# Role of the Kidney in Regulating the Metabolism of HDL in Rabbits: Evidence That Iodination Alters the Catabolism of Apolipoprotein A-I by the Kidney†

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ABSTRACT: To evaluate the factors that regulate HDL catabolism in vivo, we have measured the clearance of human apoA-I from rabbit plasma by following the isotopic decay of $^{125}$I-apoA-I and the clearance of unlabeled apoA-I using a radioimmunometric assay (RIA). We show that the clearance of unlabeled apoA-I is 3-fold slower than that of $^{125}$I-apoA-I. The mass clearance of iodinated apoA-I, as determined by RIA, is superimposable with the isotopic clearance of $^{125}$I-apoA-I. The data demonstrate that iodination of tyrosine residues alters the apoA-I molecule in a manner that promotes an accelerated catabolism. The clearance from rabbit plasma of unmodified apoA-I on $HDL_3$ and a reconstituted HDL particle (LpA-I) were very similar and about 3−4-fold slower than that for $^{125}$I-apoA-I on the lipoproteins. Therefore, HDL turnover in the rabbit is much slower than that estimated from tracer kinetic studies. To determine the role of the kidney in HDL metabolism, the kinetics of unmodified apoA-I and LpA-I were reevaluated in animals after a unilateral nephrectomy. Removal of one kidney was associated with a 40−50% reduction in creatinine clearance rates and a 34% decrease in the clearance rate of unlabeled apoA-I and LpA-I particles. In contrast, the clearance of $^{125}$I-labeled molecules was much less affected by the removal of a kidney; FCR for $^{125}$I-LpA-I was reduced by <10%. The data show that the kidneys are responsible for most (70%) of the catabolism of apoA-I and HDL in vivo, while $^{125}$I-labeled apoA-I and HDL are rapidly catabolized by different tissues. Thus, the kidney is the major site for HDL catabolism in vivo. Modification of tyrosine residues on apoA-I may increase its plasma clearance rate by enhancing extra-renal degradation pathways.

In vivo kinetic studies have played an important role in evaluating the physiology and pathophysiology of lipoprotein metabolism. Most investigations of the in vivo metabolism of plasma lipoproteins conducted so far have made use of iodine-labeled plasma lipoproteins (*1*−*5*). The metabolic kinetics of apolipoprotein A-I (apoA-I)[1] in vivo has been investigated both by whole-labeling intact high-density lipoproteins (HDL) or by labeling delipidated apoA-I and then utilizing an in vitro or in vivo recombination with an HDL isolate (*2*−*5*). In the whole-labeling technique, since HDL are heterogeneous with respect to apolipoprotein (apoprotein) composition, several apoproteins are labeled simultaneously. Therefore, in these studies, the decay of radioactivity from plasma corresponds to the kinetics of several different apoproteins, obscuring apoA-I kinetics and necessitating fractionation of plasma apoproteins for detailed analysis. With specific apoprotein labeling, the metabolic kinetics of individually labeled apoproteins is quantified more easily, but limitations in apoprotein affinity/exchange have been highlighted, and different plasma kinetics are evident (*6*). The assignment of physiological significance to these kinetic investigations and a characterization of the "in vivo" metabolism of HDL depend on the assumption that the labeled material behaves physiologically like its unlabeled counterpart and that the decay of the labeled tracer mimics that of the unlabeled endogenous lipoprotein.

More recently, apoA-I metabolism has been investigated by using endogenous labeling of apoA-I with amino acids labeled with stable isotopes (*7*−*10*). Endogenous isotope enrichment has the theoretical advantage that there is no possibility of altering the nature of the apoprotein through isolation and labeling. However, since the tracer can be recycled and re-incorporated into newly synthesized proteins, the technique has major limitations in kinetic analyses of proteins with slow turnover rates, such as apoA-I (*11*). In addition, the kinetic analysis of endogenous labeling studies is more complex than that of exogenous radiotracer studies and relies upon several assumptions. These assumptions usually are derived from radiotracer studies (*10*, *12*). For

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[1] Abbreviations: apoA-I, apolipoprotein A-I; FCR, fractional catabolic rate; GGE, gradient gel electrophoresis; HDL, high-density lipoproteins, LpA-I, spherical reconstituted HDL particles; PBS, phosphate buffer; POPC, 1-palmitoyl 2-oleyl phosphatidylcholine; PR, production rate; RIA, radioimmunoassay; RBP, retinol-binding protein; RT, residence time; TC, tyramine cellobiose; VLDL, very low-density lipoprotein.

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instance, the output rates for the exchange compartments are usually adjusted to achieve a comparable residence time (RT), as obtained from the exogenous labeling of apoA-I, while maintaining an appropriate fit to the data (*9, 10*). As a consequence, the fractional catabolic rate (FCR)/RT obtained by both the exogenous apoA-I iodination and the endogenous stable isotope labeling methods are related and likely to be comparable (*9*). It is however of note that both methods rely on the assumption that $^{125}$I-apoA-I is metabolically identical to native apoA-I.

Several studies have shown that the structural properties (*13, 14*) and epitope reactivity (*13*) of $^{125}$I-labeled apoA-I are substantially different than on the unlabeled protein. Indeed, Osborne et al. (*13*) showed that radioiodinated apoA-I could be separated into two pools with different immunochemical and biophysical properties. One iodinated apoA-I pool represented molecules with which the immunochemical and biophysical properties had been altered by the iodination and which appeared to be cleared faster from plasma than the other iodinated apoA-I pool. Similarly, Ly et al. (*15*) demonstrated that the iodination of chylomicrons is associated with an increased liver uptake and a faster plasma clearance of chylomicron-bound triglycerides. Taken together, these studies suggest that iodination can perturb the interactions between the apolipoprotein and the lipid components of lipoprotein particles and may alter their metabolic behavior in a manner that may increase their catabolism.

Numerous studies have attempted to determine both the factors that regulate the catabolism of apoA-I and the tissues involved. As with turnover studies, all of these studies have utilized HDL particles with apoA-I labeled with either radioactive iodine or with $^{125}$I-tyramine cellobiose (TC). The tissue uptake data are similar for both tracers, and in a variety of different animal species, data suggest that on a per organ basis, the liver is the most important site of apoA-I uptake and degradation (*16*−*22*). Some of these investigations suggest that the kidney also plays an important role in apoA-I catabolism (*6, 16, 18*) but that the adrenals are important in selective uptake of cholesteryl ester from HDL particles, without a concomitant uptake and degradation of apoA-I (*23*).

The aim of the present work was to determine the in vivo kinetic parameters of native, unlabeled, apoA-I in HDL and reconstituted HDL structures. To that extent, we developed a radioimmunoassay (RIA) for human apoA-I and then measured the clearance of free and lipoprotein-associated apoA-I (LpA-I) from plasma in rabbits. To evaluate the role of the kidney in HDL metabolism, we have characterized the clearance of apoA-I and LpA-I in rabbits after a unilateral nephrectomy. The data show that the kidney is responsible for most of HDL catabolism in rabbits.

## MATERIALS AND METHODS

*Materials.* 1-palmitoyl 2-oleoyl phosphatidylcholine (POPC) was purchased from Avanti Polar Lipids (Birmingham, AL). The monoclonal antibody, 4A12, was purchased from Sanofi Inc (Paris). 5F6 and 4H1 were generous gifts from Yves Marcel and colleagues. All other reagents were analytical grade.

