (Ralston Purina, St. Louis, MO) or enriched in cholesterol (2% wt/wt; Purina Test Diets; Richmond, IN). Food and water was available ad libitum. The rabbits were allowed a one week period of stabilization before randomization in the following groups: Normolipidemic (Group I); 30 day cholesterol fed (Group II); and 30 day cholesterol fed/30 day normal diet (Group III). All procedures used had the prior approval of the Washington University Animal Studies committee.

Isolation of β -VLDL

Rabbits that had been maintained on 2% wt/wt cholesterol diet for at least 30 days were anesthetized with sodium pentobarbital (30 mg/kg). Blood was collected from the dorsal aorta via 18 gauge cannula. Plasma ($d < 1.008$ mg/ml) was separated and subjected to a fixed angle ultracentrifugation (L8-55 Ultracentrifuge; Beckman; Palo Alto, CA) using a 55.2 Ti rotor with the following conditions: 45,000 rpm; 18 hr; 4° C. The isolated β -VLDL were washed in EDTA/NaCl (1 mM; 0.154 M; pH 8.2) and isolated by re-ultracentrifugation in the above given conditions.

Characterization of Plasma and β -VLDL

Blood samples (≈ 1 ml) were drawn from a marginal ear vein of fasting rabbits into syringes containing EDTA (final concentration of 1.5 mg/ml). Plasma was separated by centrifugation and stored at 4° C.

Concentrations of cholesterol, unesterified cholesterol, phospholipids, and triglycerides in plasma and β -VLDL were determined with

commercially available enzyme kits (Wako Chemicals, Richmond, VA). Cholesterol ester concentrations were based on the difference between total cholesterol and unesterified cholesterol. Protein mass was determined as described by Lowry et al. (1951) with bovine serum albumin as the standard.

Conjugation of Dilactitol-¹²⁵I-Tyramine to β -VLDL

DLT was synthesized as described by Strobel et al. (1985). DLT (10 nmol) in potassium phosphate buffer (0.5 M, pH 7.7) was placed in an eppendorf tube coated with iodogen (20 μ g; Pierce Chemical Co., Rockford, IL). High concentration Na¹²⁵I (10 mCi; Amersham Radiochemical Center; Arlington Heights, IL) was added. After a 5 min incubation at room temperature, the reaction mixture was transferred to another tube containing galactose oxidase (4 units; Sigma Chemical Company; St. Louis, MO) and incubated for 45 min at 37° C to generate aldehyde residues. These residues were covalently coupled to β -VLDL in the presence of sodium cyanoborohydride (20 mM) during a 1 h incubation at 37° C. Radiolabeled DLT- β -VLDL (¹-DLT- β -VLDL) was purified by gel filtration through Sephadex G-25 2.2 ml spin columns (Isolabs, Akron, OH) and overnight dialysis against EDTA/NaCl (1 mM; 0.154 M; pH 8.4) at 4° C.

Studies In Vivo

Recipient rabbits were fasted for 16 hr before administration of radiolabeled tracer. ¹-DLT- β -VLDL (80-130 μ g protein in 1 ml containing 75-200 $\times 10^5$ cpm) was injected via the marginal ear vein. At selected intervals from 1 min to 6 hr after injection, blood (1 ml) was collected into a syringe containing EDTA (1.5 mg/ml). Plasma was separated using a microfuge and radioactivity was determined in aliquots of plasma. Kinetics of tracer clearance from plasma was calculated from total radioactivity injected and estimation of plasma volume (33 ml/kg).

For determination of tissue accumulation, recipient animals were killed by overdose of sodium pentobarbital (120 mg/kg) at selected intervals after injection of tracer. A cannula was placed in the portal vein and the liver was perfused with sodium chloride (0.15 M; 60 ml). The inferior vena cava was partially transected to facilitate removal of perfusate. Tissues (adrenals and liver) were removed, blotted lightly and weighed. Radioactivity was determined for each sample using a Packard gamma counter. Samples were stored at -20° C until homogenization.

To determine the extent of catabolism of tracer, adrenal and liver samples (100-350 mg) were homogenized in distilled water (1 ml) with an Ultra-Turrax tissumizer (Tekmar; Cincinnati, OH).

Trichloroacetic acid (TCA; final concentration of 10% wt/vol) was added to homogenate and incubated for 15 min at 4°C. Samples were then centrifuged at 2000 rpm for 15 min, supernatant was removed from precipitate. Radioactivity was determined in both TCA-precipitable and TCA-soluble fractions. All calculations were based as a percentage of radioactivity of the injected tracer.

