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Research Article

Chemical modification of lysine residues by acetoacetylation of the apoproteins of iodinated canine and human low density lipoproteins (LDL) and canine high density lipoproteins (HDL) resulted in a marked acceleration in the rate of removal of these lipoproteins from the plasma after intravenous injection into dogs. Clearance of the lipoproteins from the plasma correlated with their rapid appearance in the liver. Acetoacetylated canine ^{125}I-LDL (30-60% of the lysine residues modified) were essentially completely removed from the plasma within an hour, and > 75% of the activity cleared within 5 min. Reversal of the acetoacetylation of the lysine residues of the LDL restored to these lipoproteins a rate of clearance essentially identical to that of control LDL. Identical results were obtained with modified human LDL injected into dogs. At 10 min, when ≅ 90% of the acetoacetylated human ^{125}I-LDL had been removed from the plasma, 90% of the total injected activity could be accounted for in the liver. Furthermore, it was possible to demonstrate an enhancement in uptake and degradation of acetoacetylated LDL by canine peritoneal macrophages in vitro. The mechanism(s) responsible for the enhanced removal of the LDL and HDL in vivo and in vitro remains to be determined. By contrast, however, acetoacetylation of canine ^{125}I-apoE HDL_c did not accelerate their rate of removal from the plasma but, in fact, […]

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ABSTRACT Chemical modification of lysine residues by acetoacetylation of the apoproteins of iodinated canine and human low density lipoproteins (LDL) and canine high density lipoproteins (HDL) resulted in a marked acceleration in the rate of removal of these lipoproteins from the plasma after intravenous injection into dogs. Clearance of the lipoproteins from the plasma correlated with their rapid appearance in the liver. Acetoacetylated canine ^{125}I-LDL (30–60% of the lysine residues modified) were essentially completely removed from the plasma within an hour, and >75% of the activity cleared within 5 min. Reversal of the acetoacetylation of the lysine residues of the LDL restored to these lipoproteins a rate of clearance essentially identical to that of control LDL. Identical results were obtained with modified human LDL injected into dogs. At 10 min, when ≅90% of the acetoacetylated human ^{125}I-LDL had been removed from the plasma, 90% of the total injected activity could be accounted for in the liver. Furthermore, it was possible to demonstrate an enhancement in uptake and degradation of acetoacetylated LDL by canine peritoneal macrophages in vitro. The mechanism(s) responsible for the enhanced removal of the LDL and HDL in vivo and in vitro remains to be determined. By contrast, however, acetoacetylation of canine ^{125}I-apoE HDL$_c$ did not accelerate their rate of removal from the plasma but, in fact, retarded their clearance. Control (native) apoE HDL$_c$ were removed from the plasma (64% within 20 min) and rapidly appeared in the liver (39% at 20 min). At the same time point, only 45% of the acetoacetylated apoE HDL$_c$ were cleared from the plasma and <10% appeared in the liver. Acetoacetylation of the apoE HDL$_c$ did not enhance their uptake or degradation by macrophages. The rapid clearance from the plasma of the native apoE HDL$_c$ in normal and hypercholesterolemic dogs suggests that the liver may be a normal site for the removal of the cholesteryl ester-rich apoE HDL$_c$. The retardation in removal after acetoacetylation of apoE HDL$_c$ indicates that the uptake process may be mediated by a lysine-dependent recognition system.

INTRODUCTION

Plasma lipoproteins that contain the B or E apoproteins have been shown to bind to the same low density lipoprotein (LDL)[1] receptors on the surface of human fibroblasts (1, 2). High density lipoproteins (HDL), which lack the B and E apoproteins, do not bind to these receptors (1, 2). It has been speculated that the E apoprotein of HDL$_1$ and HDL$_c$ (lipoproteins that bind to the receptors) and the B apoprotein of LDL may have a common structural sequence responsible for their interaction with the receptors (1). Furthermore, it has been shown that modification of lysine or arginine residues of LDL and HDL$_c$ abolished their ability to react with the receptors (3, 4). This study was designed to determine if selective modification of lysine residues by acetoacetylation would alter the in vivo catabolism of plasma lipoproteins.