*Purification of apoA-I.* Human HDL ($d$ = 1.063−1.210 g/mL) was isolated from fresh plasma by sequential density gradient ultracentrifugation according to the procedure of Havel et al. (*24*). HDL was delipidated in chloroform: methanol as described (*25*). Purified apoA-I was isolated by size-exclusion chromatography on a Sephacryl S-200 HR column (*26*). ApoA-I was stored in lyophilized form at −80 °C. Prior to use, it was resolubilized in 6 M guanidine hydrochloride and 10 mM Tris, pH 7.2, and dialyzed extensively against 50 mM $NaPO_4$, pH 7.2 (PBS), and 150 mM NaCl.

*Iodination of apoA-I.* Purified apoA-I was iodinated with $Na^{125}I$ or $Na^{127}I$ using the IODO-BEAD Iodination reagent (Pierce; Rockford, IL) and manufacturer-recommended protocols. The efficiency of labeling was 52%, and the resultant specific activity of apoA-I was 1 $\mu$Ci/$\mu$g of protein. Iodinated apoA-I exhibited a normal secondary structure and surface charge. The labeled protein also appeared to have an unmodified lipid affinity, as evidenced by a normal ability to solubilize dimyristoyl phosphatidylcholine vesicles and to be complexed into sonicated reconstituted LpA-I complexes.

*Preparation of $^{125}$I-Labeled LpA-I Complexes.* Reconstituted sonicated LpA-I complexes were prepared by co-sonication of apoA-I and POPC (*27*). Briefly, specific amounts of lipids in chloroform (see Table 1 for concentrations) were dried under nitrogen in a 12 × 75 mm glass tube, and 800 $\mu$L of PBS was added. The lipid−buffer mixture was successively sonicated under nitrogen for 1 min at constant output, incubated at 37 °C for 30 min, and sonicated again for 5 min at 95% duty cycle under nitrogen. Unlabeled apoA-I (1 mg of a 1.4 mg/mL phosphate solution) and $^{125}$I-labeled apoA-I (50 $\mu$Ci) were added to the lipid mixture and co-sonicated for 4 × 1 min at 90% duty cycle under nitrogen, with 1 min cooling periods between sonications.

*Determination of LpA-I Physical Properties*. The size and the homogeneity of reconstituted particles were estimated by nondenaturing gradient gel electrophoresis on precast 8−25% gradient acrylamide gels (Pharmacia Biotech Phastgel, Baie d'Urfé, QC). Densitometric profiles were obtained by analyzing Coomassie Brilliant Blue G-stained gels on a Sharp JX 325 imaging densitometer. The mean apparent diameter was determined by comparison with protein standards (Pharmacia Biotech High Molecular Weight Protein Calibration Kit, Baie d'Urfé, QC) using a data analysis software (Onedscan, Scanalytics). The number of molecules of apoA-I per particle was determined by apoprotein cross-linking with dimethyl suberimidate as described by Swaney (*28*) and electrophoresis on 8−25% SDS−acrylamide gels to determine the extent of oligomer formation. Particle surface charge was calculated from electrophoresis on precast 0.5% agarose gels (Beckman, Paragon Lipo Kit) as previously described (*29*).

*Preparation of Exchange-Labeled $HDL_3$.* $HDL_3$ ($d$ = 1.120−1.210) was isolated from fresh fasted normolipidemic plasmas by sequential density gradient ultracentrifugation. $HDL_3$ was then labeled by apolipoprotein exchange according to the method described by Horowitz et al. (*6*). Briefly, 30 $\mu$Ci of iodinated apoA-I was incubated with 500 $\mu$g of HDL at 37 °C for 1 h. The $HDL_3$ was subsequently re-isolated by ultracentrifugation and dialyzed against PBS. The size and the homogeneity of the $HDL_3$ fraction were estimated by nondenaturing gradient gel electrophoresis on precast 8−25% gradient acrylamide gels (Pharmacia Biotech Phastgel, Baie d'Urfé, QC).

*Determination of Human apoA-I Concentration in Rabbit Plasma.* The concentration of human apoA-I in rabbit plasma during a turnover study was determined with a competitive solid phase RIA similar to that previously described (*30*). Removawells (Immulon 2, Dynatech Laboratories, MA) were coated with 100 $\mu$L of $HDL_3$ (0.2 $\mu$g in 15 mM $Na_2CO_3$, 35 mM $NaHCO_3$ and 0.02% $NaN_3$, pH 9.6), washed with 50 mM $NaPO_4$ and 0.02% $NaN_3$, pH 7.2 (buffer A), and saturated with 0.5% gelatin (Bio-Rad Laboratories, Richmond, CA) in buffer A. An antihuman apoA-I monoclonal antibody that does not cross-react with rabbit apoA-I (*31*), 4A12 (at a predetermined dilution), was mixed with serial dilutions of rabbit plasma from each time point in 50 mM $NaPO_4$, 0.02% $NaN_3$, 0.05% Tween 20, and 0.1% gelatin (buffer B) and transferred to the previously coated and saturated wells for 1 h incubation at room temperature. After three washes with buffer B without gelatin, the removawells were incubated for 1 h with an $^{125}$I-labeled antimouse IgG antibody diluted in buffer B. After three washes with buffer B, the well radioactivity was measured. A curve expressing the plasma volume as a function of the percentage of inhibition of the maximal binding of 4A12 to the $HDL_3$-coated plate was constructed for each time point, and the plasma volume required for 50% inhibition of the maximal binding of 4A12 was determined. ApoA-I concentration was determined as a percent of immunoreactive apoA-I in plasma, and results for each time point are expressed relative to the 10-min time point plasma volume. The intra-assay and inter-assay coefficients of variation of the plasma volume were 4.9% and 10.0%, respectively, in the working concentration range.

*In Vivo Metabolic Studies.* The animal protocol was reviewed and approved by the animal ethics committee of the University of Ottawa. Twenty microcuries (1 mg of apoA-I) of $^{125}$I-apoA-I, $^{125}$I-LpA-I, or $^{125}$I-$HDL_3$ was injected into the marginal ear vein of a 4.5−5.5 kg male New Zealand White rabbit. Blood samples were drawn into tubes containing 0.1 mg/mL disodium EDTA from the opposite ear at 10 min and 1, 2, 4, 6, 12, 24, 27, 30, and 48 h. The isotope decay curves were constructed by counting plasma radioactivity at each time point and by RIA as indicated above. The percentage of the initial radioactivity or plasma volume (see above) was plotted as a function of time, using the 10-min sample as the zero time. In this study, each particle was injected two to three times into different rabbits, with different preparations of apoA-I and LpA-I particles. In some experiments, a unilateral nephrectomy was performed using a retroperitoneal approach and a lateral incision. Sham surgical experiments were also performed, where a similar incision was made and the kidney was exposed but was not removed. Animals were given a 3−4 week convalescence period, after which time all animals showed a full surgical recovery and return to normal dietary and behavioral habits. Turnover studies with unlabeled and $^{125}$I-labeled apoA-I and LpA-I were performed as indicated above. Creatinine levels in urine and blood samples were determined on a Hitachi 917 automate, and glomerular filtration capacities were estimated before and after nephrectomy from creatinine clearance rates. Creatinine clearance was calculated as the ratio of the urine creatinine concentration multiplied by the urine volume to the plasma creatinine concentration and was expressed per kilogram of body weight and per minute of urine collection (*32*).

