

Role of insulin in regulation of high density lipoprotein metabolism

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Abstract The effect of alloxan-induced insulin deficiency on high density lipoprotein (HDL) metabolism was studied in rabbits. Rabbits with alloxan-induced diabetes had significantly higher ($P < 0.001$, mean \pm SEM) plasma concentrations of glucose (541 ± 13 vs. 130 ± 2 mg/dl), triglyceride (2851 ± 332 vs. 101 ± 10 mg/dl), and total plasma cholesterol (228 ± 55 vs. 42 ± 4 mg/dl) than did normal control rabbits. However, diabetic rabbits had lower plasma HDL-cholesterol (7.2 ± 1 vs. 51.3 ± 1.3 mg/dl, $P < 0.001$) and HDL apoA-I (38.3 ± 6.0 vs. 87.2 ± 4.3 mg/dl, $P < 0.001$) concentrations. HDL kinetics were compared in diabetic and normal rabbits, using either ¹²⁵I-labeled HDL or HDL labeled with ¹²⁵I-labeled apoA-I, and it was demonstrated that HDL fractional catabolic rate (FCR) was slower and residence time was longer in the diabetic rabbits when either tracer was used. The slow FCR and the low apoA-I pool size led to reduced apoA-I/HDL synthetic rate in diabetic rabbits (0.97 ± 0.11 vs. 0.34 ± 0.07 mg per kg per hr). Thus, the reduced plasma HDL-cholesterol concentrations seen in rabbits with alloxan-induced insulin deficiency was associated with a lower total apoA-I/HDL synthetic rate. Since insulin treatment restored to normal all of the changes in plasma lipoprotein concentration and kinetics seen in diabetic rabbits, it is unlikely that the phenomena observed were secondary to a nonspecific toxic effect of alloxan. **Key words:** These data strongly support the view that insulin plays an important role in regulation of HDL metabolism. — Golay, A., L. Zech, M-Z. Shi, C-Y. Jeng, Y-A. M. Chiou, G. M. Reaven, and Y-D. I. Chen. Role of insulin in regulation of high density lipoprotein metabolism. *J. Lipid Res.* 1987. **28**: 10-18.

Supplementary key words HDL kinetics • apoprotein A-I • alloxan-induced insulin deficiency • insulin • diabetes

Although the association between a reduced plasma concentration of high density lipoprotein (HDL)-cholesterol and coronary artery disease (CAD) was first described more than 30 years ago (1), wide-spread interest in this relationship has only recently been kindled as the result of epidemiologic studies that have emphasized the importance of low HDL-cholesterol levels as a risk factor in the development of CAD (2, 3). Along with general acceptance of a low plasma HDL-cholesterol concentration as a risk factor for the development of CAD has come in-

creased interest in the factors that regulate plasma HDL-cholesterol concentration. For example, we now know that manipulations as diverse as moderate alcohol consumption (4), exercise training (5), and gonadal hormone administration (6) can raise plasma HDL-cholesterol levels, whereas reduced plasma HDL-cholesterol levels are often seen in patients with either chronic renal failure (7, 8) or diabetes mellitus (9-14). These changes in plasma HDL-cholesterol concentration must be secondary to variations in relative rates of HDL production and catabolism, and it is obviously necessary to quantify HDL kinetics in order to understand why plasma HDL-cholesterol concentration changes in these various situations. Although such measurements have been made in nondiabetic normal individuals, as well as in patients with various defects in lipoprotein metabolism (15-23), relatively little information is available concerning the effect of diabetes on HDL kinetics. In this regard, we are aware of results from two studies carried out in rats with streptozotocin-induced insulin deficiency indicating that the half-time of removal of radiolabeled HDL from plasma was similar in control and diabetic rats (24, 25). However, in one of these studies (24) the plasma HDL concentrations in the diabetic rats were no different from normal, while the other study (25) was conducted in rats which were both diabetic and eating a sucrose-rich diet. Furthermore, in both cases the studies were carried out in rats, a species that might not necessarily be the best model to use in

Abbreviations: HDL, high density lipoprotein; CAD, coronary artery disease; FCR, fractional catabolic rate; TG, triglyceride; RT, residence time.

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order to gain insights relevant to the effect of diabetes on HDL metabolism in humans. Therefore, we decided it would be useful to initiate the experiments presented in this report, which were aimed at defining the role of insulin in regulation of HDL metabolism. For this purpose we produced insulin deficiency in normal rabbits by the administration of alloxan (26), and quantified the changes in plasma HDL concentration and turnover rate that developed subsequent to this intervention. The results to be presented show that plasma HDL-cholesterol levels fall following alloxan-induced insulin deficiency, associated with a decline in HDL turnover rate. These changes were restored to normal following insulin treatment, indicating that the reductions noted in HDL concentration and turnover were related to the insulin-deficient state, not to a nonspecific toxic effect of alloxan. Although these data indicate that insulin plays an important role in regulation of HDL metabolism in the rabbit, the relevance of these findings to diabetes in humans can only be speculated upon at this point.

