Effect of copper deficiency on the hepatic synthesis and rate of plasma release of cholesterol

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The relative rates of hepatic ^3H incorporation into digitonin-precipitable sterols (DPS) and release of labeled DPS into the plasma were compared between copper-deficient (CuD) and copper-adequate (CuA) rats. Following intravenous injection of ^3H_2O and Triton WR 1339, the liver and plasma ^3H-DPS radioactivities were measured at 2, 4, 6, and 8 hr, and the net total accumulation of plasma cholesterol (CH) and triglyceride (TG) was determined during the 8-hr period. In both groups, the liver synthesis of ^3H-DPS displayed a biphasic curve, with an initial slow rate up to 4 hr, followed by a rapid increase starting at 6 hr. During the latter phase, a notable increase in liver ^3H-DPS radioactivity was observed in CuD rats, but the plasma release of ^3H-DPS did not significantly change, despite the increased synthesis of ^3H-DPS in the liver. In both groups, the net total accumulation of CH in the plasma, as expressed in mmol CH/100 g body weight, increased steadily from 1.2 mmol at 2 hr to 4.7 mmol at 8 hr, while plasma TG accumulated at a much greater rate ranging from 3.3 mmol at 2 hr to 13.6 mmol at 8 hr. There was no significant difference in net total accumulation of CH or TG in the plasma between the groups at any given interval. The results provide evidence that copper deficiency stimulates the liver synthesis of cholesterol, but that it does not result in an accelerated release of newly synthesized cholesterol from the liver involving very low density lipoproteins (VLDL). Thus, the present findings suggest that the hypercholesterolemia induced in copper deficiency is not attributable to a stimulation of the hepatic release of VLDL cholesterol into the plasma.

Keywords: copper deficiency; cholesterol synthesis; release

Introduction

Since the first observation by Klevay[1] that copper deficiency causes an elevation in plasma cholesterol in rats, numerous studies dealt with the mechanisms underlying the copper deficiency-induced hypercholesterolemia. Information on this subject has been reviewed in a recent publication.[2] Lei and associates[2] postulate that the elevation in plasma cholesterol is not attributable to defective uptake of plasma lipoproteins by the liver and other tissues, but may be associated primarily with a stimulation of the hepatic synthesis and release of cholesterol to the blood plasma. In rats, copper deficiency has been shown to increase the activity of hepatic 3-hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) reductase[3] and enhance the tissue uptake or plasma clearance of plasma lipoproteins in vivo[4–6] and in cultured cells,[7] with no significant alteration in bile acid synthesis and sterol excretion.[8,9]

At present, however, whether copper deficiency causes a stimulation of the hepatic synthesis and/or release of cholesterol has not been firmly established.[10] The present study was designed to address the following specific questions. Is the hepatic synthesis of cholesterol stimulated in copper deficient rats? If so, is the hepatic release of newly synthesized cholesterol via very low density lipoprotein (VLDL) into the plasma facilitated? The results provide evidence that copper deficiency stimulates the hepatic synthesis of choles-

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terol, but does not alter the rate of plasma appearance of newly synthesized cholesterol.

Materials and methods

Animals and diets

Male rats (Fischer 344, Charles River Breeding Lab. Inc., Wilmington, MA USA) with the initial body weight of 48 ± 1 g were placed individually in plastic cages with stainless-steel wire bottoms in a windowless room and subjected to a light cycle with a 1500–0300 light and 0300–1500 dark period. Temperature and humidity of the room were controlled at 23–25° C and 55–75%, respectively. All rats were housed in an 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: (a) copper-deficient group (CuD) fed ad libitum a diet containing 0.5 mg copper/kg diet *(Table 1)*, as determined by atomic absorption spectrophotometry; and (b) a 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). Diets were formulated according to the recommendations of the American Institute of Nutrition.[11,12] All rats were given distilled deionized water via an automatic stainless-steel watering system.

