In Vivo Apoprotein Catabolism of High Density Lipoproteins in Copper-Deficient, Hypercholesterolemic Rats[1,2] (42935)

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Abstract. **High density lipoprotein (HDL) apoprotein catabolism was examined in male Sprague-Dawley rats deficient in dietary copper. Twenty-four rats were randomly divided into two groups: copper-adequate (control, 5 mg of copper/kg diet) and copper-deficient (0.6 mg of copper/kg diet). After 5 weeks, animals were administered a tracer dose of iodinated HDL protein previously isolated from donor rats that were subjected to the same dietary treatments as the test animals. Copper-deficient rats exhibited a 54% increase in plasma volume and a 26% increase in HDL protein concentration above controls. Consequently, the intravascular pool of total HDL protein was increased 2-fold. The fractional catabolic rate of total HDL protein was similar between groups. However, because of the increased intravascular HDL pool in copper-deficient animals, the absolute catabolic rate was greater (640 ± 49 μg/hr vs 316 ± 12 μg/hr in controls). Tissue uptake of total HDL protein in copper-deficient rats tended to be greater in the kidneys, spleen, and testes compared with controls; the heart exhibited a significant 2.3-fold increase. In contrast, the catabolic rate of HDL protein in the liver and adrenal gland were not different between treatment groups. That an obligatory increase in HDL protein uptake was not observed in the liver and adrenal gland (organs which are sensitive to and can further metabolize cholesterol) suggests that these organs may be regulated, possibly contributing to the observed hypercholesterolemia in this model. These data imply that total HDL apoprotein catabolism is increased in response to the increased intravascular pool of HDL in copper-deficient rats.** [P.S.E.B.M. 1989, Vol 191]

A consequence of copper deficiency in rats is the development of hypercholesterolemia. Several investigators have attempted to elucidate the mechanisms responsible for this occurrence (1–8), yet the role of dietary copper in cholesterol metabolism remains unclear. Lei (1, 2) initially proposed a shift of hepatic cholesterol to the plasma pool as a potential contributor. Subsequent studies (6) indicated that newly synthesized cholesterol appeared to clear the liver at a greater rate in rats fed a copper-deficient diet. Total hepatic cholesterol concentration is indeed lower in these animals (7, 8). Furthermore, while one report (3) suggests a transient increase in biliary steroids, another study (2) demonstrated no increase in bile acid synthesis or fecal cholesterol excretion attributable to copper deficiency.

These studies have provided valuable information on the metabolism of cholesterol in copper-deficient, hypercholesterolemic rats, yet little is known about the relationships between cholesterol and plasma lipoprotein metabolism in this animal model. Preferential increases in plasma cholesterol associated with high density lipoproteins (HDLs) have been reported (5, 9). A better understanding of HDL metabolism *in vivo* may help explain these relationships. In this report, we have examined the characteristics of HDL apoprotein removal from the plasma and subsequent tissue uptake. We demonstrate that the intravascular protein pool of HDL in copper-deficient rats is significantly increased and that the absolute catabolic rate of HDL protein removal from the plasma is also increased. Of the tissues examined, the liver did not exhibit an obligatory increase in total HDL protein uptake in copper-deficient rats. We therefore speculate that the liver may be regulated in the uptake of HDL protein and that uptake

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by other tissues, particularly the bulk tissues, must necessarily be increased in copper-deficient rats.

Materials and Methods

Animals and Diets. Male weanling rats were randomly divided into two dietary treatments: copper deficient and adequate. The basal diet was prepared according to American Institute of Nutrition specifications (AIN-76) (10), except copper was excluded from the mineral mix. Briefly, the diet was comprised of 20% casein (supplemented with 0.3% methionine), 65% glucose, 5% cellulose, 5% corn oil, and all necessary vitamins and minerals. The copper-adequate (control) diet was prepared by adding $CuCO_3$ to the basal diet to a final concentration of 5 mg of copper/kg diet. The copper-deficient diet contained 0.6 mg of copper/kg diet. Upon arrival, all animals were fed the copper-adequate diet 1 week before the start of the experiments. A pair-fed group was not included in the experimental design since feed intake was not significantly affected by copper deficiency (Table I).