METHODS AND MATERIALS

General protocol

All studies were conducted in male New Zealand white rabbits (2–3 kg, Nitabell Rabbitry, Hayward, CA). Fifteen rabbits were injected intravenously with 0.9% NaCl, and they comprised the control group. Nine rabbits were used to determine ^{125}I -labeled HDL kinetics and the others were used for measurement of turnover of HDL containing ^{125}I -labeled apoA-I. Insulin-deficient diabetes was induced by intravenous administration of a 10% solution of alloxan monohydrate freshly prepared in water (160 mg/kg). Despite the fact that rabbits were injected intraperitoneally with glucose (10 ml of a 20% solution) every 2–4 hr for the first 24 hr following alloxan administration, deaths from hypoglycemia could not be entirely avoided. Plasma glucose concentrations of the 15 rabbits used for these studies ranged between 260 and 602 mg/dl, and were constant within any given rabbit for more than 30 days, or 10 half-times of HDL turnover.

Five of the 15 rabbits with alloxan-induced diabetes were studied both before and after treatment with exogenous insulin. For this purpose a combined regimen of long-acting and short-acting insulin was used daily in an effort to achieve near normal plasma glucose concentrations for 2 weeks before repeating all baseline and turnover studies. During this period, mean (\pm SEM) plasma glucose concentrations were maintained at 165 ± 18 mg/dl.

Isolation and preparation of ^{125}I -labeled rabbit HDL

Total HDL (d 1.063–1.215 g/ml) was obtained from 200 ml of rabbit serum by sequential ultracentrifugation as described previously (27). HDL was then washed by re-centrifugation at the upper density limit, dialyzed twice against a 100-fold volume of 0.15 M saline containing 0.01% EDTA, 5 mM Tris-HCl (pH 7.4), and 0.02% sodium azide, and iodinated by a modification of the method of MacFarlane (28). Free ^{125}I was removed from iodinated HDL by dialysis in 500 ml of double-distilled water containing Dowex X8 (Bio-Rad, Richmond, CA) for 1 hr, followed by exhaustive dialysis against 0.15 M saline containing 0.01% EDTA, 5 mM Tris-HCl (pH 7.4), and 0.02% sodium azide. Specific activity of iodinated HDL varied between 100 to 300 cpm per ng of HDL protein, with minimal iodination on lipids ($1.48 \pm 0.16\%$) and $< 3\%$ of free ^{125}I present ($2.3 \pm 0.37\%$).

Preparation of HDL radiolabeled on apoA-I

Apoprotein A-I was purified from rabbit HDL by gel filtration, using a modified protocol of Swaney, Braithwaite, and Eder (29). Briefly, HDL was delipidated (30) by extracting three times with ethanol-ether 3:1. The delipidated apoproteins were then dissolved in 0.2 M Tris-HCl (pH 8.0), 5 M guanidine chloride, 0.01% EDTA, and applied to a column (1.6 \times 100 cm) of Sephacryl S-200 (Pharmacia Fine Chemicals, Piscataway, NJ) equilibrated with the same buffer. Fractions of the right side of the first large peak, containing purified apoA-I, were dialyzed free of guanidine chloride, concentrated, and aliquots were stored in a -20°C freezer. This preparation of apoA-I gave one major band by SDS polyacrylamide gel electrophoresis (Fig. 1). In each experiment, apoA-I was iodinated to a specific activity of 500 to 700 cpm/ng as described above. ^{125}I -labeled apoA-I was incorporated into HDL by incubating one part of ^{125}I -labeled apoA-I with five parts of HDL, and the A-I/HDL re-isolated by ultracentrifugation at d 1.215 g/ml. This material, herein referred to as the ^{125}I -labeled apoA-I/HDL preparation, was then used for the apoA-I/HDL turnover studies.

Slab SDS polyacrylamide gel electrophoresis

Isolated HDL was electrophoresed on 15% gel (2.7% of bis-acrylamide in total acrylamide) for 12 hr at a fixed current (10 mA per gel) using a Protean II apparatus (Bio-Rad) with water cooling (31). When the gel was stained by Coomassie brilliant blue G150, eight major apoprotein bands were seen (Fig. 1). To quantitate the mass of these eight apoproteins, each lane was then cut from the slab, scanned by a Gilford spectrophotometer, and the area under each peak was measured. Various concentrations of purified apoA-I (from 0.2 μg to 17 μg) were