Determination of sterol synthesis and release

After 9 wk of dietary treatment, rats were fasted for 18 hr prior to injection of ^3H_2O and Triton WR 1339. Each rat from both groups was injected simultaneously via the left jugular vein with a dose of ^3H_2O and Triton WR 1339. The dose injected per 100 g body weight contained 10 mCi ^3H_2O (specific activity, 66.7 mCi/ml, Amersham, Arlington Heights, IL USA) and 30 mg of Triton WR 1339 (polymeric p-isooctyl polyoxyethylene phenol; Tyloxapol, Sigma Chemical, St Louis, MO USA) in 150 μL of saline. Injection of the doses was completed during the mid-dark phase (between 0821 and 1015) of the light cycle. Immediately after dose injection, a 0.4 mL blood sample was collected from each rat via the orbital sinus for analysis of plasma cholesterol and triglyceride. These values were used as the baseline plasma concentrations of the lipids at time 0 (t_0) for each rat. Three rats at 2 hr, and 4 rats at 4, 6, and 8 hr were exsanguinated and killed under ether anesthesia. The livers were excised for determination of the ^3H radioactivity incorporated into digitonin-precipitable sterols (DPS). Blood plasma was obtained by centrifugation at 2000*g* for 30 min and used to determine the plasma release of ^3H radioactivity in DPS and the rate of accumulation of total cholesterol and triglyceride at each time interval during the 8-hr period.

Extraction of digitonin-precipitable ^3H radioactivity in the liver and plasma samples were performed as described previously.[13] One gram of finely minced liver (or 2.0 mL plasma) was saponified in 30% ethanolic KOH at 75° C for 2 hr. From the saponified sample, sterols were extracted 3 times with 5 volumes of hexane. The hexane extract was then dried under N_2 and dissolved in acetone-ethanol (1:1, vol/vol). After adding a small amount of acetic acid (125 μL), sterols were precipitated with 3.75 mL of digitonin (20 mg/mL of 50% ethanol) for 30 min at room temperature. The precipitate was centrifuged and washed twice with acetone and once with ether. The DPS was dried under N_2, cleaved in 4.0 mL of pyridine, and extracted with 12 mL of hexane. The hexane-pyridine layer was recovered by centrifugation and 25 μL of the preparation was used for cholesterol analysis. To determine the ^3H radioactivity incorporated into the DPS, an aliquot of the extract was dried, re-dissolved in 1.0 mL of benzene and mixed with scintillation fluid (Ready Solve NA, Beckman Instruments, Palo Alto, CA USA). The entire procedure performed with a tissue sample mixed with 2 μCi of ^{14}C-cholesterol yielded 97.0–98.1% recovery. To determine the total plasma ^3H-DPS radioactivity, the total plasma volumes were calculated on the basis of 3.84 mL/100 g body weight for CuD and 3.35 mL/100 g body weight for CuA rats, as determined by a radioisotopic dilution method in our previous study.[6]

Determination of plasma lipids and other analyses

Plasma cholesterol and triglyceride were determined enzymatically[14,15] using analytical reagents (Sigma Chemical, St. Louis, MO USA). Triton WR 1339 at the expected plasma concentration range (0.7–1.5%) had no effect on cholesterol analysis with undiluted plasma. Triglyceride values were slightly higher at the same concentration range, relative to those obtained without Triton WR 1339. Therefore, for triglyceride analysis, plasma samples were diluted 1:20 with deionized water, and the final concentration of the detergent in the assay mixture was estimated to be 0.0008%. All calibration standards and blanks contained the same concentration of the detergent, although no appreciable interference was observed at this level.

For copper analysis, 200 mg of finely minced liver was digested with 1.25 mL of 20% tetramethylammonium hydroxide at 60° C for 4 hr, and plasma was diluted 1:3 with deionized water prior to analysis by atomic absorption spectrophotometry (Perkin-Elmer 5000, Perkin-Elmer Corp., Norwalk, CT USA). Hematocrit was determined by centrifugation in microcapillary tubes.

Statistical analysis

Data were analyzed by *t* test using a statistical computer program (PC ANOVA, Human Systems Dynamics, Northridge, CA USA). The level of significance was determined at $P < 0.05$.

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†	3.5
Vitamin mix‡	1.0

*Purchased from Zeigler Bros., Inc. (Gardeners, PA USA).
†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.
‡Formulated according to the AIN recommendations. Biotin content was increased to 50 mg/kg of the mix.