Lipoprotein Isolation and Iodination. After 5 weeks, rats from each treatment group were fasted for 24 hr and blood was obtained by cardiac puncture. Plasma lipoproteins were separated and purified according to Rudel *et al.* (11). Plasma was adjusted to a density of 1.225 g/ml with 0.3517 g of KBr/ml plasma and overlayered with buffer solution (density, 1.225) in ultracentrifuge tubes. The plasma was centrifuged in a SW-28 rotor at 100,000*g* for 24 hr at 15°C using a Beckman L8-80M ultracentrifuge (Beckman Instruments, Fullerton, CA). The floated lipoproteins were aspirated and applied to an agarose column containing Bio-Gel A-5m (Bio-Rad, Richmond, CA), maintained at 6°C. Lipoproteins were eluted at 20 ml/hr with 150 m*M* NaCl, 0.01% EDTA, and 0.02% NaN_3 at pH 7.4. The eluate was collected in 4-ml fractions and monitored for protein concentration at 280 nm. Identification of the separated plasma components was validated by calibrating the column with rat very low density lipoprotein (VLDL), low density lipoprotein (LDL), and HDL obtained by sequential ultracentrifugation (12) and with purified molecular weight standards (Sigma Chemical Co., St. Louis, MO). HDL fractions were pooled and concentrated using CF-25 ultrafiltration cones (Amicon Corp., Danvers, MA). Protein was determined according to the procedure of Lowry *et al.* (13).

Isolated HDL from each treatment group were radiolabeled as described by Goldstein *et al.* (14), using the iodine monochloride method (15). In all iodinations, greater than 97% of the radioactivity was precipitated by trichloroacetic acid and less than 6% was associated with the lipids extracted in chloroform:methanol (2:1, v/v). The extent of radiolabel distribution among HDL apoproteins after iodination and subsequent redistribution after injection (see below) were determined by first separating the apoproteins using sodium dodecyl sulfate-polyacrylamide electrophoresis as described by Laemmli (16). The gels were scanned for protein and the bands counted for radioactivity.

Experimental Manipulation of Animals. Rats from each treatment group were administered, via the femoral vein, a tracer dose (30–40 μg) of labeled HDL. At 10 min after injection, 50-μl blood samples were taken from the tail vein for blood volume determination. Rats were killed at 1, 3, and 6 hr after injection, blood was removed by cardiac puncture, and hematocrit was determined. Plasma was obtained for further analysis. After removal of blood, the vasculature was flushed of residual blood with normal saline containing 0.01% EDTA, as described by Pittman and Taylor (17). Briefly, the inferior vena cava was cut and perfusion initiated through the left ventricle. After flushing with approximately 200 ml of saline, the liver was further perfused with an additional 20 ml through the portal vein. Immediately after perfusion, the heart, liver, spleen, adrenal glands, kidneys, and testes were removed and weighed on an analytical balance. Approximately 1-g liver samples were dried, digested in 5 ml of nitric acid (Ultrex; J. T. Baker) for 3 hr at 95°C, and assayed for copper content using atomic absorption spectrophotometry. In additional experiments, the procedure described above was repeated using HDL preparations that had been biologically screened prior to administration by first injecting into donor rats. The serum of the donor animals was collected 4 hr after injection by allowing the blood to clot and centrifuging for 20 min at 2000*g*. This preparation was designated "screened HDL" and used for injection into test animals. Biologic screening is thought to remove any radioactive iodine not associated with intact HDL particles. The rationale of this approach has been discussed in detail elsewhere (18–20).

Plasma Analyses. To obtain plasma, the blood of experimental animals (collected by cardiac puncture) was centrifuged at 2000*g* for 20 min. Plasma from each animal was adjusted to density 1.225 g/ml, overlayered, and centrifuged as described above for the isolation of lipoproteins. The top 3 ml were removed, and, in all cases, greater than 98% of the total plasma radioactivity was recovered. The floated lipoproteins were separated and purified as previously described, using the method of Rudel *et al.* (11). The fractions corresponding to VLDL, LDL, HDL, and albumin were collected and concentrated by ultrafiltration. The amount of total radioactivity recovered with each of the major lipoprotein classes was determined. In addition, the percentage of total radioactivity associated with individual HDL apoproteins was calculated. Plasma disappearance curves were then plotted for total HDL protein as well as individual HDL apoproteins. Fractional catabolic rate was estimated according to the method of Mat-

thews (21), assuming a two-compartmental model for plasma HDL clearance. The absolute catabolic rate (ACR) was calculated as fractional catabolic rate × intravascular pool of HDL protein. Plasma volume was determined by the dilution of total injected radioactivity in the blood 10 min after injection.