Dogs were used for these lipoprotein metabolism studies (5, 6). The B apoprotein is the major protein constituent of both canine and human LDL. Furthermore, cholesterol feeding of dogs induces the appearance of a plasma lipoprotein similar in some respects to LDL except that this cholesterol-induced lipoprotein, referred to as apoE HDL$_c$, contains the E apoprotein

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[1] *Abbreviations used in this paper*: HDL, high density lipoproteins; LDL, low density lipoproteins.

as the only detectable apoprotein constituent and no B apoprotein (6). Canine HDL (d = 1.087–1.21), similar to human HDL_3, contain primarily the A-I apoprotein and only small amounts of other proteins (A-II and C apoproteins). Therefore, it was possible to compare in vivo the effects of lysine modification on lipoproteins that contain primarily the B (LDL), E (HDL_c), or A-I (HDL) apoproteins.

METHODS

Lipoproteins. Normal canine LDL (d = 1.02–1.063) were isolated by ultracentrifugation from the plasma of fasted NIH foxhounds and purified by Geon-Pevikon block electrophoresis (5). Normal canine HDL (d = 1.087–1.21) were isolated by ultracentrifugation at 59,000 rpm (60 Ti rotor) for 36 h and recentrifuged at d = 1.21 for 24 h. ApoE HDL_c (d = 1.006–1.02) were isolated and purified from the plasma of dogs fed diets containing coconut oil and cholesterol, as described (6). Human LDL (d = 1.02–1.05) and HDL_3 (d = 1.125–1.21) from a normal fasted subject were isolated as described (2). Canine albumin was isolated from serum by block electrophoresis (5). All lipoproteins were isolated and used in the in vivo and in vitro studies within 2 wk after the blood was obtained. We have determined that the binding activities of canine and human LDL and canine HDL_c with human fibroblasts were unaltered by storage for up to 3 wk after isolation. Untreated (native) and modified lipoproteins within an individual experiment represented separate aliquots of the same batch of lipoproteins prepared in parallel. Therefore, within an individual experiment, the lipoproteins used were the same age and represented the same lipoprotein except for the modification or lack of modification. Canine LDL, HDL, and albumin and human LDL and HDL_3 were iodinated (^{125}I) by the iodine monochloride method (7) and HDL_c (^{125}I and ^{131}I) by the Bolton and Hunter (8) procedure (≅0.1% of lysines modified). The specific activities of the LDL and HDL_c used in all studies were similar and ranged from 100 to 196 cpm/ng of protein. Lipid labeling was <4% for canine and human LDL and <2% for the apoE HDL_c. The iodinated LDL and HDL_c were consistently >97 and 98% precipitable with TCA, respectively.

Chemical modifications. Lysine residues of the ^{125}I-labeled lipoproteins were modified by acetoacetylation with diketene as described in detail (3). Lipoprotein (1 mg in 0.1 M borate, pH 8.5) was treated with 0.2–4.0 μmol of diketene for 5 min (26°C). The reaction was stopped by dialysis against 0.2 M carbonate-bicarbonate buffer, pH 9.5. The amount of diketene required was determined empirically by measuring the extent of acetoacetylation for each lipoprotein (3). Reversal of the acetoacetylation was accomplished by incubating the modified lipoproteins with 0.5 M hydroxylamine for 16 h, as described elsewhere (3). Iodinated LDL and albumin were carbamylated with potassium cyanate as described (3). The extent of lysine modification was determined by the trinitrobenzenesulfonic acid colorimetric assay (9) and by amino acid analysis (3). The colorimetric assay gave values 7% higher than amino acid analysis, and results are reported on the basis of amino acid analysis. Control and modified lipoproteins were characterized by paper electrophoresis, apoprotein content by gel electrophoresis, and particle morphology by negative-staining electron microscopy (3, 5).

In vivo studies. Iodinated lipoproteins or albumin (0.4–1.0 mg of protein) were injected into the cephalic vein of 15–20 kg male NIH foxhounds fed a normal dog chow. For specific studies, hypercholesterolemia was produced by feeding the semisynthetic coconut oil and cholesterol diet to foxhounds as described (6). Blood samples were obtained from the jugular vein at the designated times. Liver biopsies (thin slices of <200 mg) were removed from pentobarbital-anesthetized dogs through an abdominal incision from two different sites at the designated times. Bleeding was stopped by the use of Gelfoam (Upjohn Co., Kalamazoo, Mich.). The slices of liver (1–2 mm in thickness) were washed in saline to remove blood, blotted, weighed, digested in Protosol, and counted. The total weight of the liver was determined by weighing the liver of the dog at the termination of the study after excess blood had been drained from the organ. Plasma levels were based on plasma volume of 4.5% (body weight). This was validated in several dogs by using Evans blue dye to determine plasma volume.