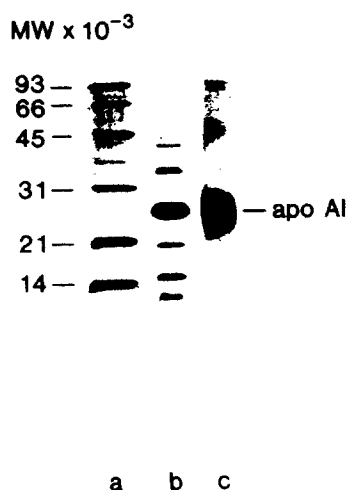


Fig. 1. Sodium dodecyl sulfate polyacrylamide gel analysis of rabbit HDL apoproteins (lane b) and purified rabbit apoprotein A-I (lane c). Rabbit HDL and apoA-I were prepared as described in Methods and Materials, and visualized in a 15% polyacrylamide slab gel by Coomassie staining. Lane a contains the protein markers, with molecular weights (MW) labeled to the left.

also run on the gel to provide a standard curve in order to convert the area under the curve into mass of apoprotein A-I. The mass of the other peptides was also estimated using the apoA-I standard curve.

The radioactivity of each apoprotein band of each sample was determined by counting the individually cut band using a 1850 gamma spectrometer (TM Analytic). Approximately 90% of the loaded radioactivity was recovered from the gel by this technique. The percent radioactivity distribution over the various apoproteins of each sample was then computed.

¹²⁵I-labeled HDL turnover and ¹²⁵I-labeled apoA-I/HDL turnover studies

Food was removed at 1800 hr the night before, and turnover studies were initiated at 0800 hr the following morning by the administration of either 200 μ g of ¹²⁵I-labeled HDL protein or ¹²⁵I-labeled apoA-I/HDL protein (approximately 10 to 30 μ Ci) per rabbit intravenously. Blood samples (5 to 6 ml) were obtained from the ear vein at 10 min, 2 hr, 4 hr, 6 hr, 8 hr, 10 hr, 12 hr, 24 hr, 30 hr, 48 hr, 52 hr, 72 hr, 78 hr, and 96 hr following the injection of radioactive material. Decay of ¹²⁵I radioactivity of plasma samples was determined in serum samples of 1 ml, using a 1850 model (Searle Nuclear, Chicago, IL) gamma spectrometer. In addition, 3 ml of the same serum samples was subjected to sequential ultracentrifugation to isolate radioactive HDL quantitatively. The labeled HDL was then washed once at d 1.215 g/ml, and the apoA-I and radioactivity in this fraction was determined. Thus, measurements of plasma ¹²⁵I radioactivity, isolated ¹²⁵I-

labeled HDL radioactivity, and ¹²⁵I-labeled apoA-I/HDL specific activity were obtained. The decay of radioactivity was followed by semilog plotting of percent disappearance of initial cpm measured at 10 min versus time, assuming a first order reaction. In all cases, the plot of the decay curve showed nonlinearity. These nonlinear curves were then resolved into two exponential components with the following equation:

$$\% \text{ radioactivity remaining} = (1 - p(5))e^{-p(1)t} + p(5)e^{-p(2)t}$$

using a SAAM27 simulator (32) on a VAX 11/780 computer system; $p(1)$ designates the rate constant of the first (early) phase, $p(2)$ that of the second (late) phase, and $p(5)$ the fraction of radioactivity whose removal is accounted for by the second phase. Fractional clearance rate (FCR) can be computed from the following equation (33):

$$\text{FCR} = \frac{1}{\frac{1 - p(5)}{p(1)} + \frac{p(5)}{p(2)}}$$

Residence time equals the total area under the disappearance curve, and is also equal to $1/\text{FCR}$.

Determination of plasma concentration of glucose, triglyceride, cholesterol, and HDL cholesterol

Glucose (34) was measured using a Beckman Glucose Analyzer II (Beckman Instruments, Palo Alto, CA). Triglyceride (35) and cholesterol (36) were measured using enzymatic methods. Three to five fasting plasma samples per rabbit were collected for plasma glucose and lipid determinations. Plasma HDL-cholesterol concentration was determined by measuring the amount of cholesterol in the infranatant of the d 1.063 g/ml fraction.

Radioimmunoassay of rabbit apoA-I

Rabbit apoA-I was measured by radioimmunoassay using a chicken anti-rabbit apoA-I antibody. Purified rabbit apoA-I was iodinated to a specific activity of 800 cpm/ng. A 1:100 dilution of chicken antibody was incubated with apoA-I standards or plasma samples for 6 hr at 4°C in a final volume of 0.25 ml with NaPi buffer saline solution containing 0.25% BSA and 10 μ g/ml aprotinin. ¹²⁵I-labeled apoA-I (200 ng) was then added and incubation continued at 4°C for 12 hr. The antibody-antigen complex was then precipitated by a goat antichick antibody. The standard curve was linear at a range from 20 ng to 500 ng. LDL fraction and fractions containing small molecular weight peptides isolated from the S-200 column did not interact with this antibody significantly.

