Interactions of high density lipoprotein subclasses (HDL_2 and HDL_c) with dog adipocytes: selective effects of cholesterol and saturated fat feeding

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Abstract Adipose tissue is a cholesterol storage organ and derives its cholesterol primarily from circulating lipoproteins. The present study shows that adipocytes isolated from canine omental fat tissue interact specifically with high density lipoprotein subfractions lacking or enriched in apolipoprotein E, namely canine high density lipoprotein-2 (HDL_2) and HDL_c, respectively. While ^{125}I-labeled HDL_2 binding was inhibited similarly by both excess unlabeled HDL_c and HDL_2, ^{125}I-labeled HDL_c interaction was inhibited by its homologous ligand only. Paired studies showed that the amount of HDL_c associated with adipocytes was significantly higher compared to HDL_2. The effect of a short-term cholesterol and saturated fat feeding on adipocyte–HDL interaction was examined using fat cells obtained from dogs before and again 3 weeks after a diet supplemented with cholesterol (1% w/w) and saturated fat (30% lard, w/w). Significant increases in body weight and omental fat cell weight occurred after fat feeding. The amount of ^{125}I-labeled HDL_2 that could be bound to adipocytes increased after the diet, whether expressed on a per cell basis ($P < 0.005$) or per unit cell surface ($P < 0.025$). The amount of cell-associated ^{125}I-labeled HDL_c, however, was not significantly affected by the cholesterol-rich diet. The characteristics of HDL_c and HDL_2 dissociation were assessed by examining the release of labeled lipoproteins from adipocytes preincubated with ^{125}I-labeled HDL_c and ^{125}I-labeled HDL_2. HDL_2 dissociation from adipocytes was significantly decreased ($P < 0.05$) following the diet and may explain in part the apparent increase in cell-associated ^{125}I-labeled HDL_2. The dissociation of ^{125}I-labeled HDL_c from adipocytes before and after the high-fat diet was similar. These results demonstrate modulation of HDL binding to dog adipocytes by dietary fat and show that cellular interactions of HDL_c and HDL_2 are regulated differently. — **Fong, B. S., J-P. Despres, P. Julien, and A. Angel.** Interactions of high density lipoprotein subclasses (HDL_2 and HDL_c) with dog adipocytes: selective effects of cholesterol and saturated fat feeding. *J. Lipid Res.* 1988. **29:** 553–561.

Supplementary key words adipocyte–lipoprotein interaction • HDL receptors

High density lipoproteins specifically bind to many cell types. Generally, their physiological function varies depending on the tissue (1–10). This may involve cholesterol delivery to the liver for bile acid formation and excretion (11), to adrenal and gonadal tissues for steroid hormone synthesis (1, 3, 4, 12), or to adipose tissue where a large storage pool is found (10, 13). Clinical interest in HDL is based upon epidemiological studies which show that plasma HDL-cholesterol correlates negatively with the incidence of coronary heart disease (14, 15). It is thought that HDL's protective effect relates to its cholesterol acceptor function as these particles promote efflux of cholesterol from peripheral tissues for transport back to the liver, a process usually known as reverse cholesterol transport (11).

Cholesterol feeding to dogs and other experimental animals (16) induces the appearance of a unique HDL subclass rich in apoE called HDL_c that is associated with the development of hypercholesterolemia and atherosclerosis (17). It has also been suggested that HDL_c is involved both in reverse cholesterol transport as well as in the delivery of cholesterol to peripheral tissues (18). The latter notion has not been directly validated by in vivo or in vitro studies of cellular binding and metabolism of this lipoprotein species. Furthermore, the cholesterol diets used in these earlier studies (16–18) were also supplemented with propylthiouracil, an antithyroid drug. Thus any effect of the diet was confounded by the effects of hypothyroidism. The present study examines the impact of a short-term cholesterol and saturated fat diet alone on the interaction of dog HDL_c and HDL_2 with isolated dog adipocytes. Adipose tissue was examined because it is a major cholesterol storage organ in vivo (13) and derives its cholesterol primarily from circulating lipoproteins and is a known peripheral site of HDL metabolism (10).

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; KRBA, Krebs-Ringer bicarbonate buffer with albumin.

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METHODS

Experimental animals and diet

Adult mongrel dogs weighing 15–25 kg were employed. Prior to experimentation, they were maintained on a commercial chow (Purina Lab Canine Diet) for at least 2 months. The cholesterol-rich diet contained by weight 1% cholesterol, 30% lard, and 69% Purina canine chow and was prepared by dissolving crystalline cholesterol (Sigma) into melted lard (Tenderflake, Canada Packers Inc.) at 80°C and adding this mixture to ground dog chow. The dogs were fed 400 g of the cholesterol-rich diet per day (5.56 kcal/g) such that the total caloric intake was similar to dogs fed the control diet.