Canine peritoneal macrophages. The method for production and isolation of peritoneal macrophages was modified from Stephenson and Osterman (10). Male foxhounds (15–20 kg) were injected with 100 ml i.p. of sterile light mineral oil (Barre Drug Co., Baltimore, Md.). After 7 d, 1 liter of sterile saline was infused into the peritoneal cavity of the anesthetized dogs, and then the fluid was removed with a stylocath (Abbott Diagnostics, North Chicago, Ill.). The cells were pelleted by centrifugation at 160 g for 5 min and then resuspended at 1×10^7 cells/ml in Dulbecco's modified Eagle medium containing 20% fetal calf serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). Cells (2 ml) were dispensed into 35-mm Petri dishes and allowed to settle for 2 h in a humidified (5–8% CO_2) incubator at 37°C. They were then washed twice with Hanks' base salt solution (GIBCO catalogue No. 310-4020, Grand Island Biological, Grand Island, N. Y.) and incubated with Dulbecco's modified Eagle medium and 20% fetal calf serum until the experiment was performed 16–20 h later. In contrast to polymorphonuclear leukocytes, macrophages rapidly become adherent to the dishes. By light and phase microscopy, the adherent cells were large, flat, motile cells, characteristic of macrophages (11), which contained translucent intracellular vacuoles, presumably ingested oil.

In vitro assays. Assays for binding and internalization and degradation were performed on the macrophages for 6 h (37°C) as reported for fibroblasts (2), except that the incubation media (1 ml/dish) contained 20% fetal calf serum. Each dish contained ≅0.1 mg of cell protein.

RESULTS

Acetoacetylation of ≅30–60% of the lysine residues of canine and human ^{125}I-LDL resulted in a markedly accelerated clearance of these lipoproteins from the plasma of the dog after intravenous injection. As shown in Fig. 1 and Table I for representative experiments, >75% of the acetoacetylated canine LDL were removed from the plasma within 5 min after injection. The activity did not reappear in the plasma (Fig. 1). By comparison, approximately one-half of the total injected dose of the control ^{125}I-LDL remained in the plasma for up to 6 h. Reversal of the acetoacetylation by hydroxylamine treatment restored to the LDL a clearance rate essentially identical to that of control LDL (Fig. 1). Analysis of the modified, reversed LDL revealed that <1% of the lysine residues remained modified. When compared with control LDL, the aceto-

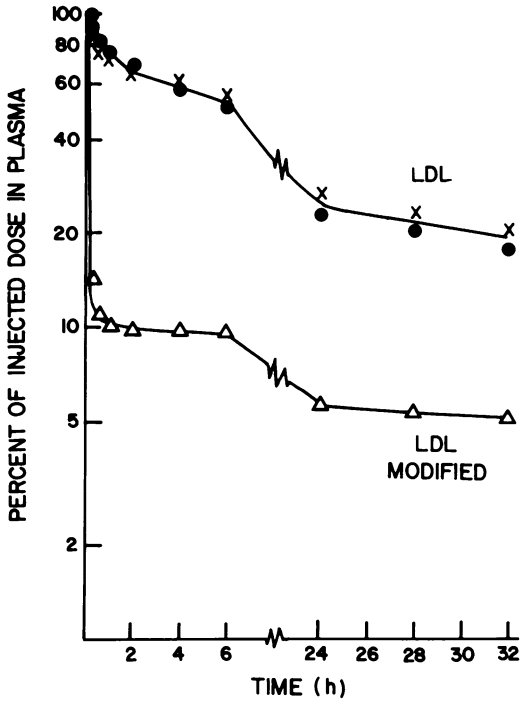

FIGURE 1 Percentage of the total injected dose of control canine ^{125}I-LDL (●), acetoacetylated ^{125}I-LDL (△) that had 56% of the lysine residues modified, and acetoacetylated-reversed ^{125}I-LDL (×) remaining in the canine plasma. 1 mg of lipoprotein protein was injected into each dog.

acetylated LDL had a similar chemical composition, apoprotein pattern, and morphologic appearance by negative-staining electron microscopy. Furthermore, as documented (3), the only detectable alteration in the acetoacetylated lipoproteins was an increased electrophoretic mobility indicative of the neutralization of the positive charge on the modified lysine residues. Additional data, compiled in Table I, document the consistent finding that acetoacetylation of >30% of the lysine residues accelerated the clearance of LDL from the plasma. These data were obtained in individual animals.

