Aortic accumulation and plasma clearance of β-VLDL and HDL: effects of diet-induced hypercholesterolemia in rabbits

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Abstract To assess the role of β-VLDL in diet-induced atherogenesis, the in vivo metabolism and aortic accumulation of ^{125}I-labeled β-VLDL were investigated in cholesterol-fed rabbits and chow-fed controls. ^{125}I-labeled HDL and ^{125}I-labeled albumin were studied for comparison. The fractional catabolic rate of ^{125}I-labeled β-VLDL was reduced in cholesterol-fed rabbits (0.011 vs 0.139 hr^{-1}), but due to the high endogenous pool, the total β-VLDL flux was very high (13.1 vs <1.1 mg/kg per 24 hr). These results suggest that elevated levels of β-VLDL during cholesterol feeding were due to an enhanced rate of synthesis, a finding confirmed in hypercholesterolemic rabbits subjected to plasmaphoresis. Following acute reduction of plasma cholesterol by plasmaphoresis, the quantitative increases in β-VLDL cholesterol concentrations (210 to 364 mg/dl) over the subsequent 24 hr were in agreement with the rise calculated from the plasma clearance kinetics of ^{125}I-labeled β-VLDL (378 mg/dl per 24 hr). Aortic accumulation of β-VLDL in hypercholesterolemic rabbits was increased >15-fold over controls. Accumulation was predominantly in the intimal atheromatous lesions. The fractional catabolic rate of ^{125}I-labeled HDL was increased during cholesterol feeding (0.037 vs 0.021 hr^{-1}). A decreased rate of synthesis appeared to be responsible for the markedly depleted plasma HDL. HDL accumulation within the aorta was attenuated >9-fold in cholesterol-fed rabbits compared to those fed normal chow. Plasma kinetics and aortic accumulation of ^{125}I-labeled albumin were similar in hypercholesterolemic and control rabbits. These data suggest that: *1*) β-VLDL accumulated preferentially in lesioned areas of the aorta; *2*) diet-induced hypercholesterolemia was due to overproduction of β-VLDL; *3*) reduced plasma levels and aortic accumulation of HDL may accentuate the cholesterol loading produced by β-VLDL. —**Daugherty, A., L. G. Lange, B. E. Sobel, and G. Schonfeld.** Aortic accumulation and plasma clearance of β-VLDL and HDL: effects of diet-induced hypercholesterolemia in rabbits. *J. Lipid Res.* 1985. **26:** 955–963.

Supplementary key words β-VLDL • HDL • plasma clearance • cholesterol feeding

In establishing the link between diet-induced hyperlipidemia and atherosclerosis, it is important to demonstrate that the diet-induced lipoproteins accumulate in lesioned areas of arterial walls. Increased plasma concentrations of LDL are associated with atherosclerosis in a variety of circumstances and the accumulation of LDL in atheromatous lesions is well established (1–3). Although plasma β-VLDL is also associated with atherosclerosis, less evidence is available on the accumulation of β-VLDL in arterial walls and the influence of hyperlipidemia on this accumulation. Such information would be particularly relevant to the genesis and progression of atherosclerosis in the high-fat, high-cholesterol diet-fed experimental animal models of atherosclerosis since β-VLDL is the major diet-induced lipoprotein in the plasmas of these animals (4–6). Because β-VLDL and LDL have different metabolic fates and receptor recognition, the vascular wall accumulation may also be different for these lipoproteins. Indeed, the distribution of cholesterol in tissues, including atherosclerotic lesions, is different in two commonly used models of lapine atherosclerosis: the Watanabe heritable hyperlipidemic rabbit, which is characterized by the accumulation primarily of LDL in plasma (7), and the diet-induced hypercholesterolemic rabbit, in which plasma cholesterol is carried predominantly by β-VLDL (8, 9).

The aim of the present investigation was to assess the role of β-VLDL in diet-induced aortic atherogenesis. Using rabbits fed a cholesterol-enriched diet, we have studied the influence of hypercholesterolemia on the intravascular metabolism of β-VLDL and on its accumulation in the aortic walls of these animals. The metabolism and aortic accumulation of HDL and albumin were studied for comparison.

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; apoE, apoprotein E; apoB, apoprotein B; apoA-I, apoprotein A-I.

METHODS

Characterization of plasma lipids and lipoproteins

Protein was quantified by the method of Lowry et al. (10) with bovine serum albumin as the standard (Fraction V, Sigma Chemical Company). Cholesterol and triglycerides were determined enzymatically with commercially available test kits (Boehringer Mannheim). Phospholipid was determined by the method of Fiske and Subbarow (11) by quantification of phosphate.

Apoprotein composition was determined with 3 to 20% sodium dodecyl sulphate polyacrylamide gels (12). Albumin, ovalbumin, chymotrypsinogen, and cytochrome c were used as molecular weight standards. Gels were stained with Coomassie blue.