Tissue Analyses. Approximately 1-g samples of each tissue were weighed and counted for radioactivity using an LKB-Wallac model 1272 gamma counter (LKB Instruments, Inc., Gaithersburg, MD). For the spleen and adrenal glands, the whole organ was used. The amount of HDL uptake was calculated as the percentage of injected radioactivity recovered in each organ at 1, 3, and 6 hr after injection. In order to determine the mass of HDL protein uptake, the ACR of HDL protein removal from plasma was multiplied by the fraction of that removal attributed to each organ (i.e., percentage of injected dose) 6 hr after injection. This calculation represents the absolute mass of HDL protein cleared/hour/organ, and is termed the organ catabolic rate. Because organ weights were altered by the copper-deficient diet, data were normalized to 100 g of body weight to allow direct comparison of organ catabolic rates between treatment groups.

Statistical Analyses. Because of the differences in body weight between animals of the two dietary treatments, data were normalized to 100 g of body weight where noted. Significant differences between treatment groups were tested at the $P < 0.05$ level using the two-tailed Student's t test. Analysis of variance was used to determine the effect of time after injection on HDL apoprotein radiolabel distribution and tissue uptake of total HDL.

Table I. Effect of Dietary Copper Deficiency on Various Physiologic Measurements[a]

Measurement	Copper adequate	Copper deficient	P^b
Body weight (g)	254 ± 4	219 ± 5	<0.001
Heart weight (g/100 g body wt)	0.419 ± 0.013	0.620 ± 0.038	<0.001
Liver weight (g/100 g body wt)	4.37 ± 0.11	5.20 ± 0.28	<0.02
Blood volume (ml/100 g body wt)	7.65 ± 0.16	8.71 ± 0.25	<0.01
Plasma volume (ml/100 g body wt)	4.05 ± 0.12	6.23 ± 0.30	<0.0001
Hematocrit (% PCV)	47.0 ± 0.9	28.8 ± 2.1	<0.0001
Liver copper (μg/g wet wt)	2.87 ± 0.34	0.83 ± 0.09	<0.0001
Feed intake (g/week)	132 ± 4	128 ± 4	NS

[a] Mean ± SE; $n = 12$.
[b] Student's two-tailed t test; NS, not significant.

Table II. Effect of Dietary Copper Deficiency on Plasma Lipoprotein Levels[a]

Lipoprotein	Copper adequate	Copper deficient	P^b
	Plasma protein concentration (mg/dl)		
HDL	115.0 ± 3.8	145.1 ± 6.5	<0.001
LDL	3.51 ± 0.45	3.29 ± 0.35	NS
VLDL	2.89 ± 0.44	3.89 ± 0.50	NS
	Intravascular protein pool size (mg/100 g body wt)		
HDL	4.50 ± 0.18	9.10 ± 0.69	<0.0001
LDL	0.140 ± 0.018	0.209 ± 0.028	<0.05
VLDL	0.118 ± 0.018	0.285 ± 0.055	<0.01

[a] Mean ± SE; $n = 12$.
[b] Student's two-tailed t test; NS, not significant.

Results

Rats fed the copper-deficient diet demonstrated a significant reduction in body weight gain, hematocrit, and liver copper concentration (Table I). Increases were observed in the relative heart and liver weights, as well as plasma volume (54% above controls). Each of these observations is indicative of copper deficiency. Feed intake was not significantly different between the dietary groups.

The total intravascular protein pool of HDL was significantly increased more than 2-fold in copper-deficient rats (Table II). This finding was attributed to both an increase in HDL protein concentration and plasma volume. The plasma protein concentration of VLDL and LDL was not significantly affected, implying that HDL metabolism was selectively altered by copper deficiency.