Before injection, 97% of the radioactivity of the control canine LDL and acetoacetylated LDL was precipitable with TCA, and >98% of the radioactivity associated with the LDL was in the B apoprotein as determined by tetramethylurea precipitation. 1 h after injection of the control LDL, the lipoproteins that remained in the plasma were reisolated and characterized. Greater than 90% of the activity that remained floated by ultracentrifugation at $d < 1.21$ and 95% was associated with the B apoprotein. With the acetoacetylated LDL, only 13% of the total injected activity remained in the plasma at 1 h, and of that only 1% floated at $d < 1.21$. The remainder was found in the $d > 1.21$ fraction, and essentially all of that activity was represented by low molecular weight material, which passed through a dialysis membrane. The dialyzable material was not further characterized but, as shown later, degradation products (^{125}I-tyrosine) rapidly appeared in the plasma of animals receiving acetoacetylated LDL. The acetoacetylated LDL, therefore, were rapidly and almost completely cleared from the plasma in <1 h.

When modified human ^{125}I-LDL were injected into dogs, accelerated removal of these lipoproteins was also observed. As shown in Fig. 2, ≅90% of the acetoacetylated human LDL (48% of the lysine residues modified) were removed from the plasma and 90% of the total injected dose could be accounted for in the liver within 10 min. At the same time point, 81 and 8% of the total control LDL were in the plasma and liver, respectively. Acetoacetylation of 20% of the lysine

TABLE I
Percentage of the Total Injected Dose Remaining in the Plasma of Each Dog

	Control LDL	Acetoacetylated-reversed LDL	Acetoacetylated LDL	Carbamylated LDL‖		
5 min	94	90	24* 4§	10		
30 min	86	83	13‡ —	5		
	Control albumin	Carbamylated albumin¶	Control canine HDL	Control human HDL_3	Acetoacetylated canine HDL**	Acetoacetylated human HDL_3
5 min	95	71	89	100	68	67
30 min	88	45	81	86	55	61

* 35% of total lysine residues modified.
‡ 32% of total injected activity in the liver.
§ Greater than 50% of the total lysine residues modified.
‖ Level of modification was not measured but expected level would be 30–40% of the lysine residues modified, as described (3).
¶ 22% of total lysine residues modified.
** 78% of total lysine residues modified.

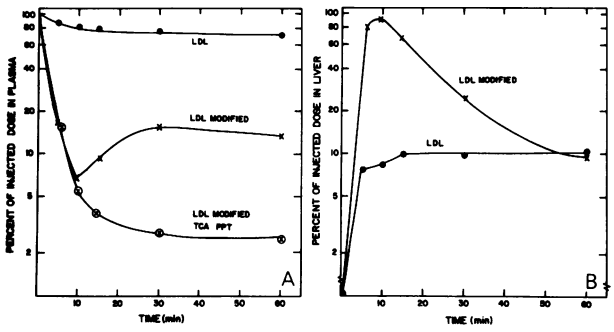

FIGURE 2 Percentage of the total injected dose of human control ^{125}I-LDL (●) and acetoacetylated LDL (×, 48% of the lysine residues modified) that remained in the plasma (A) and that appeared in the liver (B). With control LDL, >98% of the activity remaining in the plasma was TCA precipitable. With modified LDL, a significant fraction of the plasma radioactivity after 5 min was not TCA precipitable; data replotted on the basis of percent of injected dose remaining in the plasma which was TCA precipitable (⊗). The activity in the liver was calculated on the basis of the actual weight of the liver. Values obtained for the duplicate biopsies at each time point agreed within 5%. At each time point, the counts per minute per liver biopsy (100–200 mg of liver) were in excess of 1,200 cpm (range, 1,200–36,000 cpm). 1 mg of lipoprotein protein was injected.

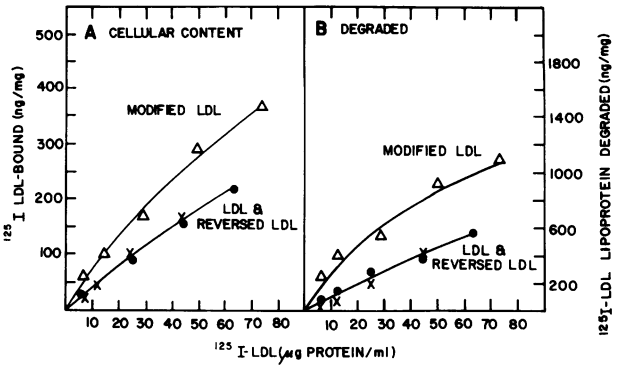

FIGURE 3 Accumulation (A) and degradation (B) of iodinated canine LDL by canine peritoneal macrophages in vitro. Control ^{125}I-LDL (●), acetoacetylated ^{125}I-LDL (△) that had 56% of the lysine residues modified, and acetoacetylated (56%)-reversed (<1% of the residues remained modified) ^{125}I-LDL (×) were compared directly in assays performed at 37°C.