Adipocyte isolation

Fat tissue biopsies were obtained under general anesthesia from the omentum of dogs prior to and 3 weeks after the cholesterol-rich diet. Experimental procedures were in accordance with the recommendations of the Canadian Council on Animal Care. Adipocytes were isolated by collagenase digestion according to the procedure of Rodbell (19) with minor modifications (20). Mean fat cell size was determined using a microscope equipped with a graduated ocular as previously described (21).

Lipoprotein isolation and labeling

Canine HDL_c was isolated from dogs fed the cholesterol-saturated fat diet for 3 weeks. The animals were fasted overnight and blood was withdrawn from a cephalic vein into a syringe containing 7.5% EDTA. After removal of VLDL from the plasma by ultracentrifugation, a fraction of density 1.006–1.063 g/ml was isolated. This fraction was separated into LDL and two HDL_c subfractions with different electrophoretic mobility by Pevikon electrophoresis (22). Dog HDL_2 was isolated at density 1.087–1.21 g/ml by sequential ultracentrifugation (23) from plasma of dogs fed the control chow diet.

All lipoproteins were analyzed by agarose gel electrophoresis (Beckman Paragon electrophoresis) and by sodium dodecyl sulfate polyacrylamide gel (10%) electrophoresis (24). Both HDL_c subfractions contained apoprotein A-I as the major apoprotein but differed in apoE content. The slower migrating HDL_c contained significantly more apoE (**Fig. 1**) and this fraction was employed in the present study. HDL_2 contained apoproteins A and C but no apoE (Fig. 1).

Lipoproteins were iodinated by the iodine monochloride method of McFarlane with minor modifications (25).

Protein and lipid determinations

Protein was measured by the procedure of Lowry et al. (26). Total cholesterol and triglycerides were determined using commercial kits purchased from Fisher and Sigma, respectively. Phospholipid content was measured using the method of Bartlett (27).

Cell lipids were extracted by the procedure of Folch, Lees, and Sloane Stanley (28). [^{3}H]Cholesterol was added to the sample at the time of extraction in order to assess recovery, which was >95% in all experiments. The extracted lipids were saponified by the method of Abell et al. (29) and total cholesterol was determined in the nonsaponified fraction as described by Chiamori and Henry (30).

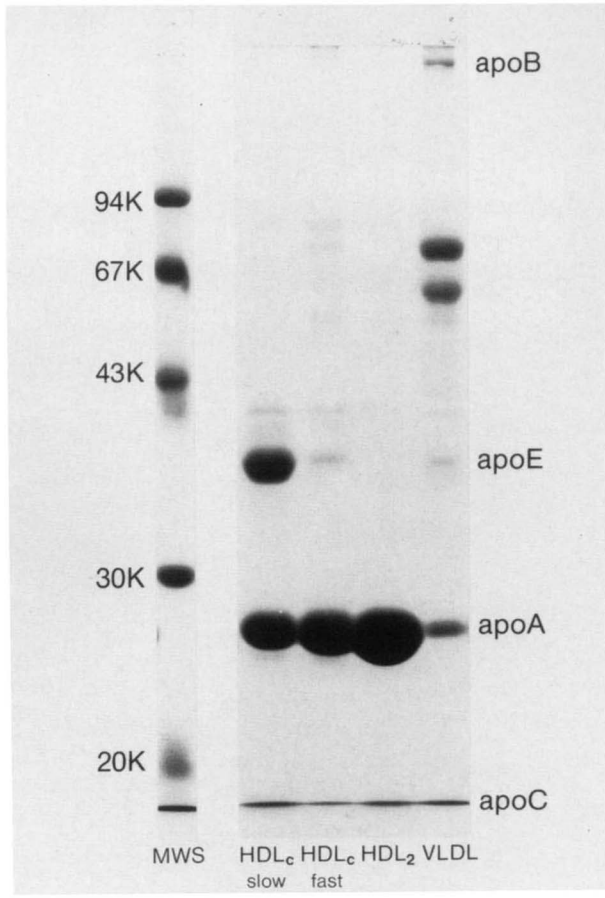

Fig. 1. Sodium dodecyl sulfate polyacrylamide (10%) gel electrophoresis of HDL_c and HDL_2 fractions of dog plasma lipoproteins. The apolipoproteins were stained with Coomassie blue and compared to VLDL and molecular weight standards (phosphorylase b, 94,000; BSA, 67,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean trypsin inhibitor, 20,000).

Assay of lipoprotein interaction with adipocytes

A modification of the procedure previously described for human adipocytes (10) was employed. Isolated adipocytes (100 mg of lipid per flask) were incubated with gentle shaking at 37°C with ^{125}I-labeled HDL_2 or HDL_c in plastic bottles in 2 ml of Krebs-Ringer bicarbonate buffer containing 5% bovine serum albumin (KRBA) and 5 mM glucose. After incubation, the medium and cells

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were separated by brief centrifugation and the cells were washed four times with 2.5 ml of KRBA at room temperature. The cells were then denatured with 5 ml of 10% trichloroacetic acid (TCA) and heated at 80°C for 10 min. The TCA precipitate was extracted twice with 5 ml of ethanol–ether 3:1 followed by one extraction with 5 ml of diethyl ether. The TCA supernatant was delipidated by extraction with 5 ml of petroleum ether. The organic extracts were pooled and used for determination of total cell lipid weight as described by Folch et al. (28). The TCA-insoluble lipid-extracted residues were dried and dissolved in 1 ml of 1 N NaOH and counted. This radioactivity represents cell-associated HDL material and includes HDL bound to the fat cell surface, lipoproteins sequestered in the plasma membranes as well as internalized particles.