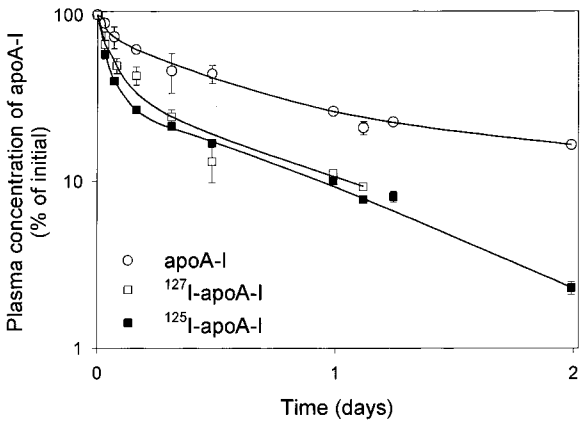


FIGURE 1: Plasma clearance curves of apoA-I. ApoA-I was purified from human plasma and iodinated with either $^{125}$I ($^{125}$I-apoA-I) or $^{127}$I ($^{127}$I-apoA-I) as described in the methods section. $^{125}$I-apoA-I (20 $\mu$Ci) was combined with unlabeled apoA-I, and then 1 mg of the $^{125}$I- or $^{127}$I-labeled mixtures was injected into rabbits. The plasma clearance of $^{125}$I-apoA-I was estimated from the plasma radioactivity decay, while those of $^{127}$I-apoA-I and unlabeled apoA-I were determined by RIA. Results are expressed as the percentage of the initial amount injected into the rabbit, as a function of time, and are mean ± SD of clearances obtained in 2−3 different rabbits.

*Kinetic Modeling and Statistical Methods.* The plasma clearance curves were analyzed according to the two-pool model of Matthews (*33*). This model assumes the existence of an intravascular pool in dynamic equilibrium with an extravascular pool. According to this model, both new input and exit of apoA-I occur from the intravascular pool. Clearance curves for each experiment were modeled separately with a biexponential equation. ApoA-I FCR was determined from the area under the curve, as calculated from Matthews' eq 33 and expressed in pools/day. The RT was calculated as the reciprocal of the FCR. Essentially identical results were obtained when the data were analyzed with SAAM-II. Correlation coefficients were determined by the method of Spearman. $p \leq 0.05$ was regarded as statistically significant.

## RESULTS

*Determination of the In Vivo Clearance of Unlabeled apoA-I by RIA.* To determine the in vivo clearance of unmodified, human apoA-I, we developed a sensitive RIA using an antihuman apoA-I monoclonal antibody, 4A12, which does not cross-react with rabbit apoA-I (*31*). Native and reconstituted HDL complexes, containing 1 mg of unlabeled apoA-I, were injected into rabbits, and plasma samples were taken at various times to determine the amount of apoA-I remaining in the plasma of the rabbit. The plasma volume required to inhibit 50% of the maximal binding of 4A12 on the $HDL_3$-coated plates was calculated for each time point by constructing a curve expressing rabbit plasma dilutions as a function of the percentage of the maximal 4A12 binding on the plate. At the various time points, curve slopes (at 50% inhibition of 4A12) for native and reconstituted HDL showed limited variation for each individual particle; intra-assay slopes variability ranged from 12 to 20% for the various particles. The reproducibility of the clearance kinetics were good, since the injection of the same particle in different rabbits gave almost superimposable results (see SD for each time point of Figures 1−3).

Table 1: Biophysical Characteristics of ApoA-I, LpA-I, and Native $HDL_3$

| particle | hydrodynamic diameter[b] (nm) | surface potential[c] (mV) |
|---|---|---|
| apoA-I | 5.6 ± 0.5 | −8.3 ± 0.1 |
| LpA-I[a] | 8.7 ± 0.1 | −8.5 ± 0.1 |
| $HDL_3$ | 9.6 ± 0.3 | −10.5 ± 0.3 |

[a] Reconstituted HDL particles (LpA-I) were prepared by co-sonication of 1-palmitoyl 2-oleyl phosphatidylcholine and apoA-I (120 mol of POPC/2 mol of apoA-I). Molar stoichiometries were determined from SDS−PAGE gels after cross-linking with dimethyl suberimate. [b] Particle diameters were determined from nondenaturating gradient gel electrophoresis. Data are mean ± SD of 3 determinations. [c] Molecular surface potentials were determined from electrokinetic analysis of agarose gels. Data are mean ± SD of three determinations.

To test whether the injection of 1 mg of apoA-I disrupted the steady state of the apoA-I pool in the rabbit plasma, we injected trace amounts of $^{125}$I-apoA-I together with increasing amounts of unlabeled apoA-I (from 250 $\mu$g to 1 mg). Similarly, LpA-I complexes were prepared from trace amounts of $^{125}$I-apoA-I, unlabeled apoA-I, and POPC, and increasing amounts (from 250 $\mu$g to 1 mg) of LpA-I were injected into rabbits. In contrast to the recent observation of Spady et al. (*22*) conducted in mice, we observed no alteration in the apoA-I and the LpA-I FCRs with increasing amounts of injected apoA-I (data not shown). This demonstrates that the apoA-I steady state in the rabbit plasma was not affected in our experimental conditions.

*Characterization of Lipoprotein Biophysical Properties.* Reconstituted LpA-I were prepared by co-sonication of POPC and apoA-I. As previously reported (*34*, *35*), the complexation of 120 molecules of POPC with apoA-I promoted the formation of complexes containing 2 molecules of apoA-I and exhibited a hydrodynamic diameter similar to that for native $HDL_3$ (Table 1). Even though the structures had a similar size, electrokinetic analysis of their electrophoretic mobilities in agarose gels showed the net charge on the lipoproteins to differ significantly. LpA-I were much less negatively charged than the $HDL_3$ and exhibited a surface potential closer to that for lipid-free apoA-I.

*Determination of the Plasma Clearance of Unlabeled and Iodinated apoA-I.* Purified human apoA-I containing trace amounts of $^{125}$I-apoA-I was injected into rabbits, and the clearance curves of unlabeled apoA-I, from an RIA, and that of $^{125}$I-apoA-I, from the plasma isotope decay, were determined. As we (*34*) and others (*36*) have previously shown, $^{125}$I-apoA-I is rapidly cleared from rabbit plasma and is essentially eliminated within a 48 h period (Figure 1). In contrast, the clearance of unlabeled apoA-I is significantly slower; only ~80% of the apoprotein is removed from the plasma of a rabbit after 2 days. This reduced clearance corresponds to an almost 3-fold reduction in plasma FCR, from 3.4 to 1.2 pools/day (Table 2).

To determine if the differences between the apoA-I and the $^{125}$I-apoA-I clearance could be due to the chemically modified apoA-I, we iodinated 1 mg of apoA-I with Na$^{127}$I, injected it into rabbits, and monitored the clearance of $^{127}$I-apoA-I from plasma by RIA. While the sensitivity of the RIA was only sufficient to accurately measure $^{127}$I-apoA-I concentrations in plasma for 30 h after its injection (Figure 1), the data show that the clearance of $^{127}$I-apoA-I was not significantly different from that of $^{125}$I-apoA-I and much faster than that for unlabeled apoA-I. This result suggests that the incorporation of iodine onto apoA-I tyrosine residues alters the apoA-I molecule in such a manner that accelerates its clearance from plasma.