Separation of lipoproteins from donor rabbits

New Zealand rabbits were obtained from one source (Eldridge, St. Louis, MO). Blood was obtained from 16-hr fasted rabbits that had been maintained on either normal chow or chow supplemented with 2% (w/w) cholesterol for 78 days (Ralston Purina Test Diets, Richmond, IN). Animals were anesthetized with pentobarbital and blood was collected via the abdominal aorta into tubes containing EDTA (1.5 mg/ml) as anticoagulant. Plasma was obtained and lipoproteins were separated using preparative ultracentrifugation. The d < 1.006 g/ml fraction from cholesterol-fed rabbits was isolated at plasma density by centrifugation using a Beckman L8-55 ultracentrifuge. Samples were centrifuged in a 60Ti rotor for 22 hr at 50,000 rpm and maintained at 14°C. This fraction was washed in 0.15 M NaCl/1 mM EDTA solution (pH 8.2) under the same centrifugation conditions noted above. β-VLDL from hypercholesterolemic rabbits had a high cholesterol content (71%) and very little triglyceride (3%); there was 6% protein and 20% phospholipid. This fraction migrated as a broad band of beta mobility on agarose electrophoresis, and sodium dodecyl sulfate polyacrylamide gel electrophoresis showed three major protein bands corresponding to apoB-100, apoB-48, and apoE. HDL (d 1.063 to 1.21 g/ml) was isolated from plasma obtained from normal chow-fed rabbits. The harvested HDL had a chemical composition similar to that described by others (protein, 41%; cholesterol 18%; triglyceride 11%; phospholipid 31%: reviewed by Chapman, ref. 13) and apoA-1 was the predominant apoprotein.

Lipoproteins were dialyzed against 2 l of 0.15 M NaCl/1 mM EDTA (pH 8.2) with three changes over 24 hr, concentrated with the use of Aquacide II, filtered through a 0.45-μm filter, and stored in a solution containing gentamycin (0.1%, w/v), sodium azide (0.2%, w/v), and chloramphenicol (0.05%, w/v).

Radioiodination

Following dialysis to remove sodium azide, β-VLDL, HDL, and albumin (bovine serum albumin, Fraction V, Sigma), were radioiodinated by the iodine monochloride method as described by McFarlane (14). Free iodide was removed by gel filtration down a Sephadex G25 column (0.9 × 30.0 cm) and subsequent exhaustive dialysis against 0.15 M NaCl, 1 mM EDTA (pH 8.2). Specific radioactivities were: HDL 145–170; albumin 162–194 (cpm/ng of protein). Two ranges of specific radioactivities were prepared for β-VLDL; one designated as high specific radioactivity (72–99 cpm/ng of protein) and the other as low specific radioactivity (41 cpm/ng of protein). Eighty-four percent of the radioactivity in ^{125}I-labeled β-VLDL was precipitated by tetramethyl urea. As assessed by sodium dodecyl sulfate polyacrylamide gel electrophoresis, the percentage labeling of apoproteins was 77% apoB, 22% apoE, and negligible amounts in C apoproteins. Seven percent of the radioiodine was coupled to lipid constituents. Hence the particle was predominantly labeled in apoB. Na^{125}I (carrier-free) was obtained from Amersham Radiochemical Centre.

Recipient animals

Recipient control New Zealand rabbits (3.0–3.8 kg males, Eldridge) were maintained on standard chow; the experimental groups were fed a diet containing 2% (w/w) cholesterol (Ralston Purina) for 78–91 days. Food and water were available ad libitum.

Prior to administration of radioiodinated lipoproteins, rabbits were fasted overnight and injected with potassium iodide (10 mg i.v.) to reduce uptake of ^{125}I by the thyroid. Food intake was restricted throughout the study. A blood sample was obtained for the assay of plasma total cholesterol and triglyceride. Rabbits fed the cholesterol-enriched diet had high plasma cholesterol concentrations, 1545 ± 216 mg/dl, (mean ± standard error of mean); of this ~1000 mg/dl was in d < 1.006 g/ml lipoproteins (see below). Plasma cholesterol concentrations were low in the normal chow-fed group (42 ± 5 mg/dl). Plasma triglyceride concentrations were not altered by the diet. Subsequently, ^{125}I-labeled lipoproteins were administered to rabbits (1.0 mg of protein in 1–2 ml, diluted in 0.15 M NaCl/1 mM EDTA) via the marginal ear vein. For each experimental day, cholesterol-fed and normal chow-fed rabbits were injected concurrently with identical donor lipoproteins. Blood samples were collected in EDTA-containing tubes from the vein of the opposite ear, at intervals of 0.08, 0.5, 1, 2, 6, and 24 hr post-injection. Blood was centrifuged and plasma was obtained. Radioactivity in whole plasma and trichloroacetic acid (TCA)-precipitated fractions was quantified using an Isoflex gamma counter. Twenty-four hr after injection, a plasma sample was obtained for lipo-

protein fractionation (d 1.006, 1.063, and 1.21 g/ml) to determine distribution of ^{125}I.