Cell-mediated degradation of ^{125}I-labeled HDL was measured by adding 1 ml of 10% TCA to 1 ml of incubation medium after removal of cells. The clear TCA-soluble supernatant was extracted with $CHCl_3$, H_2O_2, and KI to remove inorganic $^{125}I^-$. The amount of radioactivity in the $CHCl_3$-extracted TCA-soluble fraction of the medium was taken to represent cell-mediated degradation of labeled lipoproteins. Non-cell-mediated degradation of labeled ligand was measured in flasks containing medium without cells and the values were subtracted from those with cells.

In each of these experiments, HDL and fat tissues were obtained from separate animals. Plasma was obtained from the dog 2 weeks prior to the fat cell experiment and the appropriate HDL fraction was freshly isolated and used within 1 week after isolation.

Lipoprotein dissociation studies

Adipocytes were incubated for 2 hr at 37°C with 5 μg/ml ^{125}I-labeled HDL_c or ^{125}I-labeled HDL_2 under conditions similar to those described for lipoprotein uptake assay. Cells were then washed four times at room temperature with KRBA to remove unbound HDL, resuspended in 2 ml of fresh KRBA and 5 mM glucose, and incubated again at 37°C for various times. At the end of this second incubation, cells were separated from the medium and TCA-precipitable ^{125}I radioactivity that remained associated with the cell was determined as described above.

RESULTS

The apoprotein and lipid compositions of HDL_c and HDL_2 used in the present study are shown in Fig. 1 and **Table 1.** In contrast to HDL_2, HDL_c contained apoE (Fig. 1) and significantly more cholesterol, triglycerides, and phospholipids (Table 1).

TABLE 1. Composition of canine HDL_c and HDL_2

	Mass Ratios		
	Chol/Prot	TG/Prot	PL/Prot
HDL_c	2.160 ± 0.190	0.071 ± 0.014	2.365 ± 0.266
HDL_2	0.395 ± 0.016	0.013 ± 0.004	0.903 ± 0.030

All values are mean ± SEM, n = 6. Chol, cholesterol; Prot, protein; TG, triglycerides; PL, phospholipids.

The effects of a 3-week diet enriched with cholesterol and saturated fat on body weight, plasma cholesterol concentration, fat cell size, and cholesterol content of normal euthyroid dogs are shown in **Table 2.** The body weight of these animals showed a slight but significant increase ($P < 0.001$) after the short-term diet. Hypercholesterolemia was induced as the mean plasma cholesterol concentration increased about twofold and is consistent with our previously published results (22). A significant increase in omental fat cell size was also observed ($P < 0.005$) after the diet. The mean fat cell cholesterol content, determined as a weight ratio to total lipid weight (*mg/g of lipid*) was not significantly different before or after the cholesterol-saturated fat feeding. However, when fat cell cholesterol was expressed on a per cell basis (μg/10^6 cells), a significant increase ($P < 0.05$) was obtained after the high fat diet. A significant and positive correlation was also observed between this fat cell cholesterol content and fat cell weight (**Fig. 2;** $r = 0.72$, n = 16, $P < 0.01$). This result is consistent with a number of previously reported studies (see ref. 13).

In preliminary studies defining conditions, time course experiments of the interaction of dog HDL_2 and HDL_c with dog adipocytes showed that the amount of ^{125}I-labeled HDL_c and ^{125}I-labeled HDL_2 associated with fat cells from control and cholesterol-fed dogs reached a plateau by 1–2 hr of incubation. A 25-fold excess of the homologous unlabeled HDL inhibited 50–70% of the total amount of cell-associated ^{125}I-labeled HDL_c or ^{125}I-labeled HDL_2 radioactivity. TCA-soluble ^{125}I radioactivity that could not be removed by chloroform extraction, and representing small peptides, was detected in the incubation medium and amounted to less than 10% of the total cell-associated radioactivity. A trivial amount of TCA-soluble radioactivity was found in the cells, suggesting little or no intracellular degradation of ^{125}I-labeled HDL_c or ^{125}I-labeled HDL_2 in the dog adipocytes.