Results

Indices of copper status

Table 1 compares various indices of copper status of CuD and CuA rats. A significant decrease in plasma copper was noted in CuD rats, as determined at 9 wk. Characteristic of copper deficiency, plasma cholesterol was significantly elevated in CuD rats, with low hematocrit and enlarged hearts. The relative liver weight tended to increase in CuD rats, but did not significantly differ from that of the CuA group. The final body weight of CuD rats was significantly decreased. The food intake tended to decrease in CuD rats, with no significant difference between the groups throughout the experiment.

Time-dependent changes in liver and plasma ^3H-DPS radioactivity

Figure 1 shows changes in the rates of hepatic incorporation of ^3H into the DPS over the 8-hr period following injection of ^3H_2O. The upper panel compares time-course changes in the total ^3H-DPS radioactivities in the whole livers of CuD and CuA rats. The hepatic ^3H-DPS radioactivities rose slowly during the first 4 hr, with no appreciable difference between the two groups. At 6 hr, however, the liver ^3H-DPS radioactivities began to increase sharply in both groups. A significant increase in liver ^3H-DPS radioactivity was observed in CuD rats at 8 hr, compared with the CuA group at the same interval. When the relative rates of ^3H incorporation into DPS were compared on the basis of ^3H-DPS radioactivity per g liver per hr (the lower panel, *Figure 1*), there was no significant difference between the groups in the rate of ^3H incorporation at any time interval post-dosing.

Figure 2 presents the time-dependent changes in plasma ^3H-DPS radioactivity and the rate of ^3H-DPS release into the plasma in CuD and CuA rats. The upper panel shows the total ^3H-DPS radioactivity released in the plasma during the 8-hr period. At any given interval, there was no difference in total plasma ^3H-DPS radioactivity between the groups. There was no significant increase in the amount of newly synthesized DPS released into the plasma in CuD rats even at 8 hr, despite the significant increase in the liver ^3H-DPS radioactivity observed at the same interval (upper panel, *Figure 1*). The lower panel *(Figure 2)* compares the rates of ^3H-DPS release into the plasma on the basis of plasma ^3H-DPS released per 100 g body weight per hr. No difference was noted between the groups in the rate of release of ^3H-DPS into the plasma.