RESULTS AND DISCUSSION

Concentration of Lipids in Plasma

Normolipidemic rabbits (Gp I) had the expected low plasma concentrations of lipids (Table 1). Cholesterol feeding for 30 days resulted in dramatic increases cholesterol, both total and unesterified, and phospholipids, while triglycerides were not significantly altered. In Gp III, rabbits were fed a cholesterol enriched diet for 30 days, and were then returned to normal laboratory diet. Plasma concentrations of cholesterol were still markedly elevated in this group (Table 1). This very slow return to normal plasma cholesterol concentrations has been noted previously and has been attributed to a continued synthesis of cholesterol ester-rich lipoproteins from the lipid laden liver (Daugherty et al., 1986).

Group	Concentration (mg/dl)			
	TC	UC	TG	PL
I	46 ± 5	23 ± 3	159 ± 33	88 ± 19
II	813 ± 75	312 ± 32	82 ± 11	471 ± 27
III	513 ± 88	319 ± 64	58 ± 13	297 ± 41

Table 1 - Plasma concentrations of total cholesterol (TC), unesterified cholesterol (UC), triglycerides (TG), and phospholipids (PL) in the groups of rabbits. Values represent the mean ± the standard error of the means.

Kinetics of Clearance of ^{125}I -DLT- β -VLDL from Plasma

In the normolipidemic group, kinetics of clearance of ^{125}I -DLT- β -VLDL were rapid and were optimally fitted to a biexponential function. Fractional catabolic rates (FCR) for Gp I were 0.942 hr^{-1} and were reduced considerably after 30 days of cholesterol feeding to 0.083 hr^{-1} in Gp II. This dramatic decrease in FCR of β -VLDL during cholesterol feeding in rabbits has been attributed to a saturation of receptors (Kovanen et al., 1982). Gp III had been returned to standard laboratory diet for 30 days prior to the determination of the kinetics of clearance of ^{125}I -DLT- β -VLDL, however FCR was markedly retarded (0.199 hr^{-1}).

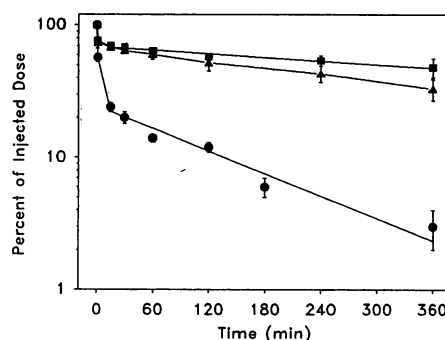


Figure 1 - Kinetics of clearance from plasma of ^{125}I -DLT- β -VLDL in rabbits fed (1) standard laboratory diet (circles); (2) cholesterol-enriched diet (squares); (3) a cholesterol-enriched diet followed by a return to a standard laboratory diet (triangles). Points represent the means of 5 observations and bars the standard error of the mean.

Tissue Accumulation and Catabolism of ^{125}I -DLT- β -VLDL

Hepatic tissue was the most prominent tissue locus for the metabolism of β -VLDL (Daugherty et al., 1985). While the contribution of the liver to the metabolism of β -VLDL, has been described previously, the kinetics of this metabolism has not been determined. Accumulation of radioactivity after the injection of ^{125}I -DLT- β -VLDL was unexpectedly rapid in the normolipidemic group (Gp I). After only 5 min post-injection, $46 \pm 8\%$ of the injected dose of radioactivity was present in the liver, and no significant changes were noted over the following 6 hr (Figure 2A). Hepatic accumulation of radioactivity was markedly reduced during cholesterol feeding in Gp II. After 5 min, only $10 \pm 2\%$ of the injected dose was present in the liver. However, in accord with the results obtained from Gp I, there was no further accumulation of radioactivity over the 6 hr of observation. Accumulation of radioactivity was also markedly decreased at 5 min in Gp III compared to Gp I with only $11 \pm 2\%$ of injected dose being present at 5 min post-injection. In contrast to the other two groups, there was some further accumulation of radioactivity over the 6 hr of observation, with $27 \pm 5\%$ of injected dose being present at the final interval of study. It should be noted that inadequate removal of plasma from the hepatic tissue would have produced errors in the calculation of the accumulation of radioactivity. This error is considered unlikely since the visually inspection of the liver indicated that there was little residual blood remaining. Also, contamination of this