RESULTS

The effect of alloxan-induced insulin deficiency on plasma glucose, triglyceride (TG), cholesterol, and HDL-

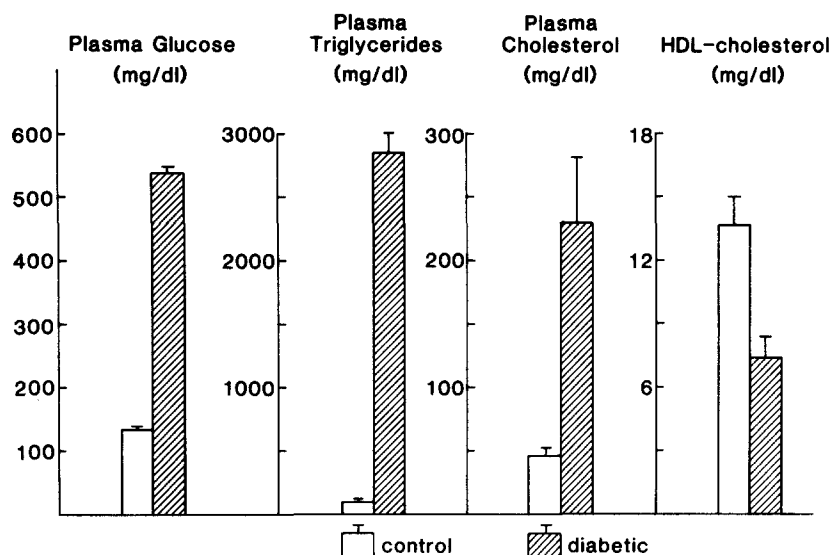


Fig. 2. Mean (\pm SEM) plasma glucose, triglyceride, cholesterol, and HDL-cholesterol concentrations measured in 15 control and 15 diabetic rabbits. All differences between data of control and diabetic rabbits are significant at $P < 0.001$ level (Student *t*-test).

cholesterol are shown in **Fig. 2**. It is obvious from these data that the diabetic rabbits were markedly ($P < 0.001$) hyperglycemic, hypertriglyceridemic, and hypercholesterolemic. In particular, plasma TG concentrations were dramatically elevated in diabetic (2851 ± 332 mg/dl) as compared to normal control rabbits (101 ± 10 mg/dl), with a range of plasma TG levels of from 897 to 4422 mg/dl in the diabetic rabbits. It was apparent from simple inspection that chylomicrons were often present in these fasting plasma samples, and undoubtedly were responsible for the extreme elevations in plasma TG concentrations observed. In addition, plasma HDL-cholesterol concentrations were reduced by approximately 50% ($P < 0.001$) in rabbits with alloxan-induced insulin deficiency (7.2 ± 1.0 vs. 15.3 ± 1.3 mg/dl).

Results of turnover studies in 12 rabbits, six normal and six with alloxan-induced insulin deficiency, are seen in **Fig. 3**. The disappearance curves of ^{125}I radioactivity were expressed both as total plasma radioactivity (**Fig. 3A**) and as ^{125}I -labeled HDL radioactivity (**Fig. 3B**), and it can be seen that the general appearance of the curves for diabetic and normal rabbits seen in **Figs. 3A** and **3B** were similar. When these data were analyzed by the SAAM 27 program, it was found that the fractional catabolic rate (FCR) was significantly lower ($0.022 \pm 0.004 \cdot \text{hr}^{-1}$ vs. $0.043 \pm 0.002 \cdot \text{hr}^{-1}$, $P < 0.01$) and the RT greater (52 ± 9.1 hr vs. 23.9 ± 1.2 hr, $P < 0.05$) in diabetic rabbits when the ^{125}I radioactivity disappearance was specifically determined in HDL. However, this was not true when calculation of FCR and RT were based on the disappearance curves of total plasma radioactivity. This latter finding

was almost certainly related to our observation that the majority ($94.6 \pm 5.5\%$) of total plasma radioactivity was present in the density range of HDL in normal rabbits, whereas only $57.6 \pm 1\%$ of plasma radioactivity was in the HDL density range in diabetic rabbits. Thus, the suggestion based on studies of normal humans (16) that total plasma radioactivity can be used to determine HDL kinetics must be interpreted with caution.