Characterization of lipoprotein accumulation in aortic segments

Rabbits were killed with an overdose of pentobarbital at 5, 24, and 72 hr after administration of radioiodinated β-VLDL, and 24 hr after administration of ^{125}I-labeled HDL and ^{125}I-labeled albumin. Cold saline was poured into the body cavity through a ventral incision and flushed through the aorta. Aortas were rapidly dissected free and extraneous fat was removed while the excised aorta was immersed in cold saline. The procedure required approximately 15 min. Aortas were cut transversely into five portions at branching arteries to obtain the following segments: *a*) descending arch and upper thoracic, *b*) lower thoracic, *c*) upper, *d*) mid, and *e*) lower abdominal, and placed in vials for determination of ^{125}I content. Tissue was blotted lightly and wet weight was determined for each segment. To control for radioactivity that may have remained in any undissected adventitia, in selected cases the grossly visible intimal lesions were scraped from the remaining tissue, tissues were reweighed, and the residual radioactivity was determined. The mass accumulations of lipoprotein-protein in aortic segments were calculated using the specific radioactivities of the radioiodinated protein tracers in plasma. Specific radioactivities were determined with respect to the lipoprotein-protein based on the protein:cholesterol ratio of the isolated lipoprotein (which was not significantly altered over the course of the experiment) and the cholesterol concentration of this fraction in plasma. In cases where endogenous pools were too low to quantify the protein:cholesterol ratio, this value was taken from the donor lipoprotein. The protein:cholesterol ratio was 1:11.8 for β-VLDL and 2.3:1 for HDL. The d < 1.006 g/ml plasma fractions of cholesterol-fed animals had cholesterol concentrations of 998 ± 84 mg/dl. Very little cholesterol (<2 mg/dl) was found in the d < 1.006 g/ml fractions of the normal chow-fed group. Due to the small pool size, and therefore the small dilution over time, the specific radioactivity of ^{125}I-labeled β-VLDL in chow-fed animals was assumed not to change over time. In agreement with Roth et al. (8), HDL cholesterol was <2 mg/dl in the d 1.063–1.21 g/ml fraction of cholesterol-fed rabbits, and was 20 ± 5 mg/dl in normal chow-fed animals. After calculation of specific radioactivity of tracer at each bleed interval, the median specific radioactivity of the labeled lipoprotein was obtained from the area under the specific radioactivity/time plot. Area under the curve was determined using a Hewlett Packard 9864A digitizer linked to an HP model 10 calculator.

The uptake of ^{125}I-labeled lipoproteins was evaluated with respect to cholesterol content of aortas. The five segments of aorta were homogenized separately. Bligh and Dyer (15) extractions were performed. For each case the organic layer was removed and dried under nitrogen. The extract was resuspended in isopropyl alcohol and the solution was enzymatically assayed for cholesterol content using commercially available kits (Boehringer Mannheim).

In some cases the spatial distribution of radioactivity in the segments of aorta was visualized by radioautography. Fresh aortic segments from a hypercholesterolemic rabbit were stained with Sudan black to identify areas of lipid deposition. In a dark room, aortic segments were overlaid with Kodak X-O mat AR film and placed in a cassette for 24 hr at −70°C. The film was subsequently developed for visualization of the distribution of radioactivity within the aortic segments.

Calculation of plasma clearance kinetics

Since the first plasma sample collection was at 5 min (0.08 hr), and thus loss of label could result in an underestimate of radioactivity in the plasma, the concentration of the label at 0 min (as cpm/ml) in plasma was calculated from a knowledge of the injected counts and by assuming that the plasma volume was 33 ml per kg body weight (16). This value was regarded as the 100% value. Radioactivity at each time interval was expressed as a percentage of this initial value. Clearance kinetics of TCA-precipitable radioactivity from plasma was analyzed by the method of Matthews (17). The apparent half-time of catabolism was calculated by the expression:

$$t_{1/2} = \frac{\ln 2}{\text{slope of elimination phase}}.$$

Fractional catabolic rate was determined as follows:

$$FCR = \frac{1}{A'/\alpha + B'/\beta}$$

where α = slope of equilibration phase, β = slope of elimination phase A′ = A/Po, B′ = B/Po, Po = 100, A = y (ordinate) intercept of equilibration phase, B = y intercept of elimination phase (17).