residues of human ^{125}I-LDL did not significantly alter their removal rate. The nearly quantitative hepatic uptake of acetoacetylated human and rat LDL (30–60% of the lysine residues modified) has been documented by studies in rats. Furthermore, the activity in the livers of the rats has been localized by autoradiography in the Kupffer cells.[2]

Modification of lysine residues of canine LDL and canine albumin by carbamylation also resulted in their accelerated clearance from the plasma (Table I). Likewise, canine HDL (d = 1.087–1.21) removal from the plasma was accelerated after acetoacetylation of 78% of the lysine residues (Table I), but the removal rate for HDL was unaltered when <40% of the lysine residues were modified. Acetoacetylation of human HDL_3 (d = 1.125–1.21) also resulted in an accelerated disappearance from the plasma (Table I).

Because the accelerated hepatic removal of modified LDL appeared to be mediated by the Kupffer cells, studies were undertaken to determine if enhanced uptake would occur in peritoneal macrophages maintained in culture. Macrophages were shown to bind and internalize (Fig. 3A) and degrade (Fig. 3B) very small quantities of normal canine LDL at levels that were ≅5% of the values observed with normal human fibroblasts. However, acetoacetylation of the LDL increased the amount of lipoprotein bound and degraded (Figs. 3 and 4). The most dramatic effects (an 8- to 10-fold enhancement) were seen with an LDL preparation that had 73% of the lysine residues modified (Fig. 4). Modified LDL that had been incubated with hydroxylamine to reverse the acetoacetylation of the lysine residues gave results identical to those of the control LDL (Fig. 3). This further indicated that the acetoacetylation had not irreversibly altered the LDL. All the acetoacetylated LDL used in the studies with the macrophages were incapable of binding to the high affinity cell receptors of human fibroblasts. It has previously been reported that acetoacetylated LDL (>20% of the lysine residues modified) were not bound and degraded by fibroblasts (3).

In striking contrast to the accelerated rate of removal of acetoacetylated canine and human LDL and canine

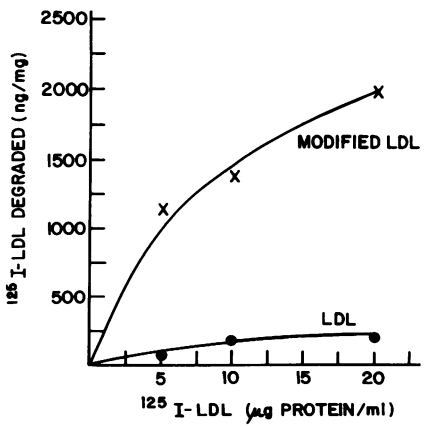

FIGURE 4 Degradation of canine LDL by macrophages. Control ^{125}I-LDL (●) were compared with acetoacetylated ^{125}I-LDL (×, 73% of the lysine residues modified).

[2] Mahley, R. W., K. H. Weisgraber, T. L. Innerarity, and H. G. Windmueller. 1979. Accelerated clearance of low-density and high-density lipoproteins and retarded clearance of E apoprotein-containing lipoproteins from the plasma of rats after modification of lysine residues. *Proc. Natl. Acad. Sci. U. S. A.* **76**: 1746–1750.

HDL from the plasma, canine apoE HDL_c modified to a similar extent (30–60% of the lysine residues modified) were cleared from the plasma much more slowly than the unmodified (control) HDL_c (Fig. 5). Control apoE HDL_c were normally removed from the plasma more rapidly than LDL (Fig. 1). The acute phase of the removal of control HDL_c could be accounted for by a rapid hepatic uptake (Fig. 5). By 20 min after the injection, 64% of the control HDL_c had been cleared from the plasma and 39% of the total injected dose could be accounted for in the liver. However, after

