Effect of copper deficiency on the plasma clearance of native and acetylated human low density lipoproteins

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The rates of plasma clearance of human native low density lipoproteins (LDL) and acetylated human low density lipoproteins (acetyl-LDL) were compared between copper-deficient (CuD) and copper-adequate (CuA) rats. Purified human LDL (d 1.02–1.063) were labeled with ^{125}I and injected to fasted recipient rats intravenously. At different time intervals plasma clearance of ^{125}I radioactivity was measured. The percent of clearance was calculated based on the total plasma volume, as determined by a radioisotopic dilution method. Native human ^{125}I-LDL were cleared at a faster rate in CuD, compared with CuA rats. The half-times ($t_{1/2}$) of ^{125}I-LDL clearance are 4.90 ± 0.20 and 5.80 ± 0.30 hours in CuD and CuA rats, respectively. The plasma trichloroacetic acid-soluble ^{125}I-radioactivity was significantly and steadily increased in CuD rats at each interval, reflecting the faster clearance and degradation of LDL in those rats. The plasma removal of ^{125}I-acetyl-LDL was faster compared with that of ^{125}I-LDL. The half-times ($t_{1/2}$) of acetyl-LDL in CuD and CuA rats are 5.20 ± 0.06 and 5.16 ± 0.08 minutes, respectively, with no significant difference between the groups. The data indicates that the uptake of LDL via the "scavenger" receptor remains unaffected in copper-deficient rats. The faster removal of the unmodified (native) LDL in CuD group suggests that the apoB,E receptor is up-regulated in copper-deficient rats and that the hypercholesterolemia observed in copper deficiency is not associated with the defective uptake of LDL by the apoB,E-receptor dependent mechanism.

Keywords: copper deficiency; cholesterol; low density lipoproteins

Introduction

Numerous studies, as reviewed in a recent publication,[1] have confirmed that copper deficiency causes a marked elevation in plasma cholesterol in both experimental animals and humans. Because of a possible etiological link between copper deficiency and the development of coronary heart disease, as postulated by Klevay,[2,3] considerable effort has been directed toward elucidation of the biochemical role of copper in lipid metabolism.

Copper-deficiency–induced hypercholesterolemia in fasted rats is characterized by a pronounced increase in plasma HDL cholesterol.[4-7] Alterations in the composition of plasma HDL and HDL subfractions also have been indicated.[4-7] Current information, obtained mostly from rat models, suggests that copper deficiency may stimulate the hepatic synthesis of cholesterol.[8-11] The hypercholesterolemic effect of copper deficiency might be due mainly to an increased net efflux of HDL cholesterol ester from the liver to the blood plasma.[12,13]

Thus far, however, no information is available concerning the effect of copper deficiency on the metabolism of LDL cholesterol, despite the fact that plasma LDL cholesterol also is significantly elevated in copper-deficient rats[4] and humans.[14,15] It is not known whether such a change in plasma LDL is associated with alterations in the mechanisms of plasma LDL uptake and clearance by the liver and extrahepatic tissues. Native LDL is removed from the circulation pri-

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marily by the apoliproteinB(apoB),E receptor. The apoB,E receptor: (a) is found on hepatocytes, smooth muscle cells, and other parenchymal cells; (b) has a very high affinity for native LDL and low affinity for acetyl-LDL; and (c) is down-regulated by intracellular cholesterol. The "scavenger" or "acetyl-LDL" receptor is distinct from the apoB,E receptor and is found primarily on phagocytic cells such as monocytes/macrophages and the Kupffer cells of the liver. The acetyl-LDL receptor has a low affinity for native LDL and high affinity for acetyl LDL or LDL chemically modified by oxidation or denaturation. Chemically modified forms of LDL may be particularly atherogenic since they have been implicated in the formation of "foam" cells found in atherosclerotic lesion. An impaired ability to catabolize modified LDL could contribute to atherogenesis. In this investigation, we have evaluated the effect of copper deficiency on the rate of clearance of native and acetyl-LDL.