Total catabolic rates were calculated as the product of the fractional catabolic rates and the pool sizes and were expressed as mg of protein/kg per 24 hr. Total catabolic rates were not determined when endogenous pools were not quantified, as in the case of the albumin study. In the cases where endogenous pools were below the limits of detection (~1 mg/dl for lipoprotein-protein), it was not possible to calculate accurate total catabolic rates. However estimates were made based on an assumed plasma concentration of 1 mg/dl. Data are presented as being less than those calculated values. These estimates, although subject to error, are generally so dramatically different

from the respective control group values that they are included for comparison.

Plasmaphoresis

Twenty-four hr after the rabbits were returned to normal chow, approximately 50 ml of blood was obtained from two hypercholesterolemic rabbits, via the ear artery, on two intervals separated by 24 hr. Blood was collected in tubes containing EDTA (1.5 mg/ml) and centrifuged to pack the erythrocytes. The plasma was decanted and replaced with the plasma expander, Hespan (American Critical Care). Following mild vortexing, the Hespan/erythrocyte mixture was returned via the marginal ear vein. Total plasma cholesterol content was determined 30 min after each plasmaphoresis and 24 hr following the plasmaphoresis.

RESULTS

Hypercholesterolemia was found to alter both the plasma clearance kinetics and the aortic accumulation of both β-VLDL and HDL, but in opposite directions. Aortic accumulation of ^{125}I-labeled β-VLDL was initially determined 5, 24, and 72 hr after injection. Aortic accumulation was greatest at 24 hr (**Fig. 1**). This time interval was subsequently used for the majority of experiments using ^{125}I-labeled β-VLDL. Aortic segments from normal chow-fed rabbits accumulated less β-VLDL (**Table 1**) and had lower cholesterol content than segments from cholesterol-fed animals (**Table 2**). Similar accumulation of β-VLDL was calculated for both high specific radioactivity ($\bar{x}$ for all segments = 33.7 ± 4.9 ng/mg wet wt/24 hr) and low specific radioactivity ($\bar{x}$ for all segments = 31.1 ± 5.4 ng/mg wet wt/24 hr) ^{125}I-labeled β-VLDL (Table 1). The aortic arch segment had the highest cholesterol content (Table 2) and most accumulation of β-VLDL (Table 1). Following processing of the complete segment, the intima was dissected and accumulation was recalculated. The dissection demonstrated that accumulation was sixfold higher in the intima than in the rest of the aortic wall. For further localization studies, en face radioautograms were performed on aortic segments from a hypercholesterolemic rabbit (**Fig. 2**). The accumulation of ^{125}I-labeled β-VLDL was much greater in regions of the atherosclerotic plaque. In some radioautograms (e.g., Fig. 2B) accumulation appeared to be greatest at the margins of atherosclerotic plaques.

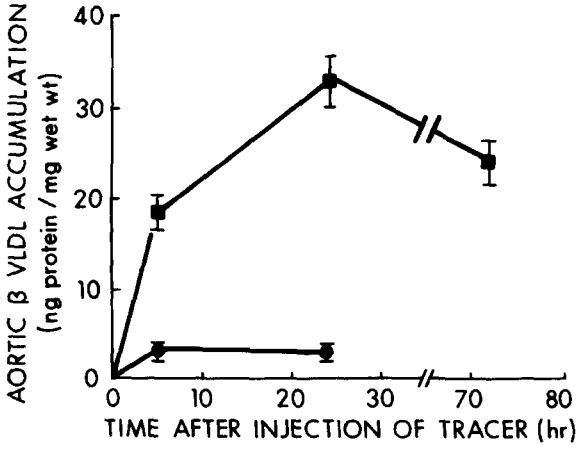

Fig. 1. The time course of aortic accumulation of β-VLDL in cholesterol-fed animals (■) and chow-fed controls (●). Points represent mean ± standard error of mean for at least four determinations.