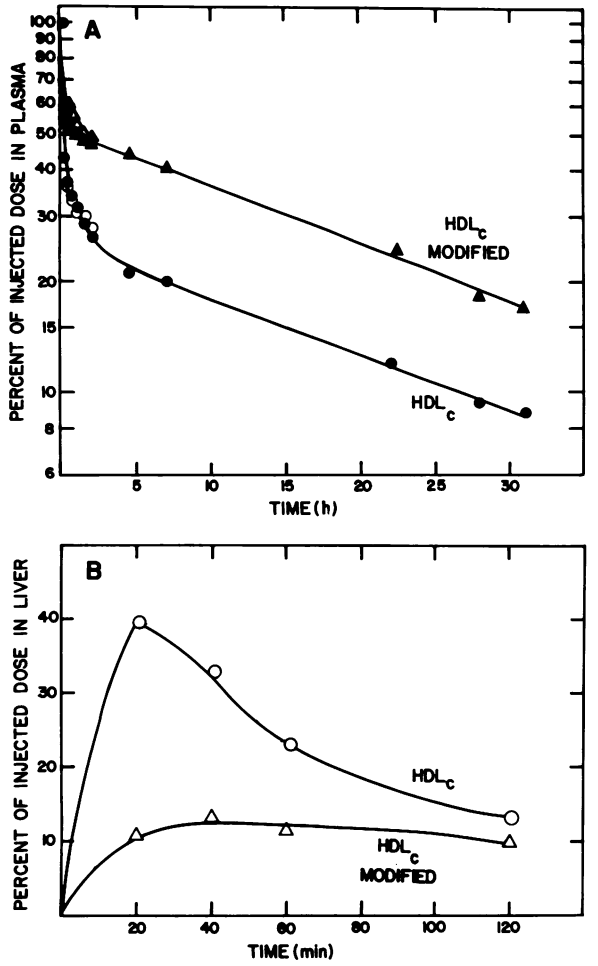

FIGURE 5 Percentage of the total injected dose of control ^{125}I-apoE HDL_c (●) and acetoacetylated ^{125}I-apoE HDL_c (▲, 60% of the lysine residues modified) that remained in the plasma with time in hours (A) and that appeared in the liver in minutes (B). Additional data from two separate dogs which received either control ^{125}I-apoE HDL_c (○) or acetoacetylated ^{125}I-apoE HDL_c (△). The activity in the liver was calculated on the basis of the actual weight of the liver. Values obtained for the duplicate biopsies at each time point agreed within 5%. At each time point, the counts per minute per liver biopsy (100–200 mg of liver) were in excess of 1,100 cpm (range, 1,100–5,700 cpm). 400 μg of lipoprotein protein was injected.

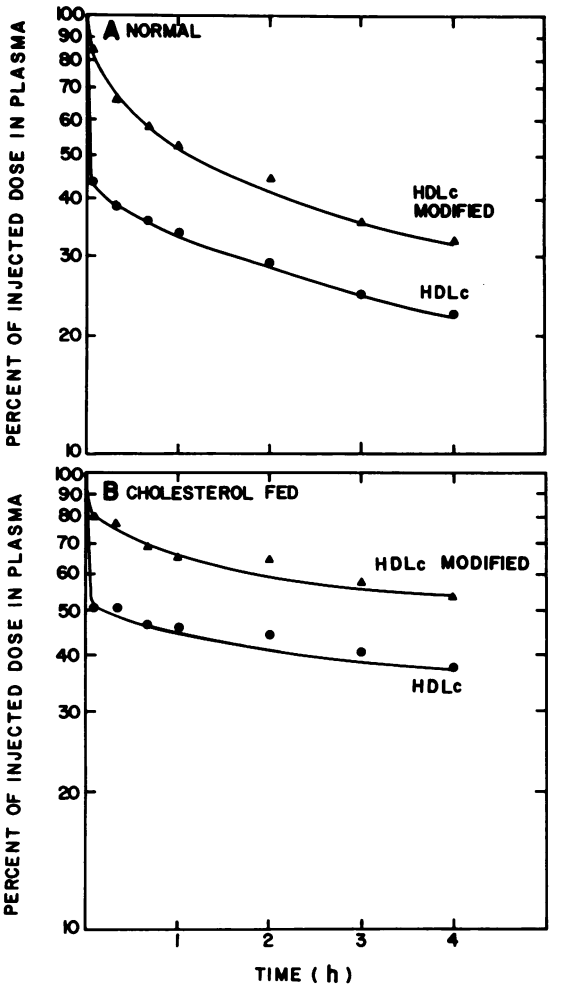

FIGURE 6 Percentage of the total injected dose of native ^{131}I-apoE HDL_c (●) and acetoacetylated ^{125}I-apoE HDL_c (▲) that remained in the plasma of a normolipidemic (A) or a hypercholesterolemic (B) dog. The native ^{131}I-HDL_c (100 cpm/ng of protein) and the acetoacetylated ^{125}I-HDL_c (166 cpm/ng of protein) were injected simultaneously into each dog (0.4 mg of each lipoprotein based on protein). The hypercholesterolemic dog had a plasma cholesterol of ≅400 mg/dl after being on diet ≅30 d.

acetoacetylation, HDL_c removal was markedly retarded (Fig. 5), and at several time points after injection, ≅10% of the total activity was present in the liver. These results suggested that a large fraction of the apoE HDL_c was normally taken up by the liver and that lysine modification retarded the recognition and(or) removal process.