Materials and methods

Animals and diet

Male rats (Fischer 344, Charles River Breeding Lab. Inc., Wilmington, MA, USA) with initial body weight ranging from 35–40 g were placed individually in plastic cages with stainless-steel wire bottoms in a windowless room and subjected to a 12-hr light cycle. Temperature and humidity of the room were controlled at 23–25° C and 55%–65%, respectively. All rats were housed in the Oral Roberts University Biomedical Research Center animal care facility, fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

All experiments consisted of the following two treatment groups: (1) copper-deficient group (CuD) fed ad libitum a diet containing 0.5 mg copper/kg diet (Zeigler Bros., Inc., Gardners, PA, USA) *(Table 1)*, as determined by atomic absorption spectrophotometry; and (2) copper-adequate control group (CuA) fed the same diet but supplemented with 7.0 mg copper/kg as the carbonate (total 7.5 mg/kg). All rats were given distilled deionized water via an automatic stainless-steel watering system.

Table 1 Composition of copper-deficient diet

Ingredient	% Diet
Egg white solids	20.0
DL-Methionine	0.3
Glucose (Celelose)	36.0
Corn starch	30.9
Corn oil	5.0
Cellulose, powder	3.0
Choline-Cl (Cholfeed, 50%)	0.3
Mineral mix[a]	3.5
Vitamin mix[b]	1.0

[a] Formulated according to the recommendations of the American Institute of Nutrition (AIN), without copper. The diet prepared as above contained 0.5 mg of copper/kg diet and was used as copper-deficient (CuD) diet.
[b] Formulated according to the AIN recommendations. Biotin content was increased to 50 mg/kg of the mix.

Isolation, acetylation and labeling of human plasma LDL

Blood samples were taken from a healthy normocholesterolemic male subject after an overnight fast. EDTA (0.5 mg/mL blood) was used as an anticoagulant and to prevent in vitro oxidation of LDL. Plasma low density lipoproteins (LDL; d 1.019–1.063) were isolated by ultracentrifugation[16] and further purified by chromatography on a Sepharose CL-4B column (Pharmacia, Piscataway, NJ, USA).[17] Acetylation of LDL was carried out by the method of Basu et al.[18] One mL of LDL (2 mg protein) in 8 mmol/L phosphate buffer, pH 7.5, containing 150 mmol/L EDTA was added to 1 mL of saturated solution of sodium acetate with continuous stirring in an ice bath. To this mixture 3 mg of acetic anhydride was added in multiple small portions using a microcapillary tube over a 1-hr period and stirred for an additional 30 min. The acetyl-LDL preparation was dialyzed for 18 hr at 4° C against 150 mmol/L NaCl, 0.2 mmol/L EDTA, pH 7.4. The native LDL and acetyl-LDL were labeled with ^{125}I using a modification of the iodine monochloride method.[19] The labeled LDL were immediately chromatographed on a Sepharose CL-4B column (1.2 × 8 cm) to remove most of the unbound iodine. The labeled LDL preparations were then dialyzed against 150 mmol/L NaCl, 0.2 mmol/L EDTA, pH 7.4 to remove any residual unbound iodine. Sodium dodecylsulfate polyacrylamide (5%) gel electrophoresis showed apoB as the only protein detectable in the purified LDL. The labeled LDL fractions were filtered through a 0.45-μ sterile Millipore filter immediately prior to dosing. Most (98%) of the ^{125}I radioactivity of the final LDL preparations was precipitable in 10% trichloroacetic acid (TCA) and 4%–5% was extractable into lipid solvents.

Determination of the rate of plasma LDL clearance

Experiment 1. Plasma clearance of LDL via the apoB,E receptor-dependent mechanism was studied by using the native ^{125}I-LDL, as prepared above. At 13 wk of dietary treatment, copper deficiency in CuD rats was confirmed by low plasma copper and hypercholesterolemia and rats were fasted for 12 hr prior to dose injection. Six rats from each of the treatment groups were injected via the jugular vein with a dose/rat of ^{125}I-LDL containing 1.88 × 10^5 cpm ^{125}I radioactivity (20.6 μg protein) in 200 μL of 150 mmol/L NaCl (pH 7.4). After 1, 2, 4, and 6 hours, blood samples (0.5 mL) were collected under ether anesthesia via the orbital sinus using heparinized microcapillary tubes. Plasma was obtained by centrifugation at 1500*g* for 30 minutes.