Since not all ^{125}I radioactivity within the HDL fraction was associated with apoA-I, the HDL turnover data represented the summation of catabolic behavior of apoA-I and other apoproteins in HDL. Consequently, it is possible that the data summarized in **Fig. 3** may not reflect differences in HDL turnover per se, but rather differences in the distribution of the radioactivity among the various HDL apoproteins in plasma of normal and diabetic rabbits. To assess this possibility, samples were obtained for electrophoresis daily for 4 days. An example of the electrophoretograms of HDL apoproteins is shown in **Fig. 1**. The mean percent distributions of mass and radioactivity of eight different apoproteins of HDL from normal and diabetic rabbits are shown in **Table 1**. As expected, the major component of HDL was apoA-I, whether assessed by mass or by radioactivity. Furthermore, the relative distribution of the various apoproteins contained in HDL was similar in normal and diabetic rabbits.

In order to more precisely quantify the effect of insulin deficiency on HDL metabolism, we measured apoA-I/HDL turnover directly, using HDL specifically labeled with ^{125}I -labeled apoA-I, in normal and diabetic rabbits. The disappearance curves of ^{125}I -labeled apoA-I/HDL from

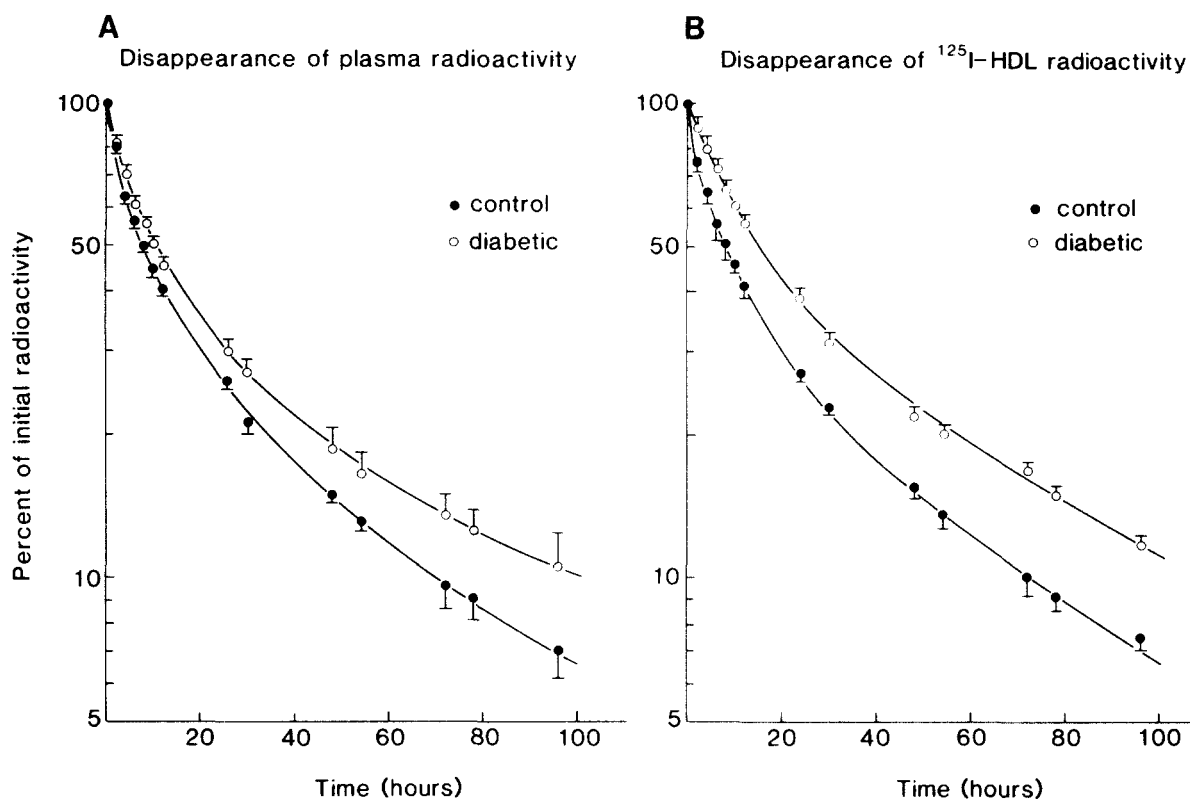


Fig. 3. Mean (\pm SEM) decay curves of ^{125}I plasma radioactivity (A) and ^{125}I -labeled HDL radioactivity (B) from six control and six diabetic rabbits injected with 200 μg of ^{125}I -labeled rabbit HDL.

whole plasma and from the HDL density range of these rabbits are seen in **Figs. 4A and 4B**. As before, the decay curves seemed to be prolonged in the diabetic rabbits.

Calculation of FCR and RT using ^{125}I -labeled apoA-I/HDL as the tracer are shown in **Table 2**. It is apparent in this instance that significant differences in FCR and RT of diabetic rabbits were again observed, and these changes were independent of whether radioactivity disappearance curves were based upon total plasma or HDL decay. This finding is consistent with the observation that recovery of ^{125}I -labeled apoA-I/HDL radioactivity was similar in the plasma and the HDL density range, averaging $95.0 \pm 4.6\%$ in normal and $90.8 \pm 5.8\%$ in diabetic rabbits.