In order to verify that the radiolabel was not being exchanged or converted to other plasma components in appreciable amounts, the redistribution of radioactivity among plasma components was determined. In all cases, less than 1.6% of the injected dose was recovered in plasma fractions other than HDL during the 6-hr experimental period and was therefore considered negligible (data not shown). Analysis of the HDL apoprotein label distribution (Table III) demonstrated that 85–90% of the total radioactivity was associated with apoprotein (apo) A-I, apo E, and the C apoproteins. The radiolabel associated with the major apoproteins was not significantly altered during the 6-hr experimental period in either treatment group.

The first objective of this study was to determine the characteristics of HDL protein removal from the circulation. The plasma clearance of total HDL-associated radioactivity is illustrated in Figure 1. The disappearance curves appeared to be biexponential (i.e., exhibiting a rapidly disappearing component and a slower, straight-line component) for both treatment groups. Although this pattern is typical of an equilibration of tracer within a two-pool kinetic model (22), it has been suggested (18, 20) that the rapid component could be due to the preferential removal of denatured protein in the iodinated HDL preparation. To test whether the initial component could be removed by prior screening of the HDL, the experiment was re-

peated using biologically screened HDL as a tracer. Disappearance curves of screened and nonscreened HDL from both treatment groups were nearly identical (data not shown), suggesting that the rapid exponential in this study reflected an equilibration of tracer rather than the removal of denatured protein (19).

The plasma disappearance of individual HDL apoproteins was also determined and circulating half-life calculated (Fig. 2). The major apoproteins (apo A-I, apo E, and C apoproteins) were cleared from the plasma of both treatment groups at similar rates, with the half-life ranging from 8.8 to 12.4 hr. These data were not significantly different and concur with previously reported values for circulating half-life of HDL apopro-

Table III. HDL Apoprotein Radiolabel Distribution[a]

HDL Apoprotein[b]	Time after injection		
	1 hr	3 hr	6 hr
	% of total radioactivity		
Apo A-I			
Cu(+)	54.1 ± 4.0	52.5 ± 1.7	58.2 ± 1.4
Cu(−)	52.1 ± 1.6	59.3 ± 1.8	53.3 ± 0.9
Apo Cs			
Cu(+)	29.2 ± 3.0	29.5 ± 1.4	26.8 ± 0.9
Cu(−)	24.7 ± 1.8	23.5 ± 2.2	25.2 ± 3.7
Apo E			
Cu(+)	5.40 ± 0.46	6.53 ± 0.46	5.83 ± 0.17
Cu(−)	8.73 ± 0.57	7.25 ± 0.37	7.38 ± 0.81
Apo A-IV			
Cu(+)	3.63 ± 0.24	3.70 ± 0.26	3.15 ± 0.18
Cu(−)	4.08 ± 1.03	2.58 ± 0.13	2.83 ± 0.25
Apo A-II			
Cu(+)	2.65 ± 0.19	2.48 ± 0.14	2.63 ± 0.16
Cu(−)	3.35 ± 0.62	2.23 ± 0.06	3.00 ± 0.34

[a] Mean ± SE; $n = 4$.
[b] Cu(+), copper adequate; Cu(−), copper deficient.

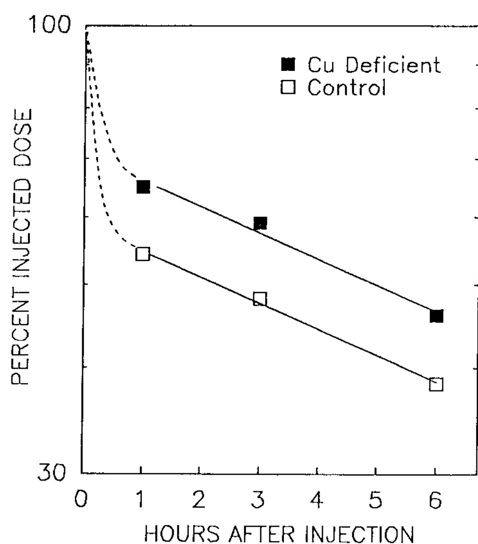

Figure 1. Plasma disappearance of total HDL in copper-deficient and control rats. Values are expressed as the percentage of injected radioactivity recovered with the plasma HDL fraction. Each point represents the mean value of four rats.