To separate plasma ^{125}I radioactivity into the TCA-precipitable (non-degraded) fraction and TCA-soluble (degraded and released) fraction, 200 μL plasma was precipitated with 200 μL of 20% TCA. The precipitate was centrifuged and then washed with 200 μL of 10% TCA. The supernatants were combined and the ^{125}I radioactivity was counted. The pellets were digested with 800 μL of 20% tetramethylammonium hydroxide (TMAH) at 60° C for approximately 4 hr until completely dissolved. The ^{125}I radioactivity was counted in a Beckman Gamma 8000 counter (Beckman Instruments, Fullerton, CA, USA). At 6 hr, the rats were exsanguinated under ether anesthesia and selected organs were removed for determination of ^{125}I radioactivity and copper status. The tissue ^{125}I radioactivities were determined after solubilization of 0.2 g of finely minced tissue in 20% TMAH as above. Tissue concentrations of copper were determined by atomic absorption spectrophotometry.

Experiment 2. Clearance of acetyl-LDL via the scavenger-receptor mechanism was examined by using acetylated ^{125}I-LDL. The rate of clearance of ^{125}I-acetyl-LDL was determined by following the same procedure as described above, but with the

following exceptions: at 7 wk, five rats from each group were injected with a dose containing 3.92×10^5 cpm of ^{125}I radioactivity (46.5 μg protein) in 250 μL of 150 mmol/L of NaCl. Blood samples were withdrawn at 5, 10, and 20 minutes after dose injection.

In both experiments, the total plasma TCA precipitable (non-degraded LDL) ^{125}I-radioactivities of native ^{125}I-LDL and ^{125}I-acetyl-LDL remaining at each time interval (t_i) were obtained by the TCA precipitable ^{125}I radioactivity at t_i per mL plasma × total plasma volume (mL). The percentage of ^{125}I-radioactivity remaining in the plasma at each interval (t_i) was calculated by the ^{125}I radioactivity at t_i ÷ ^{125}I radioactivity at t_0 × 100, where t_0 is time of dosing. The half life, the time required for ^{125}I-radioactivity to decrease to one-half of the radioactivity at t_0, was computed by the equation for exponential decay.[20]

Determination of plasma volume

In experiment 1, plasma volume was determined by using human ^{125}I-LDL. Five rats from each group, fasted for 12 hr, were injected via the jugular vein with a dose/rat of 3.40×10^5 cpm ^{125}I-LDL in 200 μL of 150 mmol/L NaCl (pH 7.4). At 5, 30, and 60 minutes, blood samples (0.3 mL) were collected from the orbital sinus and the ^{125}I-radioactivity in 100 μL plasma was counted. In experiment 2, each dose contained 0.7 μg ^{125}I-albumin (NEN, radiochemical purity > 99%) and 2.35×10^6 cpm radioactivity in 250 μL of 150 mmol/L NaCl (pH 7.4). Blood samples were taken at 5, 10, and 30 minutes after dose injection. In both experiments, the plasma ^{125}I radioactivity at the time of dosing (t_0) was determined by an extrapolation of the linear-regression curve ($r = 0.99$) for log plasma ^{125}I radioactivity versus time (t_i). Total plasma volume (mL) was calculated by the total ^{125}I radioactivity injected ÷ ^{125}I radioactivity per mL plasma at t_0. The rates ($t_{1/2}$) of plasma disappearance of human ^{125}I-LDL and bovine ^{125}I-albumin in the rats used were 5.8 hr and 5.0 hr, respectively. At 5 min postdosing, 98.0% to 98.6% of the total ^{125}I radioactivity injected was found in the plasma. The calculation error, if any, in determining the dilution of the initial ^{125}I radioactivity at t_0 by extrapolation was less than 2%.

Other analyses

Plasma cholesterol was determined by the enzymatic method of Allain et al.[21] For tissue copper analysis, 200 mg of liver, spleen, and kidney was digested with 1.25 mL of 20% tetramethylammonium hydroxide at 60° C for 4 hr prior to analysis. The copper concentrations in tissues and plasma were measured by atomic absorption spectrophotometry (Perkin-Elmer 5000, Perkin-Elmer Corp., Norwalk, CT, USA) using an air-acetylene flame.

Statistical analysis

Data were analyzed by student's t test using a statistical computer program (Statistical Analysis Software, PC ANOVA, Human Systems Dynamics, Northridge, CA). The level of significance was determined at $P < 0.05$.

Results

Copper status of rats

Various indices of the copper status of CuD and CuA groups in experiments 1 and 2 are shown in *Tables 2 and 3,* respectively. In both experiments, copper deficiency in CuD rats was confirmed by significant decreases in the copper concentrations of plasma and various organs such as liver, kidney, spleen, and testis. In addition, characteristic of copper deficiency, plasma cholesterol, plasma volume, and relative liver weight were significantly increased in CuD rats, as compared with CuA group, although there was no significant difference in body weight between the groups. The plasma volume, as expressed in mL per 100 g body weight, was increased by 15% in CuD rats, as determined by using ^{125}I-LDL and ^{125}I-albumin in experiments 1 and 2, respectively.