The kinetic constants representing the slopes of the two phases of the ^{125}I -labeled apoA-I/HDL decay curve [p(1)

and p(2)], and the fraction of ^{125}I -labeled apoA-I/HDL cleared via the second phase [p(5)], are shown in **Table 3**. A change in p(2) appeared to be the parameter primarily responsible for the changes in apoA-I/HDL turnover seen in diabetic rabbits.

In order to determine total apoA-I/HDL turnover rate from the FCR of apoA-I/HDL, it was necessary to determine HDL apoA-I pool size, i.e., $\text{FCR} \times \text{pool size} = \text{total turnover}$. HDL apoA-I pool size can be estimated by measuring both plasma HDL apoA-I concentration and plasma volume. The latter measurement was accomplished by determining the volume of distribution of ^{125}I -labeled bovine serum albumin in normal and diabetic rabbits, and resulted in a value of 39.8 ± 0.2 ml/kg in control rabbits and 38.1 ± 3.3 ml/kg in diabetic rabbits. Plasma HDL apoA-I concentration was determined by

TABLE 1. Mean (\pm SEM) percent distribution of mass and radioactivity of apoproteins in HDL from control and diabetic rabbits

Group (n)	Distribution	A-IV	E	A-I	C Apoproteins/A-II				
Control (3)	Mass	2.9 ± 0.4	5.6 ± 1.2	62.5 ± 1.7	2.9 ± 0.4	8.4 ± 0.8	4.7 ± 0.4	5.7 ± 0.6	8.5 ± 1.9
Control	Radioactivity	1.2 ± 0.2	0.8 ± 0.1	78.1 ± 1.8	1.9 ± 0.1	9.6 ± 0.3	2.3 ± 0.3	5.5 ± 0.4	0.5 ± 0.1
Diabetic (3)	Mass	3.0 ± 0.2	7.3 ± 1.0	58.3 ± 0.9	2.7 ± 0.1	8.4 ± 0.4	5.2 ± 0.5	5.9 ± 0.4	6.7 ± 0.9
Diabetic	Radioactivity	1.2 ± 0.3	0.8 ± 0.2	75.7 ± 1.8	2.2 ± 0.1	10.5 ± 0.4	2.6 ± 0.4	5.9 ± 0.4	0.5 ± 0.1