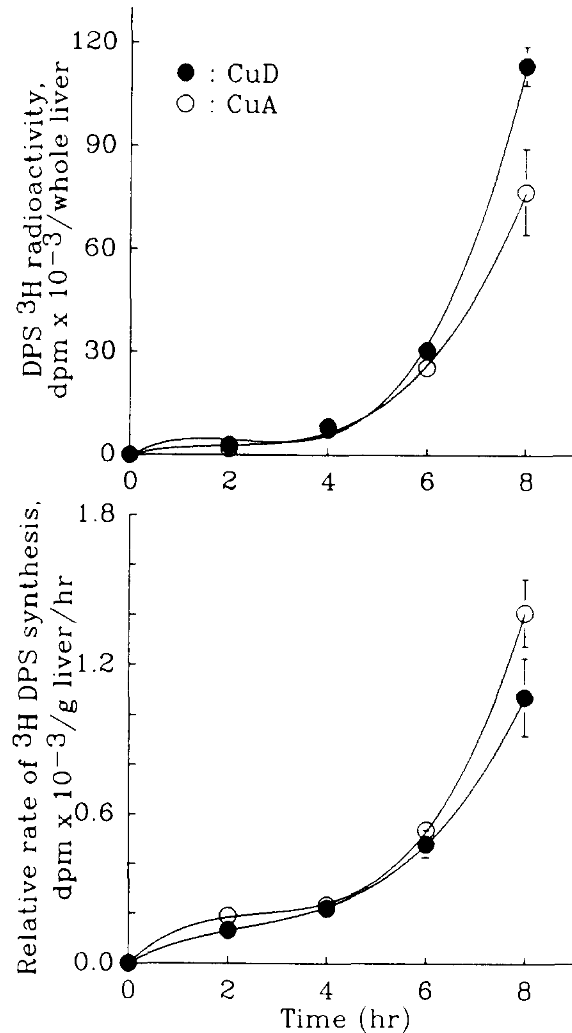

Figure 1 Time-course changes in the rates of hepatic incorporation of ^3H into the digitonin-precipitable sterols (DPS) following injection of ^3H_2O. Upper panel: time-course changes in the total ^3H-DPS radioactivities in the whole livers of CuD and CuA rats. The hepatic ^3H-DPS radioactivities rose in a biphasic manner. A significant increase in liver ^3H-DPS radioactivity was observed in CuD rats at 8 hr. Lower panel: comparison of the relative rates of ^3H incorporation into DPS on the basis of ^3H-DPS radioactivity per g liver per hr. No significant difference was noted between the groups in the rate of ^3H incorporation at any time interval post-dosing.

Table 2 Plasma copper and other indices of copper status*

	CuD	CuA
Plasma Cu (μmol/L)	1.68 ± 0.47[a]	20.78 ± 0.63[b]
Plasma cholesterol (mmol/L)	2.64 ± 0.07[a]	2.13 ± 0.08[b]
Hematocrit (%)	41.5 ± 0.4[a]	45.6 ± 0.3[b]
Heart weight (g/100 g b.w.)	0.38 ± 0.01[a]	0.30 ± 0.00[b]
Liver weight (g/100 g b.w.)	4.53 ± 0.17	3.97 ± 0.17
Body weight (g)	235 ± 5[a]	252 ± 3[b]

*Mean ± SEM (n = 15, except for plasma Cu, n = 7). Values in the same row not sharing a common superscript are significantly different ($P < 0.05$).

Time-dependent changes in plasma cholesterol and triglyceride

Figure 3 shows the cumulative net increase in plasma cholesterol (CH) and triglyceride (TG). The cumulative net increase of plasma lipid was determined by the difference between the levels of a lipid in the total volume of plasma at the given interval (t_i) and at the

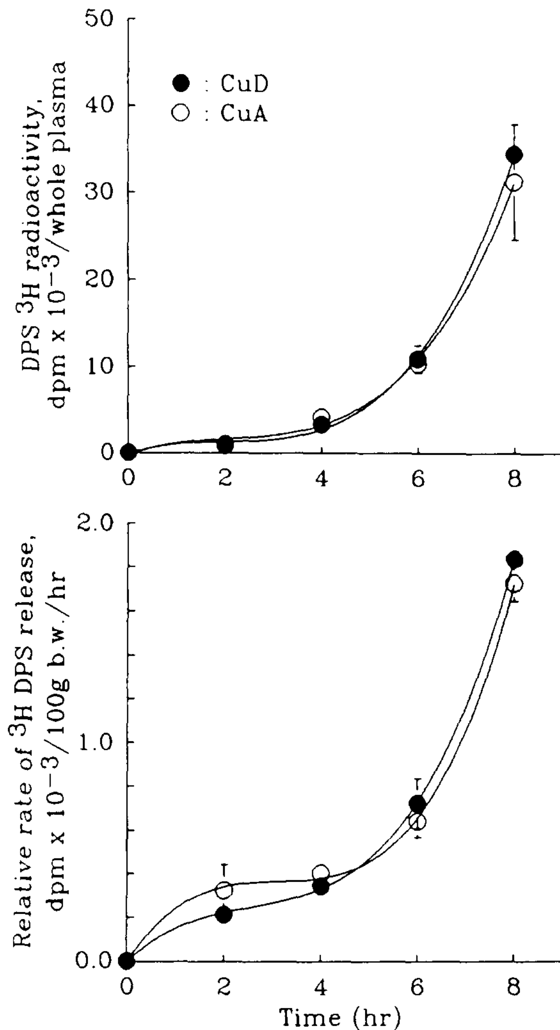

Figure 2 Time-dependent changes in plasma ^{3}H-DPS radioactivity and the rate of ^{3}H-DPS release into the plasma. Upper panel: total ^{3}H-DPS radioactivity released in the plasma during the 8-hr period. At any given interval there was no difference in total plasma ^{3}H-DPS radioactivity between the groups. Lower panel: rates of ^{3}H-DPS release into the plasma on the basis of plasma ^{3}H-DPS released per 100 g body weight per hr. No difference was noted between the groups at any time interval.