Table 2 Tissue concentrations of copper (Cu) and other indices of Cu status of Cu-deficient and Cu-adequate rats at wk 13 of dietary treatment (experiment 1)

	Cu-deficient	Cu-adequate
Plasma Cu (μmol/L)	1.6 ± 0.5*[a]	24.4 ± 0.5†
Liver Cu (nmol/g)	17.0 ± 1.6*	53.7 ± 1.6†
Kidney Cu (nmol/g)	36.0 ± 0.8*	106.6 ± 4.9†
Spleen Cu (nmol/g)	4.4 ± 0.5*	18.4 ± 0.5†
Plasma cholesterol (mmol/L)	2.97 ± 0.05*	2.59 ± 0.05†
Plasma volume (ml/100g b.w.)[b]	4.15 ± 0.17*	3.60 ± 0.15†
Liver weight (g/100g b.w.)	3.43 ± 0.15*	2.74 ± 0.01†
Body weight (g/rat)	258 ± 3	264 ± 4

[a] Mean ± SEM of 6 rats per group. Values in the same row for each parameter not sharing a common superscript are significantly different ($P < 0.05$).
[b] Determined by the radioisotopic dilution of ^{125}I-LDL (refer to Materials and methods).
b.w., body weight.

Table 3 Tissue concentrations of copper (Cu) and other indices of Cu status of Cu-deficient and Cu-adequate rats at wk 7 of dietary treatment (experiment 2)

	Cu-deficient	Cu-adequate
Plasma Cu (μmol/L)	1.7 ± 0.3*[a]	22.5 ± 1.9†
Liver Cu (nmol/g)	30.2 ± 3.1*	65.2 ± 1.7†
Kidney Cu (nmol/g)	42.5 ± 0.8*	94.1 ± 2.7†
Spleen Cu (nmol/g)	5.2 ± 0.5*	22.8 ± 0.5†
Testis Cu (nmol/g)	13.7 ± 0.6*	27.5 ± 1.6†
Plasma cholesterol (mmol/L)	1.78 ± 0.10*	1.22 ± 0.05†
Plasma volume (ml/100g b.w.)[b]	3.84 ± 0.17*	3.35 ± 0.03†
Liver weight (g/100g b.w.)	3.48 ± 0.08*	3.17 ± 0.07†
Body weight (g/rat)	172 ± 5	179 ± 4

[a] Mean ± SEM of 5 rats per group. Values in the same row for each parameter not sharing a common superscript are significantly different ($P < 0.05$).
[b] Determined by the radioisotopic dilution of ^{125}I-albumin (refer to Materials and methods).
b.w., body weight.

Rates of plasma clearance of native ^{125}I-LDL and acetylated ^{125}I-LDL

Figure 1 presents the time-course changes in the plasma ^{125}I-radioactivities associated with the TCA-precipitable and TCA-soluble fractions after injection of a dose of native ^{125}I-LDL (experiment 1). The clearance curve for the TCA-precipitable ^{125}I radioactivity (*Figure 1;* upper panel) indicated that ^{125}I-LDL was removed at a significantly faster rate in CuD rats compared with controls. The rates of ^{125}I clearance