The effect of HDL_c and HDL_2 on each other's interaction with dog adipocytes was examined in cross-inhibition studies. **Fig. 3** shows that while excess unlabeled HDL_c inhibited the amount of cell-associated ^{125}I-labeled HDL_c radioactivity by 55%, HDL_2 at 50-fold protein excess was much less effective against ^{125}I-labeled HDL_c interaction

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TABLE 2. Effect of cholesterol and saturated fat feeding on body weight, plasma cholesterol level, fat cell size, and cholesterol content

Variable (unit)	Before Diet	After Diet	*P* Value (*t*-Test)
Body weight (kg)	22.90 ± 1.42	23.50 ± 1.40	<0.001
Plasma chol level (mg%)	129 ± 4.97	270 ± 15.9	<0.001
Fat cell weight (μg of lipid/cell)	0.30 ± 0.05	0.42 ± 0.06	<0.005
Fat cell chol (mg/g of lipid)	1.12 ± 0.13	1.16 ± 0.10	ns
Fat cell chol (μg/10^6 cells)	322 ± 50	425 ± 58	<0.05

Fat cell diameter was determined using a microscope equipped with a graduated ocular. Fat cell volume was calculated from each diameter using the equation of a sphere and fat cell weight was derived from adipocyte volume using the density of triolein (0.915). Fat cell cholesterol was determined in the nonsaponified fraction of total lipid extracted from isolated cells as described in Methods. All values are mean ± SEM, n = 8.

with adipocytes (Fig. 3a). In contrast, unlabeled HDL_c inhibited ^{125}I-labeled HDL_2 interaction as effectively as unlabeled HDL_2 (Fig. 3b).

The effect of the short-term cholesterol and saturated fat diet on HDL_2 and HDL_c interaction with dog fat cells was investigated by using adipocytes from the same animal before and 3 weeks after feeding. **Fig. 4** shows concentration-dependent interaction of ^{125}I-labeled HDL_c and ^{125}I-labeled HDL_2 with the dog adipocytes. While the amounts of cell-associated ^{125}I-labeled HDL_c before and after the high fat diet were similar (Fig. 4a), significant increases in ^{125}I-labeled HDL_2 cell-associated radioactivity were found after the high fat diet at all concentrations of ligand (Fig. 4b). The affinity constant (K_m) and maximal binding capacity (V_{max}) were derived from double reciprocal plots of HDL_2 cell-associated radioactivity versus HDL_2 concentration. Results from three different experiments showed increases in K_m of HDL_2 interaction from 37.57 ± 4.13 to 93.20 ± 20.05 μg/ml and in V_{max} from 0.72 ± 0.25 to 2.25 ± 0.42 μg/10^6 cells (mean ± SEM, n = 3) after the high fat diet.

In another series of validation experiments using six other animals, the effect of the diet on HDL_2 versus HDL_c interaction was further examined. In these studies,

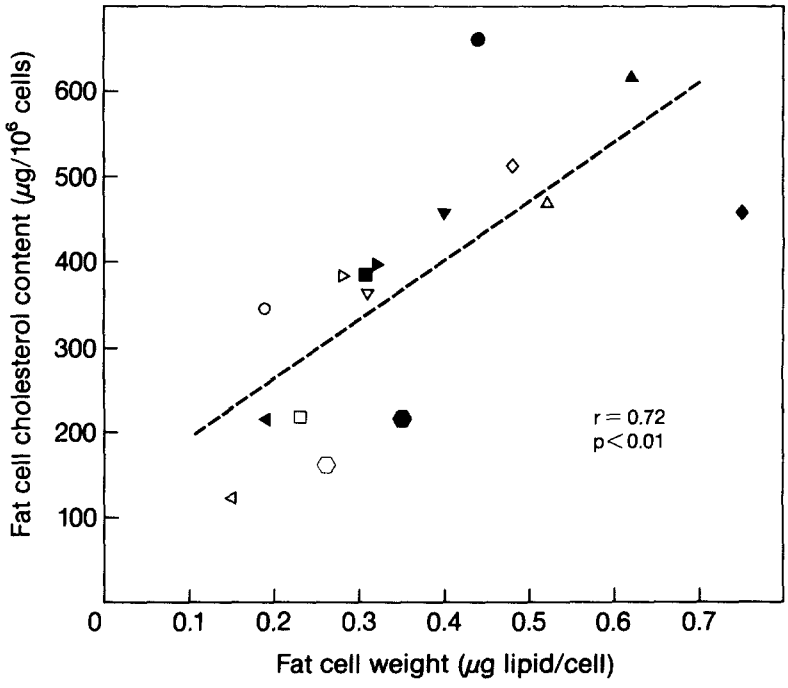

Fig. 2. Correlation between fat cell cholesterol content and fat cell weight. The mean fat cell diameter of at least 500 fat cells was determined under a microscope equipped with a graduated ocular. Fat cell volume was calculated by using the equation of a sphere and the density of triolein (0.915 g/ml) was used in the calculation of fat cell weight. Adipocytes obtained from the same dog were represented by the same symbol (open symbols: before and solid symbols: after cholesterol-saturated fat feeding).