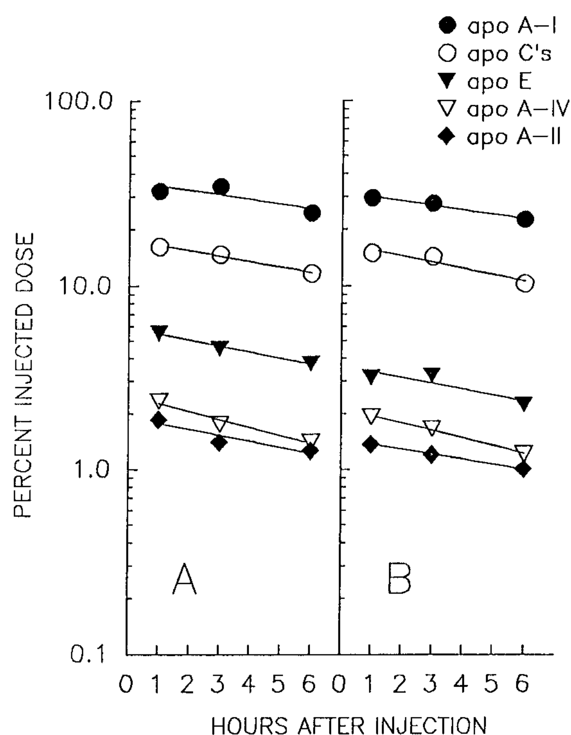

Figure 2. Plasma disappearance of HDL apoproteins in copper-deficient (A) and control (B) rats. Values are expressed as the percentage of injected radioactivity associated with individual HDL apoproteins. The points were calculated from the data in Table IV and the total radioactivity recovered with the plasma HDL fraction. Each point represents the mean value of four rats.

teins (23–25). Based on these observations, plasma clearance of total HDL protein was used as an indicator of treatment differences and in calculating tissue uptake of HDL protein. The circulating half-life of total HDL protein in both the copper-deficient and control animals was 9.9 hr (Fig. 1). However, because of the increased intravascular protein pool of HDL in copper deficiency, total mass removal of HDL protein from the plasma was calculated as ACR. The ACR of copper-deficient rats (640 ± 49 μg/hr) was 2-fold greater than that of control animals (316 ± 12 μg/hr), when normalized to 100 g of body wt.

The second objective of this experiment was to determine possible sites of HDL protein uptake by measuring radioactivity associated with various organs. Table IV shows the percentage of the total injected radioactivity recovered in each organ 6 hr after injection. Animals were thoroughly perfused prior to removal of tissues so that any residual radioactivity remaining in the plasma would not contribute significantly to the label associated with tissues. In all tissues sampled, except for the heart, the amount of recovered radioactivity was lower in copper-deficient rats. A direct comparison of these data between treatment groups, however, is inappropriate since the amount of injected radioactivity remaining in the plasma was greater in copper-deficient rats (refer to Fig. 1) due to the difference in pool size of HDL protein. Therefore, in order

to make a valid treatment comparison, the absolute rate of HDL protein removal from the plasma attributed to each organ was determined, in which the intravascular pool of HDL protein was considered (Fig. 3). This method of data expression represents the mass of total HDL protein cleared/hour/organ and was adapted from a method described previously (26). The liver was quantitatively the most important site of HDL protein uptake of the tissues sampled. The rate at which HDL protein was cleared by the liver was nearly identical between treatment groups (approximately 18 μg/hr per 100 g of body wt). The heart of copper-deficient rats exhibited a significant 2.3-fold increase in the rate of HDL protein uptake. HDL protein clearance in the kidneys, testes, and spleen tended to be greater in copper-deficient rats, although not significantly. When tissue uptake of HDL protein was expressed as μg/hr/g of tissue (data not shown), the adrenal gland demonstrated greater uptake activity than the other tissues in both treatment groups.