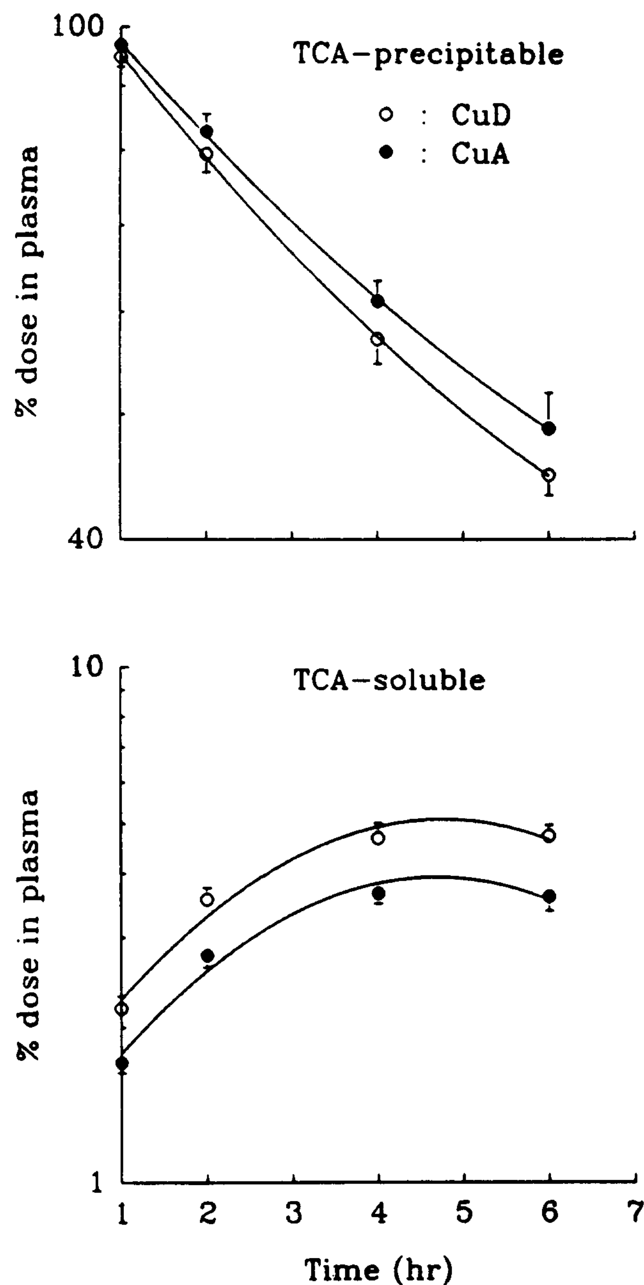

Figure 1 Time-dependent clearance of ^{125}I-labeled human LDL in copper-deficient (CuD) and copper-adequate (CuA) rats. Upper panel: percent of the injected dose of ^{125}I-radioactivity remaining in the plasma in the TCA-precipitable fraction at specified time intervals. The rates of ^{125}I clearance ($t_{1/2}$) were 4.9 ± 0.2 and 5.8 ± 0.3 hr for CuD and CuA groups, respectively. Lower panel: percent of the injected dose of ^{125}I radioactivity appearing in the plasma in the TCA-soluble fraction, which represents the ^{125}I radioactivity released subsequent to tissue uptake and degradation of ^{125}I-LDL. At each time interval, the plasma ^{125}I radioactivity appearing in the TCA-soluble fraction was significantly ($P < 0.05$) greater in CuD rats.

($t_{1/2}$) during the 6-hr period, as computed by the equation described earlier for exponential change,[20] were 4.9 ± 0.2 and 5.8 ± 0.3 hr for CuD and CuA groups, respectively. The curve for the TCA-soluble ^{125}I radioactivities (*Figure 1;* lower panel), representing the fraction of the injected dose of ^{125}I-LDL, which was metabolized and released into the plasma, showed a steady increase in a curvilinear manner in both CuD and CuA rats. At each time interval, the plasma ^{125}I radioactivity appearing in the TCA-soluble fraction was significantly ($P < 0.05$) greater in CuD rats, suggesting a faster rate of uptake and degradation of ^{125}I-LDL in these rats. No significant differences were noted between CuD and CuA rats in the distribution of ^{125}I-radioactivity in the liver, spleen, and kidneys. At 6 hr after injection of ^{125}I-LDL, only about 7% of total injected ^{125}I radioactivity was found in the liver, 0.6%–0.7% in the spleen and 0.65% in the kidneys in both CuD and CuA rats *(Table 4).*

Table 4 ^{125}I-radioactivity in selected organs at 6 hr after injection of native ^{125}I-LDL

	Liver	Spleen	Right kidney	Left kidney
	Total ^{125}I, % dose[a]			
CuD	7.04 ± 0.48	0.61 ± 0.03	0.64 ± 0.04	0.65 ± 0.05
CuA	6.93 ± 1.60	0.72 ± 0.01	0.66 ± 0.05	0.65 ± 0.04

[a] Mean ± SEM of 6 rats per group.