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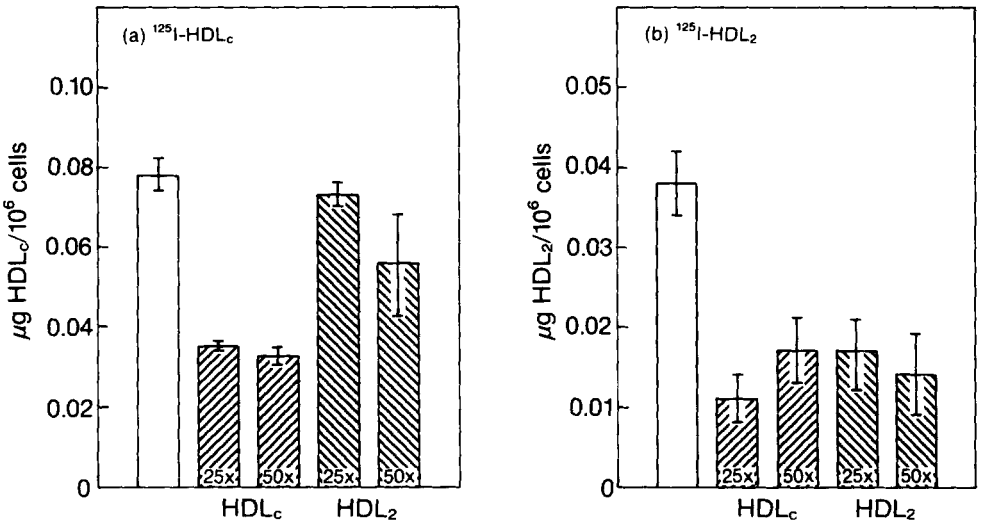

Fig. 3. Effect of excess unlabeled HDL$_c$ and HDL$_2$ on ^{125}I-labeled HDL$_c$ and ^{125}I-labeled HDL$_2$ interaction with dog adipocytes. Isolated adipocytes from the same dog were incubated with 5 μg of protein/ml ^{125}I-labeled HDL$_c$ or HDL$_2$ in the absence (open bar) or presence (hatched bar) of the indicated amount of unlabeled HDL for 2 hr at 37°C. TCA-precipitable, cell-associated ^{125}I-labeled HDL$_c$ and ^{125}I-labeled HDL$_2$ activity was assayed as described in Methods. Each point is the average ± range of duplicate assays.

a ligand concentration of 5 μg/ml was employed and the results of these studies are summarized in **Fig. 5.** The interaction of ^{125}I-labeled HDL$_c$ with dog adipocytes was not significantly altered after 3 weeks of the high fat diet (0.17 ± 0.03 μg HDL$_c$ protein/2 hr · 10^6 cells before the diet vs. 0.23 ± 0.03 μg HDL$_c$ protein/2 hr · 10^6 cells after the diet, mean ± SEM, n = 7). In contrast, a twofold increase in ^{125}I-labeled HDL$_2$ binding was observed