During the processing of the plasma samples to determine plasma clearance kinetics of ^{125}I-labeled β--VLDL it became apparent that a considerable proportion of radioactivity was present as TCA-soluble constituents in normal chow-fed rabbits (**Fig. 3**). Therefore all plasma decay kinetic parameters in both groups of animals were calculated on the basis of TCA-precipitable radioactivity. The fractional catabolic rate of ^{125}I-labeled β-VLDL in normal chow-fed rabbits (0.139 hr^{-1}) was reduced after cholesterol feeding (0.011 hr^{-1}, **Table 3**). Ninety-one percent of ^{125}I-labeled β-VLDL remained in the d < 1.006 g/ml fraction in cholesterol-fed rabbits after 24 hr (**Table 4**). Both the high and low specific radioactivity ^{125}I-labeled β-VLDL preparations gave similar plasma clearance kinetics (Table 3), as would be expected if the particle was acting as a true tracer and not being modified by the extent of radioiodination. The decrease in fractional catabolic rate was accompanied by a marked rise in the half-time of elimination (28.4 hr vs 90.4 hr for normal chow- and cholesterol-fed rabbits, respectively) although the rates of equilibration between intra- and extravascular pools were similar (Table 3). Because of the large increase in plasma d < 1.006 g/ml β-migrating lipoprotein in the hypercholesterolemic group, the total flux of β-VLDL was very high (13.1 mg protein/kg per 24 hr) compared to an estimated <1.1 mg protein/kg per 24 hr for controls. Assuming a steady state of synthesis and catabolism of β-VLDL over the time of the experiment and in view of the high endogenous pool, these data suggest that the high levels of β-VLDL in plasma during cholesterol feeding of the rabbits were due to an increased rate of synthesis. To confirm this, two cholesterol-fed rabbits were returned to normal chow and plasmaphoresed 24 and 48 hr later. Plasma cholesterol was dramatically reduced by plasmaphoresis (**Fig. 4**). However, plasma cholesterol concentrations increased by 210 and 364 mg/dl in the subsequent 24 hr. These values were close to the rate of synthesis calculated from the plasma clearance curve for ^{125}I-labeled β-VLDL (increases of 379 mg/dl cholesterol were expected, based on the total catabolic rate of β-VLDL being 13.1 mg protein/kg per 24 hr and the lipoprotein protein:cholesterol ratio of 1:11.8).

TABLE 1. Aortic accumulation of β-VLDL and HDL protein over 24 hr

Injected ^{125}I-Labeled Tracer	Diet	Aortic Section				
		DA and UT	LT	UA	MA	LA
		ng protein/mg wet wt per 24 hr				
β-VLDL (HSA)	NC (4)	4.2 ± 0.5[a]	3.2 ± 0.8	2.8 ± 0.3	1.8 ± 0.3	2.0 ± 0.2
β-VLDL (HSA)	CF (4)	51.1 ± 6.1	33.9 ± 6.0	33.0 ± 6.8	21.1 ± 3.2	29.2 ± 5.2
β-VLDL (LSA)	CF (3)	52.2 ± 4.8	27.9 ± 5.4	25.1 ± 4.1	24.2 ± 2.2	31.1 ± 5.4
HDL	NC (3)	3.7 ± 0.5	3.2 ± 0.4	3.9 ± 0.4	2.6 ± 0.4	3.5 ± 0.3
HDL	CF (3)	0.5 ± 0.1	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.4 ± 0.1

DA, descending aorta; UT, upper thoracic; LT, lower thoracic; UA, upper abdominal; MA, mid-abdominal; LA, lower abdominal; NC, normal chow; CF, cholesterol-fed; HSA, high specific radioactivity; LSA, low specific radioactivity; number in parentheses = number of animals.
[a]Mean ± SE.

Using ^{125}I-labeled HDL to trace the accumulation of endogenous HDL in aortas, it was calculated that 3.4 ± 0.2 ng of protein/mg wet wt per 24 hr ($\bar{x}$ for all segments) of HDL protein was present in aortas from normal chow-fed animals (Table 1). This accumulation was decreased in the hypercholesterolemic group ($\bar{x}$ for all fractions = 0.4 ± 0.1 ng of protein/mg wet wt per 24 hr).

In contrast to ^{125}I-labeled β-VLDL, the fractional rate of catabolism of ^{125}I-labeled HDL was accelerated by cholesterol feeding (Table 3). There were no significant differences between quantity of radioactivity in whole plasma aliquots and the TCA-precipitable portion. Also, considerable redistribution of the ^{125}I between lipoprotein fractions was present in the cholesterol-fed group (Table 2). Although the fractional catabolic rate was accelerated following cholesterol feeding, due to the severe depletion of HDL (8), there was a much lower total flux (estimated as <0.3 mg protein/kg per 24 hr) compared to normal chow-fed animals (10.5 mg protein/kg per 24 hr, Table 3). Using the rationale of steady states of synthesis and catabolism described earlier, the virtual depletion of plasma HDL appeared to be due to a depression of synthesis.

To investigate the effect of hypercholesterolemia on proteins other than lipoproteins, ^{125}I-labeled albumin was used as a tracer. Hypercholesterolemia did not affect the fractional catabolic rate of ^{125}I-labeled albumin (Table 3) nor did it influence the aortic accumulation of the protein. Aortic accumulation of ^{125}I-labeled albumin in three recipient rabbits, expressed as cpm/mg wet wt, was 19.2 ± 2.7 and 19.8 ± 2.6 for normal chow-fed rabbits and cholesterol-fed rabbits, respectively. Pool size of albumin in plasma was not determined, but these data may be taken as comparable accumulation values for both groups since plasma protein concentration is not altered by cholesterol feeding (18).