Table 5 Effect of copper deficiency on the rate of clearance of ^{125}I-acetyl LDL from the plasma

	Time interval (min) after ^{125}I-LDL dose			Half-life (min)
	5	10	20	
	TCA precipitable, % dose[a]			
CuD	36.0 ± 1.2	13.6 ± 0.5	6.09 ± 0.23	5.20 ± 0.06
CuA	31.4 ± 0.6	12.5 ± 0.4	6.32 ± 0.22	5.16 ± 0.08
	TCA soluble, % dose			
CuD	0.14 ± 0.01	0.74 ± 0.09	4.93 ± 0.36	
CuA	0.11 ± 0.01	0.56 ± 0.04	4.38 ± 0.07	

[a] Mean ± SEM of 5 rats per group.

Table 5 shows the time-course clearance of ^{125}I-acetyl LDL from the plasma in both CuD and CuA rats. In contrast to native ^{125}I-LDL, the removal of ^{125}I-acetyl LDL was extremely fast. Within 5 min after a dose of ^{125}I-acetyl LDL, 64%–69% of the total injected dose was removed from the plasma. In both groups, approximately 94% of the dose was cleared at 20 min. No difference was observed in the rate of clearance between the groups with the half life ($t_{1/2}$) of about 5.20 min in both groups. Also, there was no difference between the groups in the TCA soluble ^{125}I radioactivity appearing in the plasma (*Table 5*) or in the distribution of ^{125}I radioactivity in selected organs at 20 min after a dose of ^{125}I-acetyl LDL (*Table 6*). At 20 min, 42%–49% of the total injected dose was found in the liver and 1.1%–1.3% in the spleen. Only trace amounts of ^{125}I radioactivity were found in the kidney and testis.

Discussion

In normal control rats, plasma cholesterol is carried primarily by HDL.[4,7,22] However, when depleted of

Table 6 ^{125}I-radioactivity in selected organs at 20 min after injection of ^{125}I-acetyl LDL

	Liver	Spleen	Kidney	Testis
		Total ^{125}I, % dose[a]		
CuD	49.1 ± 2.6	1.11 ± 0.09	0.49 ± 0.03	0.13 ± 0.01
CuA	42.3 ± 1.0	1.32 ± 0.06	0.54 ± 0.02	0.13 ± 0.01

[a] Mean ± SEM of 5 rats per group.

copper, not only is the level of total plasma cholesterol elevated, but there also appears to be a disproportionate relative increase in cholesterol associated with LDL.[4,22] An increase in plasma LDL cholesterol also has been demonstrated in a human subject with experimental copper deficiency.[14] Such an increase in LDL cholesterol has been further confirmed in another study using adult men depleted of copper, despite no significant change in total plasma cholesterol.[15] The mechanism(s) for the observed changes is not known.

The primary objective of the present study was to examine whether elevation in LDL cholesterol was associated with defects in the removal of circulating LDL. The primary mechanism for removal of LDL is by the apoB,E receptor that accounts for 60%–80% of LDL clearance from the circulation. The acetyl-LDL receptor is an alternative mechanism for removal of LDL and it is independent of the apoB,E receptor pathway. The acetyl-LDL receptor is thought to represent an important mechanism for the removal of chemically modified forms of LDL that are particularly atherogenic.[23-27] In the present study, we used native and chemically modified human LDL to probe the expression of the apoB,E receptor and the acetyl-LDL receptor in copper-deficient rats. The native LDL and acetyl-LDL were cleared by distinctly different mechanisms, as indicated by a marked difference in the rate of clearance between native ^{125}I-LDL ($t_{1/2}$: 4.9–5.8 hr) and ^{125}I-acetyl LDL ($t_{1/2}$: 5.2 min). The data showed that native ^{125}I-LDL were removed from the plasma at a significantly faster rate in copper deficient rats. The rates ($t_{1/2}$) of clearance of ^{125}I-LDL in copper-deficient and adequate rats were 4.9 ± 0.2 and 5.8 ± 0.3 hr, respectively. On the other hand, the rate of clearance of ^{125}I-acetyl LDL did not differ between the groups, with a half-life of 5 min. The rates ($t_{1/2}$) of clearance of both native and acetyl ^{125}I-LDL observed here are in close agreement with those reported by other investigators.[28,29] As previously reported,[28] the tissue distribution of ^{125}I radioactivity after injection of radioiodinated LDL did not reflect the rate of plasma clearance, presumably due to the rapid intracellular degradation of iodinated proteins and release of ^{125}I subsequent to their uptake.[30] At 6 hr after injection of ^{125}I-LDL, only about 7% of the injected ^{125}I radioactivity was found in the liver, despite the fact that the hepatocyte (parenchymal cell) is the principal site of LDL uptake mediated by the receptor-dependent pathway. When ^{125}I-acetyl LDL were injected, 42%–49% of the dose was recovered in the liver at 20 min, with less than 2% found in the spleen, kidney, and testis combined. The results are consistent with those reported by others,[19,28,29] confirming that the liver is also the major organ for LDL uptake via the acetyl-LDL receptor. Unlike native LDL, however, the major cell type which takes up acetyl LDL in vivo is shown to be the liver sinusoidal endothelial cells and Kupffer cells.[25,31]