These results were confirmed by the simultaneous injection of native ^{131}I-apoE HDL_c and acetoacetylated ^{125}I-apoE HDL_c into the same normolipidemic dog and by the measurement of the disappearance of both isotopes from the plasma (Fig. 6A). The unmodified and modified apoE HDL_c were isolated from the same plasma and were prepared in parallel for injection.

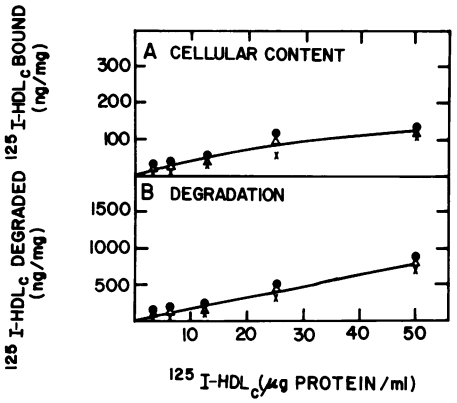

FIGURE 7 Accumulation (A) and degradation (B) of iodinated apoE HDL$_c$ by canine peritoneal macrophages. Control ^{125}I-apoE HDL$_c$ (●) were compared to ^{125}I-apoE HDL$_c$ that had 54% (×) and 64% (△) of the lysine residues modified.

By 5 min after injection, 56% of the native apoE HDL$_c$ and 15% of the acetoacetylated apoE HDL$_c$ had been removed from the plasma. After the rapid, acute phase of clearance, the rates of disappearance of the native and modified apoE HDL$_c$ from the plasma were similar (see Discussion).

Consideration was given to the possibility that the rapid removal of the unmodified (native) apoE HDL$_c$ could be a result of the absence of an HDL$_c$ pool in the plasma of normal dogs. However, the rapid clearance of the unmodified ^{131}I-apoE HDL$_c$ and the retarded clearance of the acetoacetylated ^{125}I-apoE HDL$_c$ were similarly observed when these lipoproteins were simultaneously injected into a hypercholesterolemic dog (Fig. 6B). Within 5 min, 50% of the native apoE HDL$_c$ had been cleared from the plasma, whereas only 20% of the acetoacetylated apoE HDL$_c$ was removed. After the initial few minutes, the rates of removal of the native and modified apoE HDL$_c$ appeared to be slower in the hypercholesterolemic dog than in the normal dog (see Discussion).

The native and acetoacetylated apoE HDL$_c$ were incubated with peritoneal macrophages maintained under culture conditions. As shown in Fig. 7 for a representative experiment, control apoE HDL$_c$ were not taken up and degraded by peritoneal macrophages to a significant extent by comparison with results obtained in cultured human fibroblasts (1–3). Moreover, acetoacetylation of HDL$_c$, even at 64% lysine modification, did not enhance the uptake or degradation of these lipoproteins by the macrophages (Fig. 7). We have been unable to modify >64% of the lysine residues, presumably because the other residues are inaccessible to the reagent.

DISCUSSION

Aggregated and heat or chemically denatured proteins are rapidly cleared from the plasma by the reticuloendothelial system, including Kupffer cells of the liver and other scavenger cells throughout the body. It has been suggested that modifications that increase the net negative charge on proteins may also trigger the rapid uptake of altered proteins (11, 13, 14). This study indicates that selective modification of lysine residues without aggregation or precipitation can stimulate clearance of certain proteins (LDL, HDL, and albumin) from the plasma. For several reasons, acetoacetylation with diketene is a particularly useful procedure with which to study this process. Acetoacetylation selectively modifies the lysine residues as previously shown for both LDL and HDL$_c$ (3). The positive charge on the ε-amino group of lysine is neutralized, and a net increase in the negativity of the lipoproteins is observed. Other physical and chemical properties of the lipoproteins including lipid and protein composition and particle size and morphology are unchanged (3). In addition, we have not detected an alteration in the protein conformation of LDL by circular dichroism after extensive lysine modification (unpublished data); however, although it is impossible to rule out discrete changes that may occur, there are no detectable gross alterations. Furthermore, acetoacetylation of lysine residues can be quantitatively reversed, and the modified, reversed lipoproteins have been shown to regain metabolic activity very similar to that of the native lipoproteins. As reported (3), acetoacetylated LDL are incapable of binding to the cell surface receptors of fibroblasts, but after the reversal of the modification, LDL regain nearly full binding activity. A similar reversibility of the rapid plasma clearance of LDL is presented here.