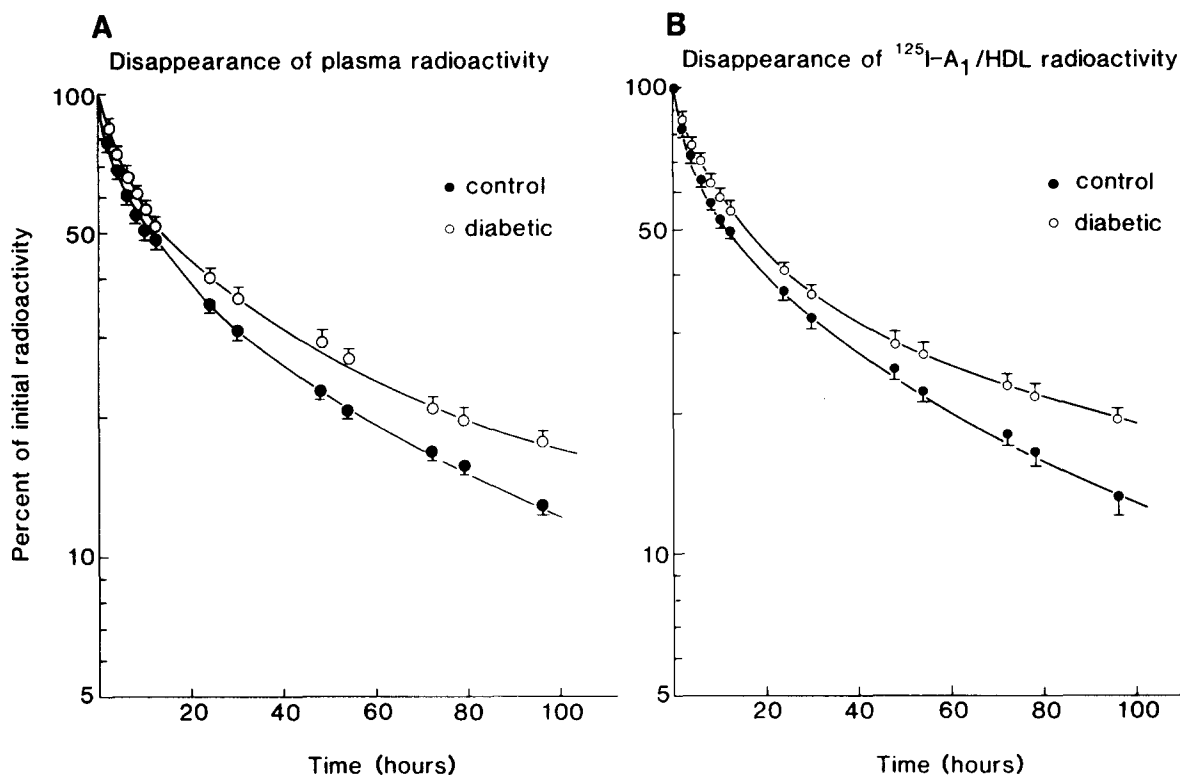


Fig. 4. Mean (\pm SEM) decay curves of ^{125}I plasma radioactivity (A) and ^{125}I -labeled apoA-I/HDL radioactivity (B) from six control and six diabetic rabbits injected with $200\ \mu\text{g}$ of ^{125}I -labeled rabbit A-I/HDL.

radioimmunoassay of apoA-I in the $d < 1.006\ \text{g/ml}$ fraction of plasma, and apoA-I levels did not vary more than 10% over the time course studied. HDL apoA-I concentration and pool size are shown in **Table 4**, and it can be seen that rabbits with alloxan-induced insulin deficiency had significantly ($P < 0.001$) lower levels of HDL apoA-I and a reduction in HDL apoA-I pool size. When the pool size and FCR of ^{125}I -labeled apoA-I/HDL were used to calculate absolute turnover rates, it was obvious from the results in **Table 4** that the synthetic rate of apoA-I/HDL was markedly reduced in rabbits with alloxan-induced insulin deficiency.

The results presented to this point have documented a series of profound changes in HDL metabolism associated

with alloxan-induced deficiency. In order to make sure that the effects noted were due to insulin deficiency, rather than to a nonspecific effect of alloxan, five diabetic rabbits were treated with insulin for 2 weeks (see Methods). As a result, mean (\pm SEM) plasma concentration of glucose ($165 \pm 18\ \text{mg/dl}$), triglyceride ($160 \pm 58\ \text{mg/dl}$), and cholesterol ($41 \pm 8\ \text{mg/dl}$) fell to levels that were not significantly different from the control values seen in **Fig. 1**. In addition, plasma HDL-cholesterol concentrations ($12.8 \pm 1.7\ \text{mg/dl}$) were restored to levels indistinguishable from normal. HDL kinetics were determined in two of the insulin-treated rabbits with ^{125}I -labeled HDL and with ^{125}I -labeled apoA-I/HDL in the remaining three rabbits. In both cases, values for FCR and RT of insulin-treated

TABLE 2. Mean (\pm SEM) values for fractional catabolic rate (FCR) and residence time (RT) with ^{125}I -labeled apoA-I/HDL as the tracer

Group (n)	Based upon Plasma Radioactivity		Based upon HDL Radioactivity	
	FCR (1/hr)	RT (hr)	FCR (1/hr)	RT (hr)
Control (6)	0.030 ± 0.001	33.9 ± 1.9	0.028 ± 0.001	35.9 ± 1.8
Diabetic (6)	0.022 ± 0.002	47.7 ± 3.9	0.023 ± 0.002	44.0 ± 3.4
P^a	< 0.01	< 0.05	< 0.05	< 0.05

^aDifference determined by Student *t*-test.

TABLE 3. Mean (\pm SEM) decay constants of 125 I-labeled apoA-I/HDL in control and diabetic rabbits

Group (n)	Decay Constants					
	Determined from Plasma Radioactivity			Determined from HDL Radioactivity		
	p(1) (1/hr)	p(2) (1/hr)	p(5) (fraction)	p(1) (1/hr)	p(2) (1/hr)	p(5) (fraction)
Control (6)	0.314 \pm 0.071	0.018 \pm 0.001	0.580 \pm 0.026	0.312 \pm 0.067	0.019 \pm 0.001	0.631 \pm 0.039
Diabetic (6)	0.213 \pm 0.020	0.013 \pm 0.001	0.573 \pm 0.049	0.322 \pm 0.060	0.014 \pm 0.002	0.589 \pm 0.058
<i>P</i> ^a	NS	< 0.01	NS	NS	< 0.05	NS

^aDifference determined by Student *t*-test; NS, not significant (*P* > 0.05).

rabbits were restored to the normal range, due to a return to normal of the kinetic constant p(2). The fact that all facets of HDL metabolism were normalized with insulin treatment strongly supports the view that the changes noted in the rabbits with alloxan-induced diabetes were secondary to insulin deficiency, and not a nonspecific result of alloxan toxicity.