time (t_0) of dosing (baseline value at t_i). Following injection of Triton WR 1339, the net accumulation of CH in the plasma, as expressed in mmol CH/100 g body weight, increased steadily from 1.2 mmol at 2 hr to 4.7 mmol at 8 hr, while plasma TG accumulated at a much greater rate ranging from 3.3 mmol at 2 hr to 13.6 mmol at 8 hr. There was no significant difference in net total accumulation of CH or TG in the plasma between the groups at any given interval. The net total accumulation of TG tended to be greater in CuA rats, but the difference was not significant ($P > 0.05$). Throughout the 8-hr period, the rate of plasma release of CH or TG, as expressed in mmol lipid/hr/100 g body weight, remained virtually unchanged in both groups *(Figure 4)*. No difference was noted between CuD and CuA groups in the rate of release of CH or TG into the plasma *(Figure 4)*.

Discussion

Previously, it has been postulated that the primary mechanism underlying the hypercholesterolemia induced by copper deficiency may be attributable to an accelerated hepatic synthesis and release of cholesterol into the plasma.[2] The hypothesis is mainly based on the following indirect evidence: increased activity of liver 3-hydroxy-3-methylglutaryl Coenzyme A (HMG CoA) reductase,[3] enhanced tissue uptake of plasma

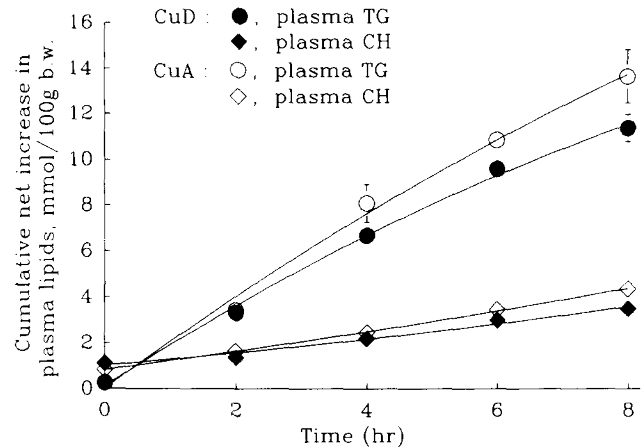

Figure 3 The cumulative net increase in plasma cholesterol (CH) and triglyceride (TG). The cumulative net increase of plasma lipid was determined by the difference between the levels of a lipid in the total volume of plasma at the given interval (t_i) and at the time (t_0) of dosing (baseline value at t_i). The net accumulation of CH in the plasma increased steadily from 1.2 mmol at 2 hr to 4.7 mmol at 8 hr, while plasma TG accumulated at a much greater rate ranging from 3.3 mmol at 2 hr to 13.6 mmol at 8 hr. There was no significant difference in net total accumulation of CH or TG in the plasma between the groups at any given interval.

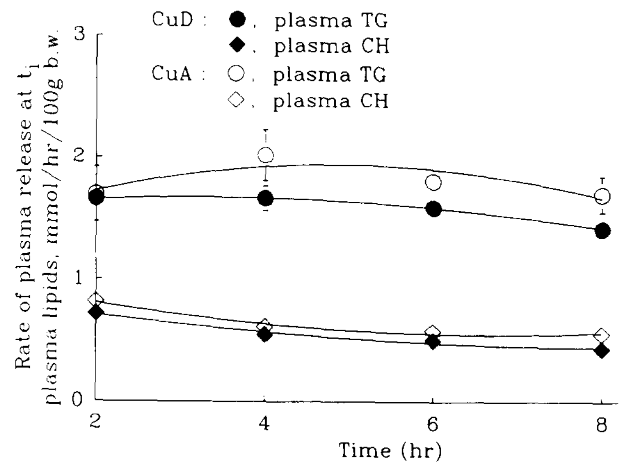

Figure 4 Comparison of the rates of plasma release of CH or TG, as expressed in mmol lipid/hr/100 g body weight in CuD and CuA rats. Throughout the 8-hr period, no difference was noted between the groups in the rate of release of CH or TG into the plasma.

lipoproteins, as demonstrated in vivo[4-6] and in cultured cell models,[7] and no change in bile acid synthesis and excretion[8,9] in copper-deficient rats. At present, however, no direct evidence is available concerning whether the hepatic synthesis and/or release of cholesterol is accelerated in copper deficiency.