DISCUSSION

In this study we investigated the influence of diet-induced hypercholesterolemia in rabbits on the in vivo accumulation in aortic wall and plasma clearance of ^{125}I-labeled β-VLDL. Currently, there is considerable interest in the possible role of β-VLDL in atherogenesis. Mahley, Weisgraber, and Innerarity (5) observed that those dogs that developed pronounced atherosclerosis when fed a high-cholesterol, high-fat diet had β-VLDL present in their plasma, whereas nonresponders lacked this lipopro-

TABLE 2. Aortic cholesterol content of normal chow-fed (NC) and cholesterol-fed (CF) recipient rabbits

Injected ^{125}I-Labeled Tracer	Diet	Aortic Sections				
		DA and UT	LT	UA	MA	LA
		mg/g wet wt				
β-VLDL	NF (4)	2.8 ± 0.2[a]	1.9 ± 0.1	3.6 ± 0.4	2.3 ± 0.9	2.1 ± 1.0
β-VLDL	CF (4)	27.5 ± 7.0	12.0 ± 1.2	14.0 ± 3.0	20.0 ± 2.1	13.8 ± 2.0
HDL	NF (3)	2.9 ± 0.6	2.8 ± 0.6	4.2 ± 1.2	3.0 ± 0.4	3.4 ± 0.3
HDL	CF (3)	30.1 ± 7.5	23.9 ± 2.5	27.9 ± 3.2	25.9 ± 5.4	23.8 ± 1.2

DA, descending aorta; UT, upper thoracic; LT, lower thoracic; UA, upper abdominal; MA, mid-abdominal; LA, lower abdominal; number in parentheses = number of animals.
[a]Mean ± SE.

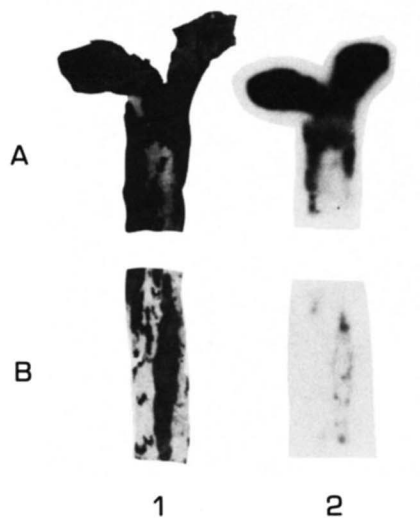

Fig. 2. Sudan-stained arterial segments from aortic arch and upper thoracic (1A) and lower thoracic (1B) regions. The accompanying en face radioautograms (2) were performed as described in the Methods section.

tein. Further work (19) has demonstrated that β-VLDL can cause massive cholesteryl ester overloading in macrophages, this cell type being the potential precursor of lipid-laden foam cells in atherosclerotic lesions. It also has been demonstrated that β-VLDL is recognized in vitro by cells that have grown out of rabbit atheromatous aortic explants (20). The current study investigated the recognition of β-VLDL by atheromatous aortas in vivo, and lends support to previous data which indicate a role for β-VLDL in progression of atherosclerosis. Following administration of tracer quantities of ^{125}I-labeled β-VLDL, we have calculated the aortic accumulation of the lipoprotein to determine whether hypercholesterolemia altered this process. There was an approximately 15-fold increase in accumulation of β-VLDL in hypercholesterolemic rabbit aortas compared to those from normal chow-fed controls. The intimal dissection demonstrated that the highest concentration of β-VLDL was in the lesion-containing parts of the vessels. Radioautographic evidence also showed that β-VLDL accumulated particularly in the atherosclerotic plaques of the aorta (Fig. 3), and in some of the atherosclerotic plaques, the radioautograms were strongly suggestive of β-VLDL being primarily located at the edges of the atheromatous lesions. These areas may represent regions of high growth, as has been demonstrated for the accumulation of radioiodinated LDL at the edge of denuded aortic endothelium (21, 22).

Since accumulation of β-VLDL was increased in aortas from hypercholesterolemic rabbits, we also measured ^{125}I-labeled albumin accumulation to observe whether hypercholesterolemia influences sequestration of a nonlipoprotein protein. Although more radioactivity was observed in atherosclerotic vessels, when radioactivity was normalized to weight, no differences in accumulation of ^{125}I were detectable 24 hr after injection of radioiodinated albumin. Further evidence that the increased presence of β-VLDL in aortas was not a generalized response to hypercholesterolemia was the fact that HDL accumulation in aortas was decreased in cholesterol-fed rabbits.

Several factors related to the vessel wall probably contributed to the accrual of β-VLDL. Cellular receptors could have played a role in *intracellular* accumulation; although it is unlikely that the LDL receptor was important, since aortic LDL receptor function is reported to be depressed during hypercholesterolemia in vivo (23) and in the presence of cholesterol in cell culture media (24). Some intracellular accumulation could have been mediated by β-VLDL (19, 25, 26) receptors, as these appear to be less susceptible to regulation by intracellular cholesterol deposition. An unknown proportion of the aortic accumulation was probably *extracellular* as well (27, 28).