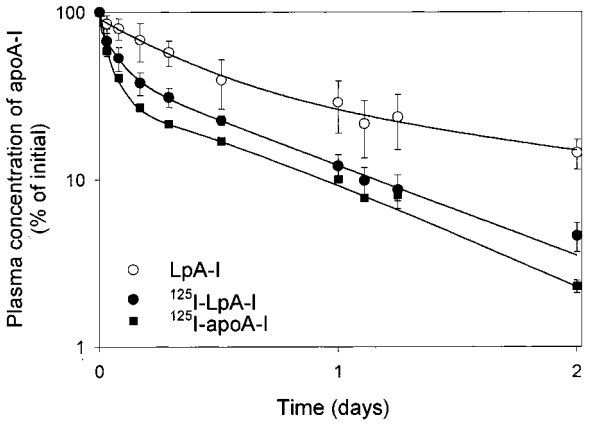


FIGURE 2: Plasma clearance curves of reconstituted HDL complexes (LpA-I). LpA-I were prepared by the co-sonication of trace amounts of $^{125}$I-labeled apoA-I, unlabeled apoA-I, and POPC. The molar ratio of POPC and apoA-I in the LpA-I complexes was 120:2. LpA-I were injected into rabbits, and the plasma clearance curve of iodinated apoA-I on the LpA-I complex ($^{125}$I-LpA-I) was determined from the plasma radioactivity decay curve, while that of unlabeled apoA-I on the LpA-I complex was determined by RIA (LpA-I). The plasma clearance curve of lipid-free iodinated apoA-I ($^{125}$I-apoA-I) from Figure 1 is shown as a reference. Results are expressed as the percentage of the initial amount injected into the rabbit, as a function of time, and are mean ± SD of clearances obtained in 2−3 different rabbits.

*Determination of the Plasma Clearance of Unlabeled and Iodinated HDL.* Figure 2 shows that the clearance curve of $^{125}$I-LpA-I was slightly slower than that for $^{125}$I-apoA-I, but the difference was not statistically significant (2.8 ± 0.5 vs 3.4 ± 0.2 pools/day, respectively) (Table 2). The clearance of apoA-I on the sonicated LpA-I complex was also determined by RIA, and its clearance was much slower than that for $^{125}$I-LpA-I (Figure 2 and Table 2), similar to that observed for apoA-I and $^{125}$I-apoA-I. The FCR values determined from the RIA were roughly one-third of the FCR values for $^{125}$I-LpA-I and $^{125}$I-apoA-I (2.8 ± 0.5 vs 0.9 ± 0.3 pools/day for LpA-I and 3.4 ± 0.2 vs 1.2 ± 0.1 pools/day for apoA-I). The differences were statistically significant for both LpA-I and apoA-I ($p < 0.5$ in both cases).

As indicated in Figure 3, exchange-labeled $^{125}$I-$HDL_3$ clearance was comparable to that observed for the $^{125}$I-LpA-I particle; approximately 4% of the initial radioactivity was present in plasma after 48 h for both particles. These results are consistent with what we have previously observed (*34*) and also comparable to other published work (*36*). As observed for apoA-I and LpA-I, $HDL_3$ clearance, as determined from an RIA, was substantially slower as compared to that of $^{125}$I-$HDL_3$ (FCR values were 0.8 ± 0.1 vs 3.1 ± 0.5 pools/day, respectively, $p < 0.01$) (Table 2). This is also apparent on the immunoblot shown on Figure 4. Plasma samples from a rabbit injected with $^{125}$I-$HDL_3$ were electrophoresed on 0.5% agarose gels, and electrophoretic mobilities of unlabeled and $^{125}$I-apoA-I were monitored both by autoradiography and by immunoblotting the gels with antihuman apoA-I monoclonal antibodies 5F6 and 4H1. As previously reported (*34*), autoradiography of agarose gels showed that apoA-I on LpA-I complexes and $HDL_3$ exhibit

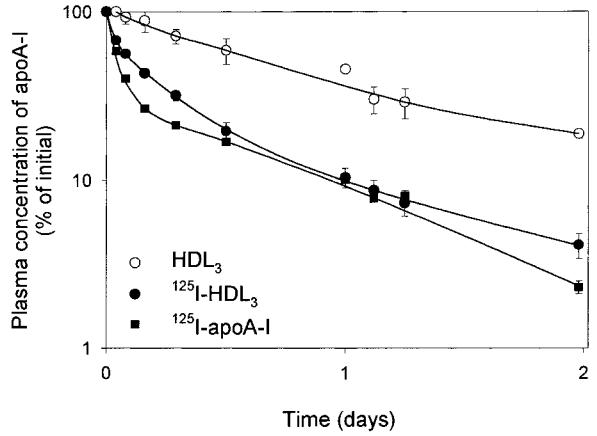


FIGURE 3: Plasma clearance curves of native $HDL_3$. $HDL_3$ (1.063 < $d$ < 1.210 g/mL) was isolated from fresh normolipidemic human plasma, exchanged-labeled with iodinated human apoA-I, and injected into rabbits. The plasma clearance curve of iodinated apoA-I on the $HDL_3$ particles ($^{125}$I-$HDL_3$) was determined from the plasma radioactivity decay curve, while that of unlabeled apoA-I on the $HDL_3$ particles was determined by RIA ($HDL_3$). The plasma clearance curve of lipid-free iodinated apoA-I ($^{125}$I-apoA-I) from Figure 1 is shown as a reference. Results are expressed as the percentage of the initial amount injected into the rabbit, as a function of time, and are mean ± SD of clearances obtained in 2−3 different rabbits.

Table 2: Fraction Catabolic Rate of apoA-I, LpA-I, and Native $HDL_3$ in Rabbits

| | FCR[a] with two kidneys (pools/day) | | FCR with one kidney (pools/day) | |
|---|---|---|---|---|
| particle | apoA-I | $^{125}$I-apoA-I | apoA-I | $^{125}$I-apoA-I |
| apoA-I | 1.2 ± 0.1 | 3.4 ± 0.2 | 0.8 ± 0.1 | 2.7 ± 0.1 |
| LpA-I | 0.9 ± 0.3 | 2.8 ± 0.5 | 0.6 ± 0.1 | 2.5 ± 0.8 |
| $HDL_3$ | 0.8 ± 0.1 | 3.1 ± 0.5 | ND[b] | ND |

[a] The FCR of unlabeled apoA-I was determined by RIA and that of $^{125}$I-apoA-I was determined from the plasma isotope decay curve. Analyses were determined before (two kidneys) and after (one kidney) unilateral nephrectomy. Data are mean ± SD of 2−3 experiments in different rabbits. [b] ND, not determined.

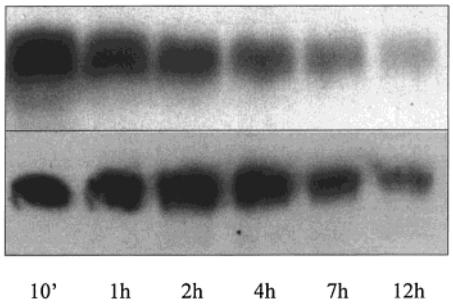


FIGURE 4: Charge characteristics of exchanged-labeled native $HDL_3$ particles after injection in rabbits. Native $HDL_3$ was $^{125}$I-exchanged labeled and injected into rabbits, and then aliquots of plasma were sampled over a 12-h period and electrophoresed on 0.5% agarose gels. Shown is an autoradiograph (top panel) and an immunoblot (bottom panel), prepared with antihuman apolipoprotein A-I antibodies (4H1 and 5F6), of the agarose gels. The electrophoretic mobility of $HDL_3$-bound apoA-I is shown for samples taken between 10 min and 12 h after injection.

no modification in their electrophoretic mobilities. This shows that the complexes remain structurally intact, and since the particle size also does not appear to change (*34*), the data suggest that no major remodeling occurs over the turnover period. Immunoblots of the gels showed essentially the same result for both LpA-I and $HDL_3$ (Figure 4). Comparison of the autoradiograph and immunoblot for the $HDL_3$ particle, however, shows that the isotopic clearance is more rapid than the mass clearance. Similar observations were made with LpA-I (data not shown), which indicates that $^{125}$I-apoA-I is cleared faster than unlabeled apoA-I both on native $HDL_3$ and on reconstituted LpA-I complexes.