In the present study, we investigated the effect of copper deficiency on the hepatic synthesis and release by injecting rats with ^3H_2O and Triton WR 1339, a non-ionic detergent. The detergent has been used to inhibit lipoprotein lipase and shown not to alter the hepatic synthesis[16] and release[17] of lipids in rats. The method allows the triglyceride-rich lipoproteins (VLDL) released from the liver to accumulate in the plasma.[16-18] By injecting ^3H_2O and Triton WR 1339 intravenously, we monitored the rates of hepatic incorporation of the tracer (^3H) into DPS, and the rates of accumulation of the newly synthesized cholesterol (^3H-DPS) and total cholesterol in the blood plasma. The present study provides two important findings. First, copper deficiency produces a significant increase in the liver synthesis of sterols. This is evidenced by the higher total hepatic ^3H-DPS radioactivity in CuD rats, relative to the CuA animals *(Figure 1)*. This increase is largely due to the hypertrophy of the liver associated with copper deficiency *(Table 2)*. Second, copper deficiency does not facilitate the hepatic release of newly synthesized sterols into the plasma. This conclusion is based on the observation that the rate of plasma release of ^3H-DPS or the total amount of ^3H-DPS accumulated in the plasma over the 8-hr period did not differ ($P > 0.05$) between the two groups *(Figure 2)*.

The significantly higher ^3H-DPS radioactivity in the livers of the CuD rats shown here is concurrent with the increased liver HMG CoA reductase activity, as observed by others.[3,19] Recently, Yount et al.[10] also measured the hepatic synthesis of cholesterol by using ^3H_2O as the tracer. The data[10] showed no difference in the rate of hepatic incorporation of ^3H into DPS between CuD rats and controls, despite a two-fold increase in liver HMG CoA reductase activity in CuD rats, as reported earlier.[3] The discrepancy may be explained partly by the difference in the duration of tracer (^3H_2O) incorporation. The investigators[10] determined the hepatic ^3H-DPS radioactivity at 1 hr after ^3H_2O injection. In the present study, the ^3H incorporation into DPS was measured at the intervals of 2, 4, 6, and 8 hr. From our data *(Figure 1)*, at 1 hr after ^3H_2O injection, no difference in hepatic ^3H-DPS radioactivity was evident between CuD and control groups. A significant increase in the total liver ^3H-DPS radioactivity in CuD rats was detectable only at 8 hr. Yount et al.[10] speculated that the failure to observe an increase in liver ^3H-DPS radioactivity in CuD animals might be due to an increased release of the newly synthesized sterol from the liver into the plasma in CuD rats.[20] In the present study, throughout the 8-hr period, the total ^3H-DPS radioactivity released into the plasma of CuD rats did not differ from that of controls, despite a sharp increase in the hepatic ^3H-DPS radioactivity in CuD rats at 8 hr *(Figure 2)*. The total plasma ^3H-DPS radioactivities in both CuD and control rats rose with time in a close parallel to the liver ^3H-DPS radioactivities up to 6 hr. In addition, no significant differences were noted between the two groups in the net total amount of cholesterol released into the plasma and in the rate of plasma release of cholesterol during the 8-hr period *(Figures 3* and *4)*. It should be emphasized that in the present experiment we aimed at examining the hepatic release of newly synthesized cholesterol via VLDL. The peripheral lipolysis of triglyceride-rich lipoproteins (VLDL) released into the plasma was effectively blocked by Triton. This was shown by the steady increase in plasma triglyceride ranging from 3.3 mmol at 2 hr to 13.6 mmol at 8 hr, and in plasma cholesterol from 1.2 to 4.7 mmol *(Figure 3)*.