Although apoproteins exchange between lipoprotein fractions (29), over 90% of ^{125}I remained in the d < 1.006 g/ml fraction even after 24 hr; thus the vast majority of aortic accumulation of radioactivity in the hypercholesterolemic rabbit was due to transport via β-VLDL. To assure ourselves that ^{125}I-labeled β-VLDL was acting as

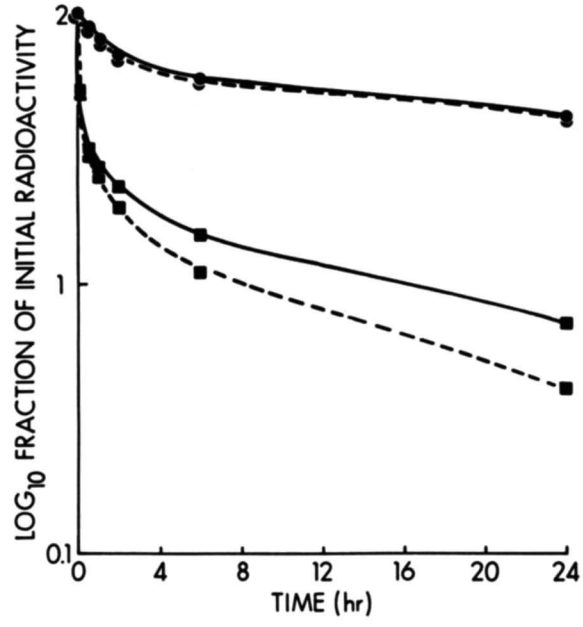

Fig. 3. Plasma clearance curves for ^{125}I-labeled β-VLDL from cholesterol-fed rabbits (n = 4, ●) and chow-fed controls (n = 4, ■). Solid lines represent radioactivity in whole plasma aliquots. Dashed lines represent the TCA-precipitable portion of these aliquots. The curves for whole and TCA-precipitable radioactivity of plasma were not significantly different in cholesterol-fed rabbits, in contrast to those fed normal chow.

TABLE 3. Kinetic analysis of the plasma clearance of labeled tracer in normal chow-fed (NC) and in cholesterol-fed rabbits (CF)

Injected ^{125}I-Labeled Tracer	Diet	Kinetic Parameters			
		$t_{\frac{1}{2a}}$	$t_{\frac{1}{2b}}$	FCR	TCR
		hr	*hr*	hr^{-1}	*mg/kg per day*
β-VLDL (HSA)	NC (4)	1.7	28.4	0.139	<1.1
β-VLDL (HSA)	CF (4)	1.9	90.4	0.011	13.1
β-VLDL (LSA)	CF (2)	2.0	84.1	0.013	15.2
HDL	NC (3)	1.6	56.8	0.021	10.5
HDL	CF (3)	1.6	44.6	0.037	<0.3
Albumin	NC (3)	2.6	54.2	0.015	
Albumin	CF (3)	1.8	62.4	0.015	

FCR, fractional catabolic rate; TCR, total catabolic rate; $t_{\frac{1}{2a}}$, rate of equilibrium between intra- and extravascular pools; $t_{\frac{1}{2b}}$, rate of clearance from plasma; HSA, high specific radioactivity; LSA, low specific radioactivity; number in parentheses = number of animals.

a true tracer of the endogenous particle, radioiodinated β-VLDL of two ranges of specific radioactivities were synthesized. The comparable calculated accumulations of endogenous β-VLDL using lipoproteins of different specific radioactivities confirmed that the label acted as a true tracer. The calculated aortic accumulations of β-VLDL probably underestimated true rates of accumulation because degradation products of β-VLDL probably effluxed from the vessel wall. Attachment of a label to β-VLDL that remains within the vessel wall would help to quantify more accurately the extent of β-VLDL accumulation in vessel walls. However, our timed experiments demonstrated that accumulation of β-VLDL peaked at 24 hr, suggesting that this was an appropriate time point for comparing the control and atherosclerotic animals. Thus, the data certainly are valid in comparing control animals and diet-induced hypercholesterolemic animals.

Although our study of plasma clearance kinetics of ^{125}I-labeled β-VLDL demonstrated a reduction in the fractional catabolic rate following cholesterol feeding (30), by virtue of the large plasma pool size, the total flux of β-VLDL was very high during hypercholesterolemia. This implied that the elevated plasma concentration of this lipoprotein must have been due to an increased rate of synthesis of this lipoprotein. The rapid reaccumulation of cholesterol in plasmas of hypercholesterolemic rabbits following the reduction produced by plasmaphoresis is in accord with this hypothesis. Elevated flux of a plasma lipoprotein has also been observed in the Watanabe heritable hyperlipidemic rabbit model of atherosclerosis (31).