The present results demonstrate that the rate of plasma LDL clearance mediated by the apoB,E receptor is significantly increased in copper-deficient rats, whereas the removal of acetyl-LDL via the scavenger receptor remains essentially unchanged. This conclusion is further strengthened by the significant steady and continual rise in plasma ^{125}I radioactivity appearing in the TCA-soluble fraction after injection of ^{125}I-LDL in those rats (*Figure 1*, lower panel). The increase was maintained during the 6-hr period after injection of the labeled LDL, reflecting the increased rate of uptake and degradation of LDL in these rats. Thus, the results indicate that the hypercholesterolemia induced by copper deficiency is not due to the impaired clearance of plasma LDL by either the apoB,E receptor or the acetyl-LDL receptor. In an earlier study by Lefevre et al.,[6] it was proposed that the elevation in plasma cholesterol in copper-deficient rats might be largely due to a reduction in hepatic binding of apoE-poor HDL leading to their progressive conversion to apoE-rich HDL_1 particles, which then accumulate in the plasma. Hassel et al.[32] reported that the in vitro binding of apoE-free HDL by a hepatic membrane preparation from copper-deficient rats was significantly reduced. However, the data suggested that the reduced binding was attributable to the inherent molecular characteristics of the apoE-free HDL rather than to a reduction in membrane binding site numbers.[32] Recently, Zhang and Lei,[33] using cultured liver parenchymal cells, provided evidence that the cellular uptake of apoE-free HDL was markedly increased in the cultured hepatocytes obtained from copper deficient rats. In addition, Hassel et al.[34] observed that apoE-rich HDL binding to hepatic plasma membranes prepared from copper deficient rats was significantly increased. Consistent with this observation, we demonstrated, in an in vivo experiment[35] using lymphatic chylomicrons labeled with ^{3}H-retinyl ester, that the apoE-dependent uptake of chylomicron remnants by the liver was not impaired in copper deficient rats, but significantly increased during the initial phase of chylomicron metabolism. Carr and Lei[36,37] also observed increases in the in vivo clearance of HDL apoproteins and cholesterol ester in copper deficient rats. Thus, the observations made from the in vivo experiments[35-37] indicate that the tissue uptake (or clearance) of HDL in general is enhanced in copper deficiency.

The data presented here clearly indicate that LDL is also removed at a faster rate in copper-deficient rats. The possibility exists that the expression of the apoB,E-receptor is up-regulated in copper-deficient rats. The up-regulation of the LDL receptor is in line

with the observations of the low concentration of unesterified cholesterol in the liver,[9,11,38] stimulation of hepatic 3-hydroxy-3-methylglutaryl Coenzyme A reductase, and increased liver synthesis of cholesterol in copper-deficient rats.[10,11] In conjunction with the increased uptake and clearance of HDL as reported by others, our data support the concept, put forth by Lei and associates,[13,33] that the hypercholesterolemia induced by copper deficiency is not due to defects in the uptake of plasma lipoproteins by the liver and other tissues. Alternatively, the primary mechanism responsible for disturbed cholesterol homeostasis in copper deficiency may involve a stimulation of hepatic synthesis[10,11,39] and increased rate of secretion of cholesterol from the liver to the blood plasma, which may exceed the rate of tissue uptake of plasma cholesterol.[9,12,13] Such an alteration may explain the elevation in plasma LDL in copper-deficient rats[4] and humans,[14,15] despite the faster clearance of LDL, as demonstrated by the present study. However, the specific metabolic signal or event(s) that triggers the stimulation of hepatic synthesis and release of cholesterol is yet to be elucidated. Further studies are needed to quantitatively verify the postulated imbalance between the hepatic output and recycling (uptake) of plasma lipoproteins in copper deficiency, which leads to an elevation in plasma cholesterol.

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