Table IV. Percentage of Total Radioactivity Recovered in Tissues 6 Hr after Injection of Iodinated HDL[a]

Organ	Copper adequate	Copper deficient
	% injected dose recovered	
Liver	5.72 ± 0.70	2.91 ± 0.68
Kidneys	1.48 ± 0.07	0.97 ± 0.19
Testes	0.80 ± 0.07	0.61 ± 0.15
Heart	0.36 ± 0.06	0.42 ± 0.07
Spleen	0.30 ± 0.02	0.19 ± 0.01
Adrenals	0.084 ± 0.008	0.051 ± 0.011

[a] Mean ± SE; $n = 4$.

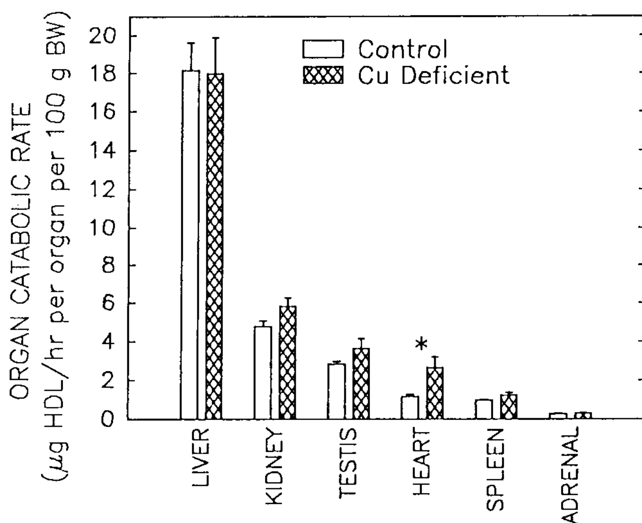

Figure 3. Organ catabolic rate of total HDL in copper-deficient and control rats. Determined by the plasma (absolute catabolic rate) × (fraction of total radioactivity recovered in each organ 6 hr after injection). This value represents the mass of plasma HDL cleared/hr/organ per 100 g body wt. *$P < 0.05$; Student's two-tailed t test.

Discussion

Cardiac hypertrophy (7, 9, 27), reduced liver copper (1, 3, 8), and growth depression (1, 9, 28) are well established indicators of copper deficiency and were observed in this study. Most notable was the increase in plasma volume, apparently as a result of reduced hematocrit. Nearly identical increases in plasma volume of copper-deficient rats have been previously reported in our laboratory using the Evan's Blue dye dilution method (9). These data confirm that rats fed the experimental diet were indeed copper deficient.

Analysis of plasma disappearance curves of total HDL protein in control and copper-deficient animals demonstrated a circulating half-life of 9.9 hr. These values, based on the second exponential of the curve, concur well with previously reported values for the clearance of total iodinated HDL (18–20). The clearance of the major HDL apoproteins did not occur at significantly different rates in either treatment group, which supports previous reports (24, 25). Alternatively, Eisenberg *et al.* (23) suggested that clearance of HDL apoproteins may occur at different rates, although their results were not tested statistically and appeared to be similar to the studies in which significant differences were not detected (24, 25). Dissimilar clearance rates of individual apoproteins, although not significantly different, probably reflect the heterogeneity of the total HDL population (29), in which certain subpopulations may be cleared from the plasma at slightly different rates.

While turnover data provide kinetic information on the fractional clearance of the intravascular HDL protein pool, the absolute mass of HDL protein removed from the plasma cannot be determined unless the pool size is considered (22). Copper deficiency in rats has been reported in our laboratory to greatly increase the HDL protein pool by both increased plasma volume (9) and HDL concentration (9, 28). The present study confirms these observations. Thus, when pool size was considered, our results demonstrate that total mass of HDL protein was removed from plasma at twice the rate in copper-deficient rats. Consequently, the use of fractional catabolic rate alone as an indicator of treatment differences is insufficient and plasma pool size must necessarily be determined.

The slopes of the plasma disappearance curves of both treatment groups were similar, although the percentage of radioactivity remaining in the plasma at each time interval was greater in copper-deficient rats. This difference can be attributed to the dilution of tracer during initial equilibration. Since the injected dose was similar between treatments and because the HDL protein pool was twice as large in the copper-deficient group, the dilution of labeled HDL in the intravascular compartment was greater than controls. As a result, a greater percentage of radioactivity remained in the plasma after initial equilibration with the extravascular

spaces. This observation further indicates that the rapid component does not necessarily reflect any physiologic catabolic event and may simply be an inherent property of the tracer-labeling technique.