*Effect of Unilateral Nephrectomy on apoA-I Clearance Rates.* To determine if the kidneys play a role in the increased rate of clearance of $^{125}$I-LpA-I, lipoprotein turnovers were undertaken in rabbits 3−5 weeks after a unilateral nephrectomy. Plasma clearance curves for $^{125}$I-labeled and unlabeled apoA-I and LpA-I were determined in nephrectomized animals and compared to that for control rabbits. Unilateral nephrectomy was associated with a major decrease in the plasma glomerular filtration capacity, which remained low throughout the study. Plasma creatinine clearance rates were reduced by 47 ± 7% 1 week after the surgery and 40 ± 10% 8 weeks post-surgery (mean ± SD). After the 4-week convalescence, the plasma lipids and relative lipoprotein levels in the nephrectomized rabbits all appeared normal. Sham animals also showed no acute or long-term change in HDL levels, and the plasma clearance of $^{125}$I-apoA-I was identical to that observed for control animals (data not shown). The plasma clearance of $^{125}$I-LpA-I was only minimally affected by the removal of one kidney; however, the clearance of the unlabeled LpA-I was reduced significantly (Figure 5A). Clearance rates for unlabeled LpA-I and apoA-I, as determined by RIA, were reduced after a unilateral nephrectomy and corresponded to a 33% reduction in the FCR values (Figure 5A,B and Table 2). Clearance rates for both $^{125}$I-LpA-I and $^{125}$I-apoA-I were much less affected by the removal of one kidney and corresponded to only a 10−20% reduction in the FCR values. The data demonstrate that the kidneys play a major role in the clearance of apoA-I and HDL from the plasma and may be responsible for up to 70% of the catabolism of apoA-I. It appears that the kidneys play a small role in the clearance of iodinated LpA-I and that extra-renal tissues account for most of the catabolism of $^{125}$I-labeled apoA-I and LpA-I.

To illustrate the contribution of the kidney in apoA-I and HDL clearance from plasma, we calculated the renal-dependent clearance rate (renal FCR) and the nonrenal-dependent clearance rate (nonrenal FCR) for free apoA-I and LpA-I (Figure 6). As expected, nonrenal FCRs were faster for $^{125}$I-apoA-I and $^{125}$I-LpA-I as compared to those for the unmodified apoA-I and LpA-I. Nonrenal FCR values were nearly identical for apoA-I and LpA-I, while nonrenal FCR for $^{125}$I-apoA-I and $^{125}$I-LpA-I were also similar but 4-fold faster. Interestingly, the faster plasma FCR value of free $^{125}$I-apoA-I relative to $^{125}$I-LpA-I appears to be due to an increase in the renal FCR of $^{125}$I-apoA-I (Figure 6). With the labeled molecules, the nonrenal clearance compartment appears saturated at about 2 pools/day. This is consistent with the work of Spady and colleagues, which showed that receptor-dependent hepatic clearance of labeled apoA-I is saturable and exhibits an almost 4-fold reduced maximal transport rate than for the kidneys in the hamster (*20*). The data suggest that the elevated $^{125}$I-apoA-I plasma clearance, relative to $^{125}$I-LpA-I, is due to an increased renal catabolism of the labeled apolipoprotein. The same relationship, albeit not as

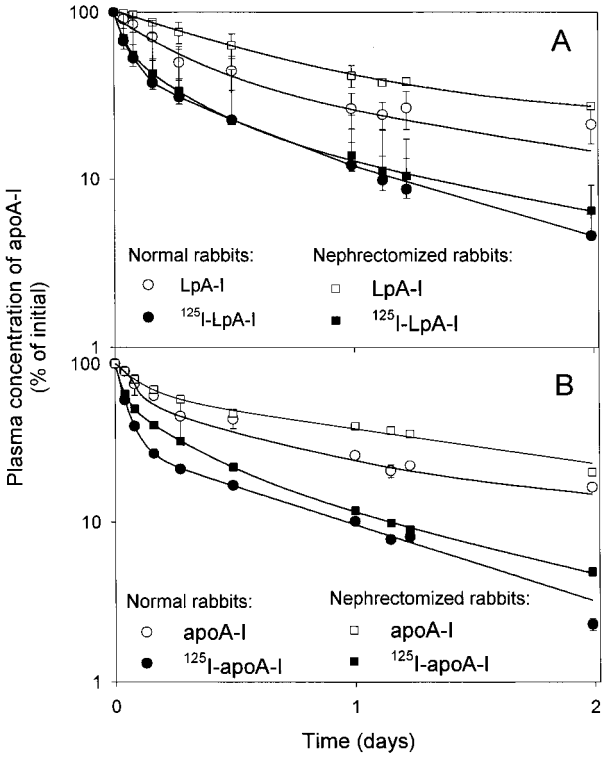


FIGURE 5: Effect of unilateral nephrectomy on plasma clearance curves of reconstituted complexes (LpA-I). LpA-I were prepared by the co-sonication of trace amounts of $^{125}$I-labeled apoA-I, unlabeled apoA-I, and POPC. $^{125}$I-labeled LpA-I (panel A) or apoA-I (panel B) were injected into uninephrectomized rabbits, and the plasma clearance curve of iodinated apoA-I ($^{125}$I-LpA-I or $^{125}$I-apoA-I) was determined from the plasma radioactivity decay curve, while that of unlabeled apoA-I was determined by RIA (LpA-I or apoA-I). Results are expressed as the percentage of the initial amount injected into the rabbit, as a function of time, and are mean ± SD of clearances obtained in 2−3 different rabbits.

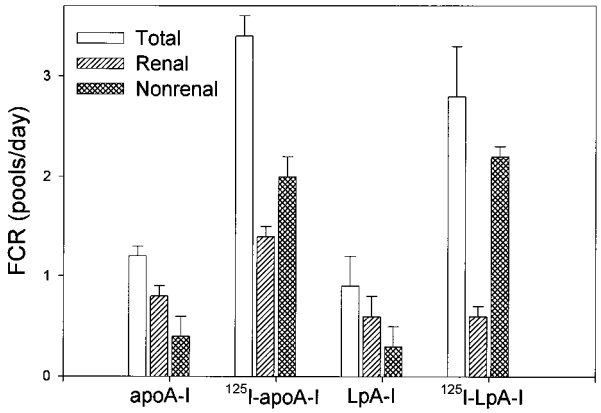


FIGURE 6: Role of the kidney and other tissues in the clearance of apoA-I and reconstituted LpA-I complexes. The contribution of the kidney and other tissues in the clearance of apoA-I is shown, relative to the total FCR of $^{125}$I-labeled and unlabeled apoA-I and LpA-I (Table 2). Renal FCR values were calculated from the difference between the total FCR and the FCR after unilateral nephrectomy and prorated to represent the contribution of both kidneys. The component of the total FCR representing clearance by other tissues (nonrenal FCR) was obtained by subtracting the renal FCR from the total FCR values. Data are mean ± SD of two to three experiments.

pronounced, is evident for the unlabeled molecules, that is, the increased FCR of apoA-I relative to LpA-I appears primarily due to an increased renal clearance. This result is consistent with earlier works, which have suggested that kidneys play an important role in apoA-I catabolism (*6*, *18*).