Rapid plasma clearance of acetoacetylated ^{125}I-LDL (essentially complete in less than an hour) and the appearance of activity in the liver were correlated with an enhancement in uptake and degradation of these modified LDL by peritoneal macrophages. Whether or not recognition of acetoacetylated LDL by the macrophages and by the liver is mediated by a common process and whether or not the stimulus for uptake by either system is an alteration in positive charge is open to speculation. However, it has been shown that Kupffer cells are primarily responsible for hepatic uptake.[2] In agreement with this study, Goldstein et al. (14) have reported that acetylated and maleylated LDL are taken up and degraded by peritoneal macrophages. Uptake of ^{125}I-acetyl-LDL is mediated by high affinity, trypsin- and pronase-sensitive binding sites on the surface of macrophages that recognize acetyl-LDL but not native LDL.

In contrast to the results obtained with LDL and HDL, acetoacetylation of apoE HDL$_c$ produced a dramatically different response. Modification of ^{125}I-HDL$_c$ retarded their clearance from the plasma and reduced the amount of activity in the liver. In fact, native HDL$_c$ were rapidly cleared (>60% of the total

injected dose within an hour) by comparison to native LDL and acetoacetylated HDL_c, and rapidly appeared in the liver. A marked reduction in hepatic uptake of acetoacetylated HDL_c has been confirmed, as well, in the rat.[2] Acetoacetylation of HDL_c did not enhance the uptake or degradation of these lipoproteins by peritoneal macrophages.

Several tentative conclusions are suggested by the data. The observation that the unmodified apoE HDL_c were rapidly removed from the plasma and appeared in the liver within minutes of injection suggests that the liver may be a normal site for clearance of apoE HDL_c. The retardation in removal by acetoacetylation of the apoE HDL_c indicates that the E apoprotein might be involved in the hepatic removal process and that the process may be mediated by a lysine-dependent recognition system. Previously, we have demonstrated that the lysine residues of the apoE HDL_c play a functional role in the interaction of these lipoproteins with the cell surface receptors of fibroblasts (3). However, it should be pointed out that after the acute phase (0–60 min after injection) the rates of clearance of the unmodified (native) and the modified apoE HDL_c were similar. The most likely explanation for this is that the apoE of the HDL_c redistributes with time to other lipoproteins and is no longer associated exclusively with the cholesteryl ester-rich apoE HDL_c. Once redistribution occurs (the time required for this to occur to a significant extent remains unknown), then the rate of removal of the iodinated apoE from the plasma would depend upon the actual class of lipoproteins with which the label is associated. We have previously demonstrated that the apoE of various lipoproteins does redistribute after injection into rats (15). This does not negate the significance of our observation that acetoacetylation of the E apoprotein acutely retarded the uptake of the modified apoE HDL_c as compared with the more rapid uptake of the unmodified apoE HDL_c. However, it is difficult to prove that the clearance of the unmodified apoE HDL_c by the liver during the first few minutes was associated exclusively with the uptake of the apoE HDL_c particles. Recognition and uptake by the liver might require that the lipoprotein have a critical mass of the E apoprotein, as occurs in the cholesteryl ester-rich apoE HDL_c.

The similarity in the data obtained acutely in the hypercholesterolemic dog as compared to the results in a normolipidemic dog indicates that the pool size of plasma HDL_c does not have a significant effect on the rapid uptake process (<1 h after injection). However, between 1 and 4 h, the overall rate of removal of both the unmodified and modified apoE HDL_c was slower in the hypercholesterolemic dog than in the normal dog. This is consistent with the idea of redistribution of the apoE to a larger pool of apoE containing lipoproteins. The cholesterol-fed dog had a plasma cholesterol level of ≅400 mg/dl and an abundance of plasma HDL_c, as reported (6). These results in the acute phase of the study support the conclusion that acetoacetylation of 30% or more of the lysine residues of apoE HDL_c interferes with the hepatic removal process.

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