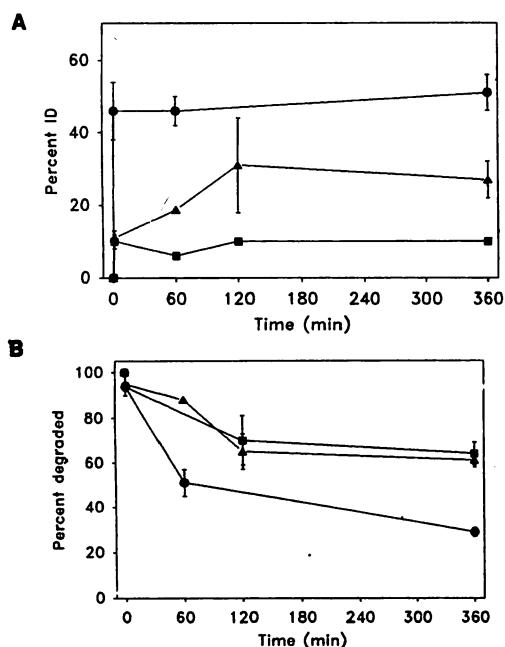


Figure 2 - (A) Kinetics of accumulation of radioactivity in hepatic tissue at selected intervals after the injection of ^3H -DLT- β -VLDL in rabbits fed (1) standard laboratory diet (circles); (2) cholesterol-enriched diet (squares); (3) a cholesterol-enriched diet followed by a return to a standard laboratory diet (triangles). **(B)** Rate of the appearance of degradation products in hepatic tissue in groups as described above. Points represent the means of 5 observations and bars the standard error of the mean.

type would have led to apparent higher accumulation of radioactivity in the groups where clearance from plasma was markedly retarded (i.e., Gp II and III), whereas the results indicate that Gp I has a much greater accumulation of radioactivity.

Degradation products of ^3H -DLT labeled proteins are retained intracellularly for protracted intervals which enables the quantification of the extent of catabolism in addition to determination of accumulation. As can be seen in Figure 2B, the accumulation of degradation products was decreased in both Gps II and III compared to the normolipidemic group.

The adrenal has a negligible contribution to the overall metabolism of β -VLDL in the whole animal. However, when data are normalized to the mass of

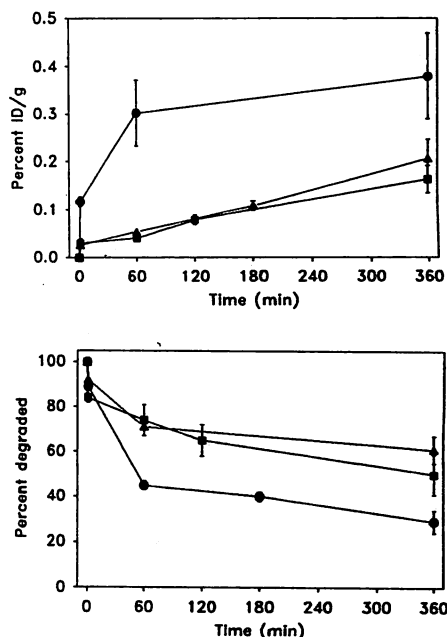


Figure 3 - (A) Kinetics of accumulation of radioactivity in adrenal tissue at selected intervals after the injection of ^3H -DLT- β -VLDL in rabbits fed (1) standard laboratory diet (circles); (2) cholesterol-enriched diet (squares); (3) a cholesterol-enriched diet followed by a return to a standard laboratory diet (triangles). **(B)** Rate of the appearance of degradation products in adrenal tissue in groups as described above. Points represent the means of 5 observations and bars the standard error of the mean.

the tissue, the adrenals are the most avid metabolic tissue for β -VLDL (Daugherty et al, 1985). In the normolipidemic group, there was detectable accumulation at 5 min (Figure 3A). However, in contrast to the accumulation in the liver, there was a markedly increased accumulation of radioactivity 1 hr after injection of tracer. No significant further accumulation occurred after the 1 hr interval. Accumulation of radioactivity was decreased in the adrenal tissue of both Gp II and III to a similar extent. While the accumulation of radioactivity was decreased, there was continued accretion over the whole of the 6 hr of observation.

As for hepatic tissue, the accumulation of degradation products in adrenal tissue was retarded by hypercholesterolemia (Figure 3B).

In summary, the results of this study demonstrate that the hepatic accumulation of radioactivity after the injection of radiolabeled β -VLDL was unexpectedly rapid, and this rapidity was not influenced by feeding a cholesterol enriched diet. However, the extent of accumulation of radioactivity was decreased by cholesterol feeding. Also, the kinetics of accumulation of radioactivity in the adrenals are dissimilar to those of the liver in both the normolipidemic and hypercholesterolemic states

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