## DISCUSSION

The in vivo metabolism of plasma apoA-I has been investigated by two fundamentally different techniques: (1) the exogenous labeling of the protein of interest with a radioactive tracer, usually $^{125}$I or $^{131}$I and (2) the endogenous labeling of the protein following the administration of a stable isotopically labeled amino acid. While an earlier study by Ikewaki and colleagues suggested that the determination of apoA-I RT by both exogenous and endogenous labeling techniques gives similar results (*9*), more recent reports raise questions as to the accuracy of turnover studies for apoA-I using endogenous labeling techniques (*10*, *11*). The use of amino acids labeled with stable isotopes to endogenously label proteins of interest has been of tremendous value in kinetic analyses of rapidly catabolized proteins, such as VLDL-apoB (*37*). However, in a more recent report, Fisher et al. showed that endogenous labeling with stable isotopes, such as $^{3}$H-leucine, is unsatisfactory for measuring RT for proteins with a slower turnover, such as apoA-I (*11*). In fact, recycling of the tracer into the plasma leucine pool as well as its reincorporation into newly synthesized proteins within the hepatocytes obscures the kinetics of the more slowly turning-over proteins (*11*). Their conclusion was that, although stable isotopes are excellent tracers to examine the secretion and early events in the metabolism of apoA-I, they cannot be used to evaluate the prolonged RT of the predominant apoA-I pool, which is better measured with radioiodinated tracers. These concerns question the interpretation of endogenous labeling kinetic studies and instead lend support to the use of exogenous labeling with radioactive tracers to study the RT of proteins with a slow turnover rate, such as apoA-I.

While exogenous labeling kinetic studies have been used to derive much of the in vivo metabolic data to date, some reports have also raised concerns over the validity of these studies. The primary assumption in these studies is that the structure and the function of the labeled molecule have not been modified to an extent that may perturb its in vivo metabolic behavior. Studies in two different laboratories in the mid-1980s have suggested quite the contrary for iodine-labeled apoA-I (*13*, *14*). Both studies showed that apoA-I radioiodinated using the McFarlane technique exhibits significantly abnormal structural and metabolic properties. While this technique has been thought to be the least damaging to the labeled protein, in the past few years, novel commercial techniques have become available, which claim to be even more gentle and yet efficiently label protein tyrosine residues with $^{125}$I. In the present work, we have utilized the Iodo-Bead iodination reagent to label apoA-I tyrosine residues with $^{125}$I. This tracer-labeled apoA-I was slightly less immunoreactive with the mAb 4A12 but exhibited completely normal secondary structure, lipid affinity, and electrostatic properties. The Iodo-Bead-labeled apoprotein gave identical kinetic parameters to those obtained from apoA-I iodinated by the McFarlane method (*34*, *36*). However, we now demonstrate that this iodinated apoA-I is cleared nearly 3 times faster from plasma than an unlabeled molecule. While this abnormal metabolism of iodinated apoA-I may be due to a nonspecific structural modification

of the protein, several lines of evidence suggest that it is the specific modification of tyrosine residues that affects the conformation and in vivo metabolism of iodinated apoA-I. Our data are consistent with a number of studies that have shown that modification of tyrosine residues can have major consequences in the metabolism of HDL. Brinton et al. showed that while many chemical modifications (i.e., reduction and alkylation, acetylation, or cyclohexadione treatment) of HDL have little or no effect on the binding of HDL to cultured fibroblasts, modification of apoA-I tyrosine residues with tetranitromethane (TNM) significantly decreased HDL binding and inhibited cholesterol efflux from the cultured cells (*38*). Chacko et al. later confirmed that this effect was specifically due to the nitration of tyrosine residues and not to cross-linking of the apoproteins (*39*). Chemical modification of tyrosine residues in HDL apoproteins by TNM has been shown to directly increase the rate of clearance of HDL from plasma in rats and to promote an increased hepatic uptake of the modified lipoprotein by a unique scavenger receptor on liver endothelial cells (*40*). More recent studies show that TNM-modified HDL can compete with acetyl-LDL for binding to scavenger receptors on rat luteal cells (*41*). Iodination of tyrosine residues in apoA-I may have similar consequences and perturb interactions at cell membranes in a manner that may affect the metabolism of HDL particles and promote an increased scavenger clearance of the modified molecules.

Chemical modification of apoA-I in this study appears to have no major effect on its lipid binding and affinity. $^{125}$I-apoA-I readily formed recombinant HDL and also appeared to remain associated with the lipoprotein during the turnover in vivo. Analysis of rabbit plasmas postinjection by electrophoresis on 0.5% agarose gels, and then either immunoblotting the gel with antihuman apoA-I antibodies or autoradiography, showed essentially superimposable results (Figure 4). Isotopically labeled apoA-I on HDL comigrated with the same electrophoretic mobility/charge as lipoproteins with unlabeled apoA-I. At no time during the lipoprotein turnover was there any evidence of a dissociated $^{125}$I-apoA-I or of the formation of a differently charged species of HDL. This result shows that the tracer remains with the lipoprotein particle with which it is incorporated. Also, as we have previously reported (*34*), these data also show that there is very little in vivo remodeling of the labeled or unlabeled lipoprotein during the turnover period. No change in the size or the charge of the injectate over the turnover period indicates that structural remodeling of HDL must occur over a longer time frame. It also shows that apoA-I does not get selectively removed or degraded in vivo, and this suggests that it is the entire tracer-labeled particle that is degraded, more rapidly than those prepared from unlabeled apoA-I. Significant differences between the isotopic and the mass clearance rates were observed for both LpA-I and $HDL_3$. $^{125}$I-apoA-I on both lipoprotein structures was cleared at similar rates, much as we have previously reported (*34*). Unmodified apoA-I on the complexes was also cleared similarly for the different lipoproteins but at rates that were 3−4-fold slower than for the tracers. Since the principal differences between the isotopic and the mass clearance curves are in the early time points, this may suggest that a large fraction of the iodinated protein (tyrosine residues) is extensively modified and rapidly cleared, while a small fraction may be cleared similarly to the native protein. The data show that sonicated LpA-I closely mimic the metabolic behavior of native HDL particles in vivo. In addition, these data show that previous kinetic studies, which have utilized exogenously labeled proteins, may have underestimated the plasma half-life of lipoprotein particles. Our results show that the delayed mass clearance, relative to that for $^{125}$I-apoA-I, corresponds to an increase in the plasma RT of apoA-I in a rabbit from 7 to 20 h. Increases in plasma RT for the LpA-I and $HDL_3$ particles appear even greater, from 8 to 30 h. This shows that HDL turnover in the rabbit is much slower than previously thought and suggests that all isotope-based measures of lipoprotein turnover in vivo may be erroneous.