To determine the effects of copper deficiency on HDL protein uptake by specific tissues, a single tracer dose of iodinated HDL was administered and tissues removed at subsequent time intervals. The validity of this approach could be substantiated only after certain criteria were met. First, relatively short time intervals were used to avoid significant "leakage" of radiolabel from cells in which degradation occurs. Stein *et al.* (20) demonstrated that leakage of radioactivity from tissues (e.g., liver, spleen, and adrenal gland) could be detected between 6 and 12 hr after injection of iodinated HDL. Preliminary experiments in our laboratory (unpublished data) concur with these observations, in which the recovery of radiolabel decreased by 12 hr after injection in all tissues studied. Therefore, tissue-associated radioactivity was determined no longer than 6 hr after injection to ensure maximal recovery. The second requirement for validation of this technique was to determine whether the radiolabel of HDL was redistributed among other plasma components in appreciable amounts. If this were to occur, the radioactivity recovered in tissues could represent uptake of other lipoprotein classes in the plasma. The current data suggest that intravascular exchanges of label were negligible, adding confidence that tissue-associated radioactivity represented the uptake of only HDL protein. In the case of rat LDL, a decrease in radioactivity was observed during the 6-hr period after injection. This probably reflects degradation of small amounts of HDL which could not be completely separated from the LDL fraction. The difficulty in purifying rat LDL has been well documented (30–32). Because the amount of radioactivity in the LDL fraction accounted for approximately 0.5% of the total, this effect was considered negligible. The third criterium for validation was to account for any residual plasma remaining in the tissue, which would yield an overestimation of the tissue-associated radioactivity. Whole-body perfusion (17) was used in the present study to clear all residual plasma not associated with tissues. This method seemed most appropriate since individual tissues exhibit different relative capacities in the space occupied by plasma (33). If perfusion were not performed, the plasma tissue space would have to be determined for each tissue sampled. Since whole-body perfusion removes virtually all residual plasma within the tissue space, the differences between tissues can be ignored. Finally, to determine whether the observed treatment differences were the result of alterations in the HDL particle which may have occurred during the iodination procedure, the experiment was repeated using an iodinated HDL particle which had been biologically screened for 4 hr in donor rats, as described previously (18). After reisolation of the serum and injection into recipient animals, similar results were obtained for each of the tissues listed in Table IV. Thus, the following potential sources of error were minimized in determining tissue-associated radioactivity; (i) leakage of radiolabel from cells, (ii) uptake of lipoproteins other than HDL, (iii) residual plasma radioactivity, and (iv) uptake of denatured protein.

The absolute removal of total HDL protein from the plasma was significantly increased in copper-deficient rats, indicating a requisite increase in the total organ catabolic rate. Of the organs examined in this study, the liver and adrenal gland, which are normally sensitive to cellular cholesterol and can further metabolize it (34), demonstrated no increase in the catabolic rate. These organs are highly regulated and have been shown to possess distinct catabolic mechanisms by which the uptake of HDL protein and cholesteryl ester moieties occur independently (20, 35). Although it is not the purpose of this study to elucidate the mechanisms involved in the uptake of HDL, the present data suggest that uptake in the liver and adrenal gland of copper-deficient rats may be regulated, whereas the other organs examined in this study demonstrated an obligatory increase in absolute catabolic rate of total HDL protein. Furthermore, we speculate that HDL protein uptake must necessarily be increased in the bulk tissues of copper-deficient rats to account for total plasma removal.

In this report, we have demonstrated that the absolute catabolic rate of total HDL protein removal from the plasma was dramatically increased in copper-deficient rats when the intravascular protein pool of HDL was considered. Because the clearance of total HDL protein was increased in copper deficiency, subsequent tissue catabolism was necessarily increased. The extent to which specific organs contribute to total HDL protein uptake may be dependent upon their individual response to the concentration of HDL in the plasma. Although these findings provide insight into the *in vivo* catabolism of HDL apoproteins in copper-deficient rats, the overall contribution of these degradative processes to the observed hypercholesterolemia in this model remains unclear.

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