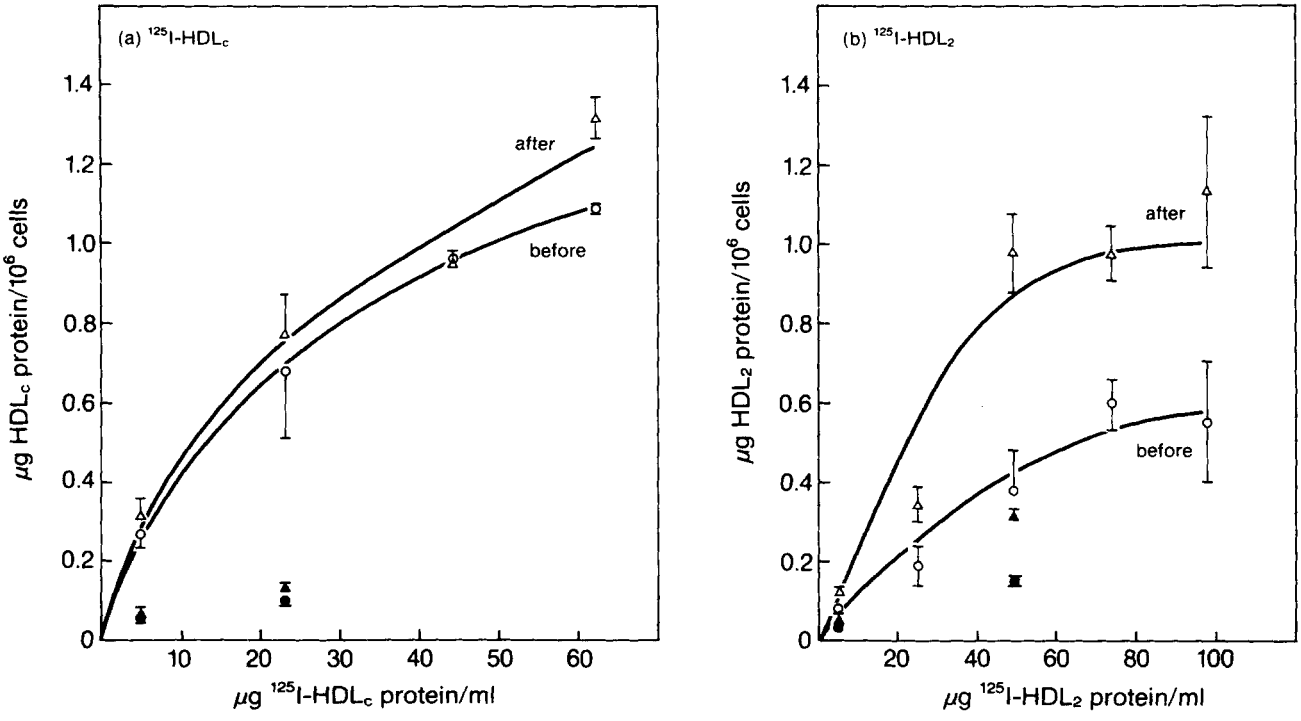

Fig. 4. Effect of cholesterol and saturated fat diet on concentration-dependent ^{125}I-labeled HDL$_c$ and ^{125}I-labeled HDL$_2$ interaction with dog adipocytes. Adipose tissue biopsies were obtained from the omentum of the same dog before and after 3 weeks of feeding on the cholesterol-saturated fat diet. Isolated adipocytes were incubated with varying concentration of ^{125}I-labeled HDL$_c$ or HDL$_2$ for 2 hr at 37°C in the presence (solid symbol) or absence (open symbol) of 25-fold excess unlabeled homologous ligand as described in Methods. Results represent TCA-precipitable, cell-associated HDL activity and each point is the mean of triplicate assays. A different dog was employed in the HDL$_c$ and HDL$_2$ studies.

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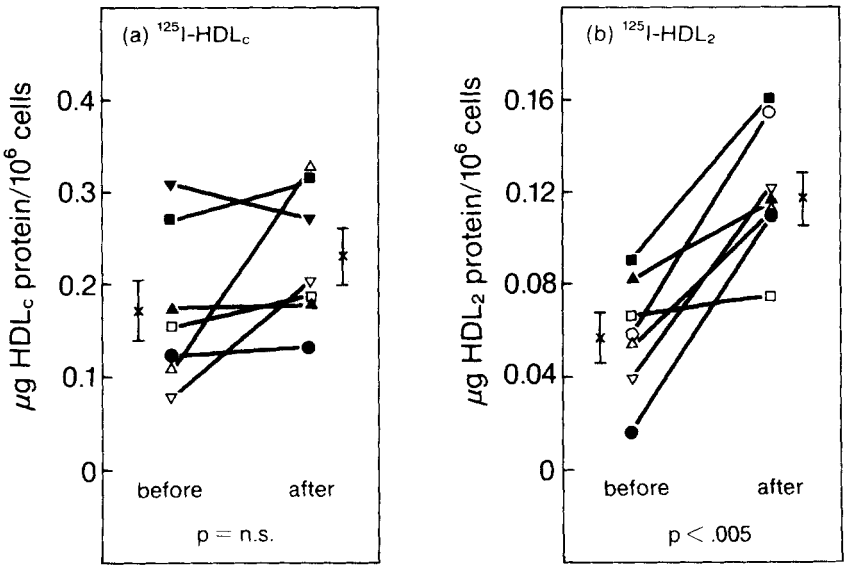

Fig. 5. Effect of cholesterol and saturated fat diet on ^{125}I-labeled HDL$_c$ and ^{125}I-labeled HDL$_2$ interaction with dog adipocytes. Assays were carried out using 5 μg of protein/ml of ^{125}I-labeled HDL$_c$ or ^{125}I-labeled HDL$_2$. Results obtained from the same dog are represented by the same symbol and dogs represented by (■) and (▲) correspond to those in Fig. 5a and Fig. 5b, respectively. Also shown are mean values ± SEM, n = 7. Interaction with HDL$_2$ was significantly increased after cholesterol feeding ($P < 0.005$ by paired t-test) while HDL$_c$ interaction was not.

(0.06 ± 0.01 μg HDL$_2$ protein/2 hr · 10^6 cells before diet vs. 0.12 ± 0.01 μg HDL$_2$ protein/2 hr · 10^6 cells after the diet, mean ± SEM, n = 7, $P < 0.005$). It was also noted that in experiments where both ^{125}I-labeled HDL$_c$ and ^{125}I-labeled HDL$_2$ cell-associated radioactivities were assayed at the same time with adipocytes from the same animal, ^{125}I-labeled HDL$_c$ binding was significantly greater than that of ^{125}I-labeled HDL$_2$ ($P < 0.005$ before diet and $P < 0.01$ after diet). Correlational analyses on fat cell HDL interaction and cholesterol content showed no significant relationship.