The organ specific clearance of apoA-I has been studied by monitoring uptake of a directly iodinated apoA-I (*42, 43*) or nondegradable $^{125}$I-tyramine cellobiose (TC)-labeled apoA-I (*16*−*22*). The TC adduct was shown to remain trapped intracellularly within the degradative tissue and to prevent leakage of the radioactive tracer (*44*). Differences between TC and directly labeled protein uptake have been reported, but recent work suggests that at early time points (i.e., 10 min), the uptake of both markers by different tissues are similar (*20*). While there is considerable variation in the absolute values reported, in general, most studies show the liver to be quantitatively the most important site for tracer-labeled apoA-I degradation (*17*−*22, 45*). Some of these studies show that the kidneys also play an important role in apoA-I clearance and that the kidney may be more active (per gram of tissue) than the liver in the clearance of this protein (*16*−*22*). Data from the present study appear somewhat consistent for tracer-labeled apoA-I. We show that the FCR of $^{125}$I-labeled apoA-I and LpA-I was reduced by about 15% after unilateral nephrectomy. This shows that the kidneys account for approximately 30% of $^{125}$I-apoA-I degradation in rabbits. This is similar to that observed in tissue uptake studies with both $^{125}$I- and TC-labeled apoA-I (*16*−*22*). In these studies, as in our own, it appears that $^{125}$I-apoA-I is predominantly degraded by the liver and the kidney. The labeled tyrosine on the apoA-I molecule may target the molecule for a scavenger receptor clearance by the hepatic reticulo-endothelial cells (*40*) and/or by renal proximal tubule cells (*16*). The protein is degraded intracellularly, and the radioactive catabolic products, iodotyrosine, are released and rapidly excreted in the urine (*1, 44, 46*−*48*).

The removal of one kidney from a rabbit had a very profound effect on the clearance of unlabeled apoA-I and LpA-I from plasma. The data show that this organ likely accounts for about 70% of plasma degradation of apoA-I and HDL. This suggests that tissue uptake studies that have utilized a tracer-labeled apoA-I may have overestimated the amount of apoA-I catabolized by the liver and greatly underestimated uptake by the kidney. In early studies with TC-labeled apoA-I, Glass et al. also showed that the kidney was a major site of apoA-I degradation and further showed that apoA-I could be found on the brush-border and in apical granules of proximal tubule epithelial cells (*16*). However, even these data should be interpreted with caution as some reports suggest that different cell trapping compounds may have unique and substantial effects on the uptake and degradation of a ligand (*48*). Therefore, while these early studies clearly implicated the kidney in apoA-I metabolism,

the extent of renal involvement in apoA-I clearance was significantly underrated. In addition, even though the general consensus has been that it is dissociated or lipid-poor apoA-I that is filtered by the kidney (*6*, *16*, *22*), we show that the renal-dependent clearance rates are similar for apoA-I and an LpA-I particle (Figure 6). This suggests that the kidney is involved in the clearance of both apoA-I and $HDL_3$ particles and that both molecules are filtered from the plasma by the glomerulus.

Numerous tissue uptake studies support the view that HDL can be filtered by the kidney and have shown that radioactively labeled HDL components can be found in the kidney cortex (*16*−*22*). In addition, some studies suggest that the kidney is capable of both filtration and reabsorption of apoA-I and small-sized HDL particles (*16*, *49*, *50*). Kozyraki et al. have recently identified a new high-affinity apoA-I receptor, cubilin, which appears to be highly expressed in kidney proximal tubule cells and may play an important role in the endocytic reabsorption of HDL particles (*51*). It is suprising, however, that 9-nm HDL molecules appear readily able to cross the glomerular barrier, while 4-nm uncharged dextran beads cannot (*52*). It appears that HDL molecules may exhibit a high renal sieving coefficient because of their unique charge and shape. A number of studies have shown that the renal sieving coefficient is very different for a protein relative to an uncharged dextran molecule (*53*). Indeed, both the molecular shape and the charge of a molecule are thought important to the ability of molecules to cross the glomerular basement membrane (*53*). The presence of negatively charged sites within the glomerular capillary walls appears to hinder the filtration of anionic molecules (*52*, *53*). This would suggest that more negatively charged HDL would be filtered more readily than the LpA-I particle. However, it is of note that both $HDL_3$ and LpA-I particles exhibit essentially identical FCR values. Since the different lipoprotein structures also differ in composition, the data suggest that HDL charge and composition do not profoundly affect its clearance from plasma. These data appear in contrast to our own observation, that more positively charged iodinated LpA-I particles are cleared faster from plasma of rabbits (*34*). However, we now know that the kidney is only minimally involved in the clearance of $^{125}$I-LpA-I from the plasma, and so this observation suggests that the increased clearance of more positively charged $^{125}$I-labeled molecules may reflect a relationship with scavenger receptor clearance pathways. The data may suggest that the renal filtration of HDL may be more dependent upon the shape or deformability of the lipoprotein than on particle charge. A similar finding has been reported for bikunin, a chondroitin-sulfate containing serum protein that has a similar charge and size to serum albumin but exhibits an 80-fold increased renal clearance rate (*54*). These authors proposed the increased glomerular permeability to be due to a unique, elongated, and flexible configuration of bikunin. Many structural investigations of apoA-I have shown that in HDL particles, this molecule may be also exhibit an adaptive and flexible tertiary structure (*35*, *55*−*57*).

In conclusion, in this study we have characterized the in vivo kinetic parameters and renal clearance capacity of $^{125}$I-labeled and unlabeled lipoprotein molecules. We show that the iodination of the apoA-I molecule affects its metabolic properties in a way that promotes an accelerated clearance from plasma. Previous lipoprotein metabolic work conducted with $^{125}$I-apoA-I may therefore have underestimated the plasma half-life of HDL particles. We show that iodination of the apoA-I molecule promotes its clearance by extra-renal tissues, most probably through a hepatic, scavenger receptor clearance pathway. In contrast, most of the native, unmodified apoA-I and HDL is catabolized by the kidneys. As such, it appears that it is the kidney that plays the most important role in the regulation of apoA-I clearance rates and plasma HDL levels.

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## REFERENCES

1. Blum, C. B., Levy, R. I., Eisenberg, S., Hall, M., III, Goebel, R. H., and Berman, M. (1977) *J. Clin. Invest. 60*, 795−807.
2. Shepherd, J., Packard, C. J., Gotto, A. M., Jr., and Taunton, O. D. (1978) *J. Lipid Res. 19*, 656−661.
3. Schaefer, E. J., Zech, L. A., Jenkins, L. L., Bronzert, T. J., Rubalcaba, E. A., Lindgren, F. T., Aamodt, R. L., and Brewer, H. B., Jr. (1982) *J. Lipid Res. 23*, 850−862.
4. Vega, G. L., Gylling, H., Nichols, A. V., and Grundy, S. M. (1991) *J. Lipid Res. 32*, 867−875.
5. Rader, D. J., Castro, G., Zech, L. A., Fruchart, J.-C., and Brewer, H. B., Jr. (1991) *J. Lipid Res. 32*, 1849−1859.
6. Horowitz, B. S., Goldberg, I. J., Merab, J., Vanni, T. M., Ramakrishnan, R., and Ginsberg, H. N. (1993) *J. Clin. Invest. 91*, 1743−1752.
7. Lichtenstein, A. H., Cohn, J. S., Hachey, D. L., Millar, J. S., Ordovas, J. M., and Schaefer, E. J. (1990) *J. Lipid Res. 31*, 1693−1701.
8. Cohn, J. S., Wagner, D. A., Cohn, S. D., Millar, J. S., and Schaefer, E. J. (1990) *J. Clin. Invest. 85*, 804−811.
9. Ikewaki, K., Rader, D. J., Schaefer, J. R., Fairwell, T., Zech, L. A., and Brewer, H. B., Jr. (1993) *J. Lipid Res. 34*, 2207−2215.
10. Fisher, W. R., Venkatakrishnan, V., Zech, L. A., Hall, C. M., Kilgore, L. L., Stacpoole, P. W., Diffenderfer, M. R., Friday, K. E., Sumner, A. E., and Marsh, J. B. (1995) *J. Lipid Res. 36*, 1618−1628.
11. Fisher, W. R., Venkatakrishnan, V., Fisher, E. S., Stacpoole, P. W., and Zech, L. A. (1997) *Metabolism 46*, 333−342.
12. Foster, D. M., Barrett, P. H. R., Toffolo, G., Beltz, W. F., and Cobelli, C. (1993) *J. Lipid Res. 34*, 2193−2205.
13. Osborne, J. C., Jr., Schaefer, E. J., Powell, G. M., Lee, N. S., and Zech, L. A. (1984) *J. Biol. Chem. 259*, 347−353.
14. Patterson, B. W., and Lee, A. M. (1986) *Biochemistry 25*, 4953−4957.
15. Ly, H. L., Mortimer, B. C., Baker, E., and Redgrave, T. G. (1992) *Biochem. J. 286* (3), 937−943.
16. Glass, C. K., Pittman, R. C., Keller, G. A., and Steinberg, D. (1983) *J. Biol. Chem. 258*, 7161−7167.
17. Glass, C., Pittman, R. C., Civen, M., and Steinberg, D. (1985) *J. Biol. Chem. 260*, 744−750.
18. Goldberg, I. J., Le, N. A., Ginsberg, H. N., Krauss, R. M., and Lindgren, F. T. (1988) *J. Clin. Invest. 81*, 561−568.
19. Kaysen, G. A., Hoye, E., and Jones, H. J. (1995) *Am. J. Physiol. 268*, F532−F540.
20. Woollett, L. A., and Spady, D. K. (1997) *J. Clin. Invest. 99*, 1704−1713.
21. Wang, N., Arai, T., Ji, Y., Rinninger, F., and Tall, A. R. (1998) *J. Biol. Chem. 273*, 32920−32926.
22. Spady, D. K., Woollett, L. A., Meidell, R. S., and Hobbs, H. H. (1998) *J. Lipid Res. 39*, 1483−1492.
23. Glass, C., Pittman, R. C., Weinstein, D. B., and Steinberg, D. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 5435−5439.