Plasma cholesterol is a product of the complex metabolic interplay of many factors, including the intestinal absorption, hepatic uptake, synthesis and release, and vascular and peripheral metabolism. It has been well documented[2] that plasma cholesterol is elevated in CuD rats, even when fed no cholesterol. Our previous study[4] showed that the intestinal absorption of cholesterol is significantly decreased in CuD rats and that the plasma clearance of cholesterol carried by chylomicrons remains unimpaired in copper deficiency. Numerous studies[21-23] using intact rats or cultured hepatocytes also have demonstrated that the hepatic uptake or clearance of plasma high density and low density lipoproteins is enhanced in copper deficiency. Therefore, a defect in hepatic uptake or clearance of plasma lipoproteins is ruled out as the underlying cause of the hypercholesterolemia.

The present data demonstrate that copper deficiency stimulates the liver synthesis of cholesterol. The data also show that the total amount and rate of hepatic export of cholesterol to the plasma via VLDL remains unchanged despite its increased synthesis in CuD rats. This observation does not necessarily suggest a defect in the hepatic release of VLDL into the plasma in CuD rats. The possibility exists that the liver synthesis of VLDL is stimulated in CuD rats, while the hepatically derived VLDL particle is relatively low in cholesterol as a result of an inadequate availability of intracellular cholesterol[3,20,24] during VLDL assembly in the liver. Under these conditions, the total amount of plasma cholesterol carried by VLDL can be reduced, although the rate of hepatic release of VLDL into the plasma is enhanced. The low amount of cholesterol packaged per VLDL particle may explain the lack of a significant change in the total amount of cholesterol released into the plasma in CuD rats, despite the increased synthesis of cholesterol in the liver, as shown by the present data. Supportive of this possibility is the recent observation of Al-Othman et al.[25] that the VLDL particle of rats carried only one-fifth the amount of cholesterol but 2.7 times the amount of triglyceride carried by that of CuA animals. In the CuD rats, they also observed a significant decrease in the plasma pool size of VLDL cholesterol and a marked (six-fold) increase in the pool size of VLDL triglyceride.

Previously,[26] it has been postulated that copper deficiency may result in impaired degradation or clearance of VLDL after their release into the plasma. In keeping with the hypothesis, a significant decrease in post-heparin lipoprotein lipase activity has been shown in CuD rats.[21,26] Such a defect in VLDL catabolism may be an important factor contributing to the hypertriglyceridemia. However, it does not appear to be the underlying cause of the hypercholesterolemia observed in copper deficiency, because not only the total hepatic output of cholesterol via VLDL remains unaltered, as demonstrated in the present study, but also the total plasma pool of cholesterol carried by VLDL is significantly reduced in copper deficient rats, as shown by others.[25]

In future studies, attention should be directed to whether copper deficiency alters the hepatic packaging and release of cholesterol via HDL, primary cholesterol carriers in the rat plasma. The mechanisms responsible for the hypercholesterolemia in copper deficiency may be associated with an imbalance between the hepatic release and re-uptake of plasma cholesterol involving plasma HDL.