DISCUSSION

This study was initiated in order to determine whether insulin plays an important role in the regulation of HDL metabolism, and the results presented have provided strong evidence that this is the case. Specifically, rabbits with alloxan-induced insulin deficiency demonstrated a fall in plasma HDL-cholesterol concentration, HDL apoA-I concentration associated with a significant reduction in the FCR and an increase in the RT of HDL, resulting in a significant lowering of apoA-I/HDL synthetic rates. In addition, both hyperglycemia and hypertriglyceridemia, characteristic of animal models of insulin deficiency (24, 37), developed in rabbits with alloxan-induced insulin deficiency. Since insulin treatment restored all of these metabolic abnormalities to normal, it appears reasonable to conclude that the changes noted were due to the state of insulin deficiency, and not to nonspecific toxic effects of the alloxan itself.

Our ability to document rather striking changes in the FCR of HDL in diabetic rabbits is at variance with results of two previous studies in the rat (24, 25). However,

although hyperglycemia and hypertriglyceridemia developed in the insulin-deficient rats studied by Van Tol (24), no change in plasma HDL-cholesterol concentration was noted. Thus, the fact that the FCR of HDL did not change may not be surprising. Similarly, the fact that the half-time ($t_{1/2}$) of disappearance from plasma of 125 I-labeled HDL was comparable in the normal and diabetic rats studies by Bar-On and Eisenberg (25) may be related to the sucrose-enriched diet fed to both groups of rats.

Alternatively, the fact that we could easily demonstrate effects of insulin deficiency on plasma HDL-cholesterol concentration and HDL kinetics may be a function of our choice of the rabbit, rather than the rat, as our experimental model. We selected the rabbit as the experimental animal based upon similarities noted in both lipoprotein metabolism and the development of atherosclerosis in rabbits and humans (38, 39). Obviously, whether the effects of insulin deficiency on HDL metabolism that we observed are unique to the rabbit, or common to other species, will only be decided by the results of future experiments.

Although it is apparent from the results presented that HDL-apoA-I disappears more slowly from the plasma of rabbits with alloxan-induced insulin deficiency, the reason for this change is unknown. On the other hand, the fact that we do not know why HDL-apoA-I catabolism is slow in rabbits with alloxan-induced insulin deficiency should not obscure the fact that the results of our turnover studies have provided interesting new descriptive information as to the effect of insulin deficiency on HDL-apoA-I kinetics. For example, it is apparent from the data presented in

TABLE 4. Mean (\pm SEM) apoA-I/HDL concentration, pool size, and total turnover rates in control and diabetic rabbits

Group (n)	ApoA-I/HDL Concentration	ApoA-I/HDL Pool Size	FCR	Turnover Rate
	mg/dl	mg/kg	1/hr	mg/kg per hr
Control (6)	87.2 \pm 4.3	34.7 \pm 1.7	0.028 \pm 0.001	0.97 \pm 0.11
Diabetic (6)	38.3 \pm 6.0	14.6 \pm 2.3	0.023 \pm 0.002	0.34 \pm 0.07
<i>P</i> ^a	< 0.001	< 0.001	< 0.05	< 0.001

^aDifference determined by Student *t*-test.

Table 3 that the prolongation in HDL-apoA-I turnover seen in diabetic rabbits was primarily due to reduction in the decay constant of the second phase, i.e., $p(2)$. One explanation proposed for the two phases of HDL-apoA-I decay from plasma is that it results from the presence of two labeled species of tracer, each with its own separate decay constant (40). If this is the case, it is obvious that the metabolism of the species represented by the second phase appears to be predominantly affected by insulin deficiency. If, on the other hand, the two phases represent two pools in different spaces that communicate, it is most likely that the first phase represents the mixing of radio-labeled HDL-apoA-I with the unlabeled pool until equilibrium is reached. The second phase is the slowest component, and represents the rate-governing step in HDL-apoA-I metabolism, i.e., irreversible removal of HDL-apoA-I from the plasma compartment. In this formulation, the effect of insulin deficiency would be to slow down the rate-limiting step, while having relatively little effect on the kinetics of the mixing of the two presumed pools.

In summary, marked hyperglycemia and hypertriglyceridemia were seen in rabbits with alloxan-induced insulin deficiency. This was associated with a fall in plasma HDL-cholesterol concentration, HDL-apoA-I concentration, and a longer RT of HDL. The prolongation of the RT of HDL in rabbits with insulin deficiency was due primarily to a decrease of the second phase decay constant. Since these changes were all restored to normal with insulin treatment of diabetic rabbits, the abnormalities noted cannot be accounted for by a nonspecific toxic effect of alloxan. As such, these data provide strong support for the view that insulin plays an important role in regulation of HDL metabolism. ■■

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