24. Havel, R. J., Eder, H. A., and Bragdon, J. H. (1955) *J. Clin. Invest. 34*, 1345−1353.
25. Anantharamaiah, G. M., and Garber, D. W. (1996) *Methods Enzymol. 263*, 267−282.
26. Brewer, H. B., Jr., Ronan, R., Meng, M., and Bishop, C. (1986) *Methods Enzymol. 128*, 223−235.
27. Sparks, D. L., Anantharamaiah, G. M., Segrest, J. P., and Phillips, M. C. (1995) *J. Biol. Chem. 270*, 5151−5157.
28. Swaney, J. B. (1986) *Methods Enzymol. 128*, 613−626.
29. Sparks, D. L., and Phillips, M. C. (1992) *J. Lipid Res. 33*, 123−130.
30. Marcel, Y. L., Jewer, D., Vezina, C., Milthorp, P., and Weech, P. K. (1987) *J. Lipid Res. 28*, 768−777.
31. Collet, X., Marcel, Y. L., Tremblay, N., Lazure, C., Milne, R. W., Perret, B., and Weech, P. K. (1997) *J. Lipid Res. 38*, 634−644.
32. Osborne, C. A., and Finco, D. R. (1995) *Canine and Feline Nephrology and Urology*, pp 222−223, Williams and Wilkins, Baltimore, MD.
33. Matthews, C. M. E. (1957) *Phys. Med. Biol. 2*, 36−53.
34. Braschi, S., Neville, T. A., Vohl, M. C., and Sparks, D. L. (1999) *J. Lipid Res. 40*, 522−532.
35. Sparks, D. L., Frank, P. G., Braschi, S., Neville, T. A., and Marcel, Y. L. (1999) *Biochemistry 38*, 1727−1735.
36. Lewis, G. F., Lamarche, B., Uffelman, K. D., Heatherington, A. C., Honig, M. A., Szeto, L. W., and Barrett, P. H. (1997) *J. Lipid Res. 38*, 1771−1778.
37. Beltz, W. F., Kesäniemi, Y. A., Miller, N. H., Fisher, W. R., Grundy, S. M., and Zech, L. A. (1990) *J. Lipid Res. 31*, 361−374.
38. Brinton, E. A., Oram, J. F., Chen, C. H., Albers, J. J., and Bierman, E. L. (1986) *J. Biol. Chem. 261*, 495−503.
39. Chacko, G. K., Lund-Katz, S., Johnson, W. J., and Karlin, J. B. (1987) *J. Lipid Res. 28*, 332−337.
40. Kleinherenbrink-Stins, M. F., Schouten, D., van der Boom, J., Brouwer, A., Knook, D. L., and van Berkel, T. J. (1989) *J. Lipid Res. 30*, 511−520.
41. Chen, Z., and Menon, K. M. (1993) *Biochim. Biophys. Acta 1150*, 79−88.
42. Roheim, P. S., Rachmilewitz, D., Stein, O., and Stein, Y. (1971) *Biochim. Biophys. Acta 248*, 315−329.
43. Eisenberg, S., Windmueller, H. G., and Levy, R. I. (1973) *J. Lipid Res. 14*, 446−458.
44. Pittman, R. C., Attie, A. D., Carew, T. E., and Steinberg, D. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5345−5349.
45. Hayek, T., Ito, Y., Azrolan, N., Verdery, R. B., Aalto-Setälä, K., Walsh, A., and Breslow, J. L. (1993) *J. Clin. Invest. 91*, 1665−1671.
46. Krohn, K. A., Knight, L. C., Harwig, J. F., and Welch, M. J. (1977) *Biochim. Biophys. Acta 490*, 497−505.
47. Atmeh, R. F. (1987) *Biochem. Med. Metab. Biol. 38*, 317−330.
48. Malaba, L., Kindberg, G. M., Norum, K. R., Berg, T., and Blomhoff, R. (1993) *Biochem. J. 291*, 187−191.
49. Segal, P., Gidez, L. I., Vega, G. L., Edelstein, D., Eder, H. A., and Roheim, P. S. (1979) *J. Lipid Res. 20*, 772−783.
50. Peterson, D. R., Hjelle, J. T., Carone, F. A., and Moore, P. A. (1984) *Kidney Int. 26*, 411−421.
51. Kozyraki, R., Fyfe, J., Kristiansen, M., Gerdes, C., Jacobsen, C., Cui, S., Christensen, E. I., Aminoff, M., de la Chapella, A., Krahe, R., Verroust, P. J., and Moestrup, S. K. (1999) *Nat. Med. (Tokyo) 5*, 656−661.
52. Guasch, A., Deen, W. M., and Myers, B. D. (1993) *J. Clin. Invest. 92*, 2274−2282.
53. Myers, B. D., and Guasch, A. (1993) *Am. J. Nephrol. 13*, 311−317.
54. Lindstrom, K. E., Blom, A., Johnsson, E., Haraldsson, B., and Fries, E. (1997) *Kidney Int. 51*, 1053−1058.
55. Scanu, A. M. (1971) *Biochem. Soc. Symp.* 29−45.
56. Marcel, Y. L., Provost, P. R., Koa, H., Raffai, E., Dac, N. V., Fruchart, J. C., and Rassart, E. (1991) *J. Biol. Chem. 266*, 3644−3653.
57. Segrest, J. P., Jones, M. K., De Loof, H., Brouillette, C. G., Venkatachalapathi, Y. V., and Anantharamaiah, G. M. (1992) *J. Lipid Res. 33*, 141−166.


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