Other studies of ^{125}I-labeled β-VLDL kinetics in cholesterol-fed rabbits showed reductions in fractional catabolic rates, although the changes were not as profound as those reported here (30, 32). This difference was likely to be due to their use of β-VLDL from unfasted animals, compared to fasted in the present study. Recently it has been realized that β-VLDL is structurally and metabol-

TABLE 4. Distribution of ^{125}I in density-fractionated plasma 24 hr after administration of radioiodinated lipoprotein in recipient normal chow-fed (NC) and in cholesterol-fed (CF) rabbits

Injected ^{125}I-Labeled Tracer	Diet	Lipoprotein Density Fractions		
		d < 1.006 g/ml	d 1.006-1.063 g/ml	d > 1.063 g/ml
		% of total counts		
β-VLDL	NC (4)[a]	49 ± 1[b]	20 ± 1	26 ± 1
β-VLDL	CF (4)	90 ± 2	8 ± 2	3
HDL	NC (3)	3 ± 1	2 ± 1	94 ± 2
HDL	CF (3)	16 ± 1	15 ± 3	70 ± 2

[a](Number of animals).
[b]Mean ± SD.

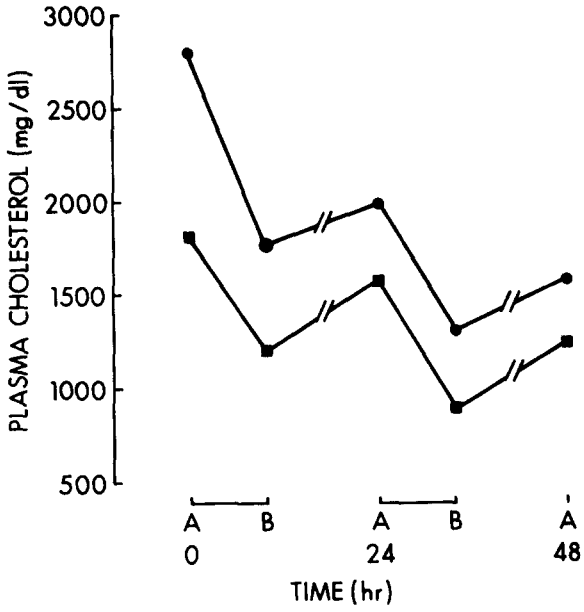

Fig. 4. Plasma cholesterol levels of two hypercholesterolemic rabbits that were subjected to plasmaphoresis at 0 and 24 hr. Plasma cholesterol levels were determined immediately prior to plasmaphoresis (A); 30 min after each procedure (B); and 24 hr after the second plasmaphoresis.

ically heterogeneous, with two populations being defined in rhesus monkeys (33), dogs, type III hyperlipidemic patients (34), and rabbits (Daugherty, A., L. G. Lange, B. E. Sobel, and G. Schonfeld, unpublished observations). The larger-sized component of β-VLDL is thought to consist of chylomicron remnants that are rapidly cleared from plasma (34). The present study used unfractionated β-VLDL, and consequently the plasma clearance kinetics shown are a composite of the two subfractions of this lipoprotein. Hence the faster rate of clearance in the aforementioned studies, compared to the present, probably reflects the use of whole β-VLDL containing a large proportion of the chylomicron remnant fraction.

Aortic accumulation of HDL has been quantified in cholesterol-fed rabbits (35), but the accumulation compared to normocholesterolemic groups has not been documented. Our study demonstrated that HDL accumulated in aortic segments of normal chow-fed animals, and hypercholesterolemia greatly reduced this effect. In fact, the small accumulation of HDL that was noted in the cholesterol-fed animals may be an overestimate, since some radioactivity was present in the d < 1.006 g/ml fraction after administration of ^{125}I-labeled HDL. Thus, small amounts of radioactivity in the aortic segments could have been delivered via β-VLDL. Based on the in vitro studies of Innerarity et al. (36), it is possible that the profound decrease in HDL accumulation within aortic tissue could have contributed to the large accumulation of β-cholesterol. Assuming that β-VLDL-cholesterol is taken up with β-VLDL-protein, a low accumulation of HDL could exacerbate the cholesterol loading into atherosclerotic lesions.

Cholesterol feeding enhanced plasma clearance kinetics of ^{125}I-labeled HDL with an increase in the fractional catabolic rate of the labeled lipoprotein. This result was consistent with the data of others (37). Measurement of the flux of HDL suggests that the severe depletion of HDL (8) in the cholesterol-fed rabbit was due to a depressed rate of synthesis.

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