Since cholesterol feeding also altered fat cell size (Table 1 and Fig. 2), the above data were also expressed per unit cell surface area. Such analysis indicates that cholesterol feeding was associated with a significant increase in HDL$_2$ interaction [(25.71 ± 4.96) × 10^{-13} μg HDL$_2$ protein/2 hr · μm^2 before the diet vs. (44.14 ± 6.58) × 10^{-13} μg HDL$_2$ protein/2 hr · μm^2 after the diet, $P < 0.025$]. There was again no significant change in HDL$_c$ uptake when analyzed on a per unit cell surface basis [(82.14 ± 15.64) × 10^{-13} μg HDL$_c$ protein/2 hr · μm^2 before the diet vs. (92.86 ± 13.19) × 10^{-13} μg HDL$_c$ protein/2 hr · μm^2 after the diet]. Thus the increase in HDL$_2$ interaction after cholesterol feeding could not be attributed simply to an increase in fat cell size.

A portion of the ^{125}I-labeled I HDL$_c$ and ^{125}I-labeled HDL$_2$ activity associated with the adipocytes was found to be reversibly bound and could be released by subsequent incubation in lipoprotein-deficient medium. Experiments were therefore carried out to determine whether the cholesterol and saturated fat diet would affect this dissociation process. **Fig. 6** shows the results of a study using adipocytes isolated from the same dog before and after the high fat diet. The initial dissociation of cell-bound ^{125}I-labeled HDL$_c$ activity was increased after the cholesterol-rich diet with a tendency to level off after 1 hr (Fig. 6a). On the other hand, dissociation of ^{125}I-labeled HDL$_2$ from control dog adipocytes leveled off after 30 min and no dissociation of ^{125}I-labeled HDL$_2$ activity was observed after cholesterol-saturated fat feeding (Fig. 6b). Further studies in a total of six dogs demonstrated that dissociation of cell-bound ^{125}I-labeled HDL$_c$ was not significantly different (**Fig. 7a**), in contrast to the dissociation of cell-bound ^{125}I-labeled HDL$_2$ (Fig. 7b) which was significantly reduced ($P < 0.05$) after the diet.

DISCUSSION

Changes in nutritional state have been shown to affect the expression of lipoprotein receptors. Prolonged fasting of adult dogs for instance induces the appearance of an apoB,E receptor in dog liver (31, 32) capable of binding HDL containing apoE. The same receptor, on the other hand, is suppressed in immature dogs by cholesterol and saturated fat feeding (2). The present study demonstrates

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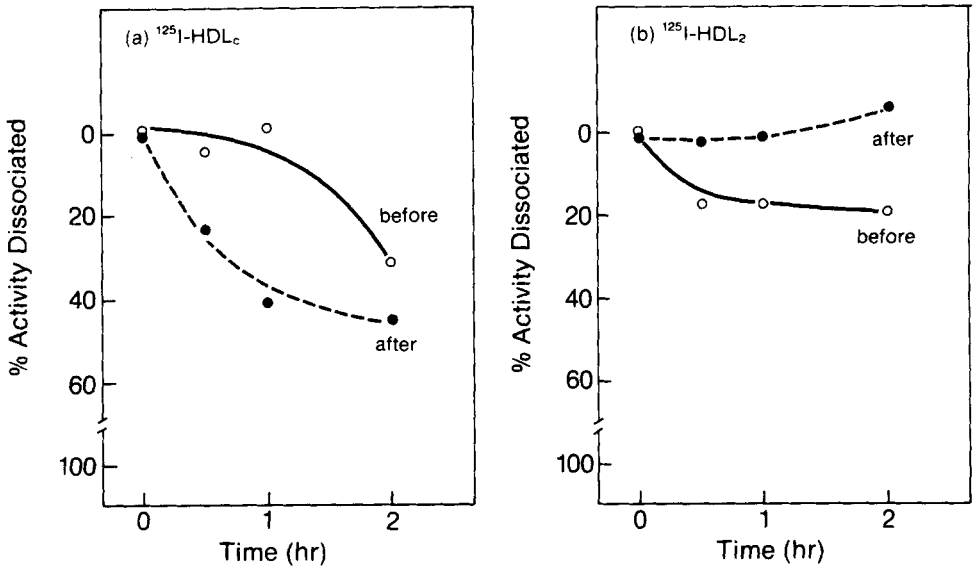

Fig. 6. Effect of cholesterol and saturated fat feeding on ^{125}I-labeled HDL$_c$ and ^{125}I-labeled HDL$_2$ dissociation from dog adipocytes. Adipocytes isolated from omental fat tissue biopsies of the same dog before (open circle) and after 3 weeks (solid circle) of cholesterol and saturated fat diet were incubated with 5μg of protein/ml ^{125}I-labeled HDL$_c$ or ^{125}I-labeled HDL$_2$ for 2 hr at 37°C. Cells were then washed four times with KRBA at room temperature to remove unbound ^{125}I-labeled HDL and resuspended in fresh KRBA. After further incubation for 0 to 2 hr at 37°C, cells were separated from medium and the amount of TCA-precipitable ^{125}I-labeled HDL radioactivity that remained associated with the cell was determined. Dissociation was calculated as the difference between this activity and that initially associated (0 time) with the cell.

that extrahepatic lipoprotein recognition sites are also affected by dietary cholesterol and saturated fat feeding. Our findings indicate that adipose tissue interaction with apoE-deficient HDL$_2$ is selectively enhanced by cholesterol and saturated fat diet. In contrast, apoE-rich HDL$_c$ interaction with dog fat cells was not affected by the fat feeding.