References

1 Klevay, L.M. (1973). Hypercholesterolemia in rats produced by an increase in the ratio of zinc to copper ingested. *Am. J. Clin. Nutr.* **26,** 1060–1068
2 Lei, K.Y. (1990). Plasma cholesterol response in copper deficiency. In *Role of Copper in Lipid Metabolism,* (K.Y. Lei, ed), p. 1–23, CRC Press, Boca Raton, FL USA
3 Yount, N.Y., McNamara, D.J., Al-Othman, A.A., and Lei, K.Y. (1990). The effect of copper deficiency on rat hepatic 3-hydroxy-3-methylglutaryl Coenzyme A reductase activity. *J. Nutr. Biochem.* **1,** 21–27
4 Koo, S.I., Lee, C.C., and Norvell, J.E. (1988). Effect of copper deficiency on the lymphatic absorption of cholesterol, plasma chylomicron clearance, and postheparin lipase activities. *Proc. Soc. Exp. Biol. Med.* **188,** 410–419
5 Carr, T.P. and Lei, K.Y. (1989). In vivo apoprotein catabolism of high density lipoproteins in copper deficient, hypercholesterolemic rats. *Proc. Soc. Exp. Biol. Med.* **191,** 370–376
6 Koo, S.I., Lee, C.C., Stone, W.L., and Scott, R.L. (1992). Effect of copper deficiency on the plasma clearance of native and acetylated human low density lipoproteins. *J. Nutr. Biochem.* **3,** 45–51
7 Zhang, J.J. and Lei, K.Y. (1990). Specific binding and uptake of apolipoprotein E-free high density lipoproteins by cultured liver parenchymal cells of copper-deficient rats. *J. Nutr. Biochem.* **1,** 291–298
8 Lei, K.Y. (1978). Oxidation, excretion, and distribution of [26-^{14}C] cholesterol in copper-deficient rats. *J. Nutr.* **108,** 232–237
9 Allen, K.G.D. and Klevay, L.M. (1978). Copper deficiency and cholesterol metabolism in the rat. *Atherosclerosis* **31,** 259–271
10 Yount, N.Y., Carr, T.P., McNamara, D.J., and Lei, K.Y. (1991). Incorporation of triated water into sterol in copper deficient rats. *Biochim. Biophys. Acta* **1082,** 79–84
11 The American Institute of Nutrition (1977). Report of the American Institute of Nutrition Ad Hoc Committee on standards for nutritional studies. *J. Nutr.* **107,** 1340–1348
12 The American Institute of Nutrition (1980). Second report of the ad hoc committee on standards for nutritional studies. *J. Nutr.* **110,** 1726
13 Jeske, D.J. and Dietschy, J.D. (1980). Regulation of rates of cholesterol synthesis in vivo in the liver and carcass of the rat measured using [^{3}H] water. *J. Lipid Res.* **21,** 364–378
14 Allain, C.C., Poon, L.S., Chan, C.S.G., Richmond, W., and Fu, P.C. (1974). Enzymatic determination of total serum cholesterol. *Clin. Chem.* **20,** 470–475
15 Bucolo, G. and David, H. (1973). Quantitative determination of serum triglyceride by the use of enzymes. *Clin. Chem.* **19,** 476–482
16 Byers, S.O., Freidman, M., and Sugiyama, T. (1963). Triton hypercholesterolemia: Cause or consequence of augmented cholesterol synthesis. *Am. J. Physiol.* **204,** 1100–1102
17 Otway, S. and Robinson, D.S. (1967). The use of a non-ionic detergent (Triton WR 1339) to determine rates of triglyceride entry into the circulation of the rat under different physiological conditions. *J. Physiol.* **190,** 321–332
18 Ishikawa, T. and Fidge, N. (1979). Changes in the concentration of plasma lipoproteins and apoproteins following the administration of Triton WR 1339 to rats. *J. Lipid Res.* **20,** 254–264
19 Valsala, P. and Kurup, P.A. (1987). Investigation on the mechanism of hypercholesterolemia observed in copper deficiency in rats. *J. Biosci.* **12,** 137–142
20 Shao, M.J.S. and Lei, K.Y. (1980). Conversion of [2-^{14}C] mevalonate into cholesterol, lanosterol and squalene in copper deficient rats. *J. Nutr.* **110,** 859–867
21 Koo, S.I., Lee, C.C., and Norvell, J.E. (1988). Effect of copper deficiency on the lymphatic absorption of cholesterol, plasma chylomicron clearance, and postheparin lipase activities. *Proc. Soc. Exp. Biol. Med.* **188,** 410–419
22 Carr, T.P. and Lei, K.Y. (1989). In vivo apoprotein catabolism of high density lipoproteins in copper deficient, hypercholesterolemic rats. *Proc. Soc. Exp. Biol. Med.* **191,** 370–376
23 Zhang, J.J. and Lei, K.Y. (1990). Specific binding and uptake of apolipoprotein E-free high density lipoproteins by cultured liver parenchymal cells of copper-deficient rats. *J. Nutr. Biochem.* **1,** 291–298
24 Harvey, P.W. and Allen, K.G.D. (1985). Lipoproteins and liver lipids in copper-deficient rats. *Nutr. Res.* **5,** 511–525
25 Al-Othman, A.A., Rosenstein, F., and Lei, K.Y. (1992). Copper deficiency alters plasma pool size, percent composition and concentration of lipoprotein components in rats. *J. Nutr.* **122,** 1199–1204
26 Lau, B.W.C. and Klevay, L.M. (1982). Post-heparin plasma lipoprotein lipase in copper-deficient rats. *J. Nutr.* **112,** 928–933