The nature of the receptors involved in HDL$_c$ and HDL$_2$ recognition in dog adipocytes is not established at this time. The differential effects of the short-term high-fat diet on HDL$_c$ versus HDL$_2$ binding (Figs. 4 and 5) and dissociation (Figs. 6 and 7) suggest the presence of separate receptors that differ in their metabolic regulation. At least two independent lipoprotein binding proteins have been isolated from dog liver membranes (33), the apoB,E receptor which is down-regulated by cholesterol feeding as already mentioned (32), and the putative apoE receptor which is more refractory to acute dietary manipulation (32). To draw an analogy with dog liver membranes, it is suggested that an apoE-type receptor may be involved in HDL$_c$ binding to dog adipocytes. The absence of a significant dietary effect on HDL$_c$ binding to adipocytes supports such a hypothesis. On the other hand, the interaction of adipocytes with HDL$_2$, which contains mainly apoA-I and lacks apoB or E, must be mediated by another HDL recognition site distinct from the apoE and the apoB,E receptors. Because of its broad specificity, this site may be the "lipoprotein binding site," a term originally suggested by Bachorik et al. (5) to describe the specific binding of apoE-free HDL to cultured porcine hepatocytes (5). Enhanced HDL$_2$ interaction with adipocytes by fat feeding as opposed to down-regulation also supports the involvement of a lipoprotein binding site other than the apoB,E and the apoE receptors.

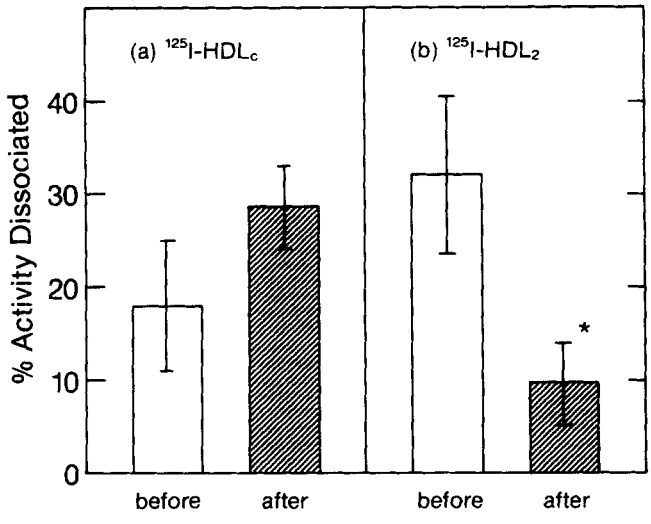

Fig. 7. Effect of cholesterol and saturated fat diet on ^{125}I-labeled HDL$_c$ and ^{125}I-labeled HDL$_2$ dissociation from dog adipocytes. Experimental conditions were as described in legend to Fig. 6. The cells were washed to remove unbound ^{125}I-labeled HDL, resuspended in fresh KRBA, and further incubated at 37°C for 1 hr. ^{125}I-labeled HDL radioactivity that remained associated with the cell was determined after this second incubation and the amount of HDL dissociation was calculated. Each point represents the mean ± SEM of results obtained from six different animals.

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In addition, the cross-competition results (Fig. 3) may be interpreted to suggest that HDL_c could bind to both HDL_c and HDL_2 receptors, while HDL_2 binds to the HDL_2 receptor only. The binding of HDL_c to the HDL_2 site might account for a small fraction of total HDL_c binding and could explain the finding that HDL_c binding exceeded that of HDL_2. This would also explain the observation that a twofold increase in HDL_2 receptor activity after cholesterol feeding was not associated with a significant increase in total HDL_c binding because the HDL_c receptor is more refractory to diet change.

The mechanism of the nutritionally induced enhancement of HDL_2 interaction with dog adipocytes following fat feeding remains to be defined. The increased binding may not simply be a result of an increased fat cell size as HDL_2 binding, whether expressed on a per cell basis or per unit cell surface area, was higher after the diet. However, in spite of a lack of significant correlation, fat cell cholesterol and HDL_2 binding were both increased after the high-fat diet. In cultured human skin fibroblasts, arterial smooth muscle cells, and aortic endothelial cells, human HDL_3 binding activity is increased after cholesterol loading which would facilitate cholesterol efflux to HDL_3 (9, 34). If dog HDL_2 also serves as a cholesterol efflux acceptor in fat tissue, then a mechanism similar to that of cultured cells may be involved and would explain enhanced HDL_2 interaction. The present study, however, was not designed to examine the role of HDL_2 in cholesterol efflux in adipocytes. The principal finding of this study is that the binding and dissociation of HDL_2 and HDL_c with dog adipocytes are modulated independently by dietary fat.

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