Impaired Biliary Excretion of Copper and Lysosomal Enzymes in Long-Evans Cinnamon Rat

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Abe, S., Yamazaki, K., Takikawa, S. and Suzuki, K. Impaired Biliary Excretion of Copper and Lysosomal Enzymes in Long-Evans Cinnamon Rat. Tohoku J. Exp. Med., 1994, 172 (4), 355-367 — Although impaired biliary excretion of copper through hepatocyte lysosomes has been postulated as a pathogenesis of Wilson’s disease, direct evidence has been lacking. Our aim was to investigate the dynamics of biliary excretion of copper and lysosomal enzymes in the Long-Evans Cinnamon (LEC) rat, a recently established rodent model of Wilson’s disease. Liver homogenate and bile were obtained from 12 week-old LEC rats (n = 7), Long-Evans Agouti rats (n = 3) and Sprague-Dawley rats (n = 8) and analyzed for copper and lysosomal enzymes. Structural integrity of hepatic lysosomes was assessed by the latency of N-acetyl-β-glucosaminidase. Compared with the controls, LEC rats exhibited a 43-fold increase in hepatic copper concentration (p <0.001), a significant increase in hepatic activities of lysosomal enzymes (p<0.001) and reduction of lysosomal latency (p <0.05). In contrast, biliary excretion of copper and lysosomal enzymes were significantly impaired in LEC rats (p<0.05). These results suggest a coupled alteration between copper and lysosomal enzymes in both the liver and bile of LEC rats (i.e., increase in liver and decrease in bile). Defective biliary excretion of copper via hepatocyte lysosomes may play a role in part in spontaneous copper accumulation in LEC rats.

The liver plays a central role in copper metabolism (Sternlieb 1980; Winge 1984). In hepatocytes, copper is incorporated into various copper-containing enzymes such as ceruloplasmin, while excess copper is bound to metallothionein to be detoxified (Gollan 1990). Most importantly, biliary excretion, presumably through hepatocyte lysosomes, represents the paramount pathway for body copper elimination and regulates the entire copper metabolism (Evans 1973; Sternlieb et al. 1973; Van Berge Henegouwen et al. 1977; Gross et al. 1989).

Wilson’s disease is an inherited disorder of copper metabolism characterized
by a marked accumulation of copper in the liver (Sternlieb 1980; Gollan 1990). Although the exact pathogenesis remains unknown, impaired biliary excretion of copper via hepatocyte lysosomes has been postulated as a central defect in Wilson’s disease (Sternlieb et al. 1973). However, information regarding the actual alteration of the “lysosome-to-bile hepatic excretory pathway (LaRusso 1989; Yamazaki and LaRusso 1989)” in spontaneous copper accumulation is fragmentary (Goldfischer 1963; Frommer 1974). Furthermore, previous experimental studies (Gross et al. 1989; Harada et al. 1993), investigating the relationship between biliary copper excretion and lysosomal exocytosis, equivocally used animals exogenously overloaded with copper which may potentially differ from spontaneous copper overload.

The Long-Evans Cinnamon (LEC) rat with hereditary hepatitis is a mutant inbred strain isolated from a closed colony of Long-Evans rats (Sasaki et al. 1985; Yoshida et al. 1991). Recent studies have demonstrated spontaneous and excessive accumulation of copper in the liver of LEC rats as well as reduced serum ceruloplasmin concentration, suggesting that LEC rats may be a new animal model of Wilson’s disease (Li et al. 1991; Okayasu et al. 1992). Indeed, hepatocytes of LEC rats exhibit lipid deposition and mitochondrial alterations, the characteristic histological change in Wilson’s disease (Li et al. 1991). Thus, the aim of the present study was to systematically investigate the biliary excretion of copper and lysosomal enzymes during spontaneous copper accumulation in LEC rats. We have demonstrated in LEC rats: i) a significant increase in copper concentration and the activities of lysosomal enzymes in the liver, ii) an impaired structural integrity of hepatic lysosomes, and iii) a significant reduction in biliary excretion of copper and lysosomal enzymes. The coupled alteration between copper and lysosomal enzymes in liver and bile suggests a role of a defective biliary excretion of copper via hepatocyte lysosomes in spontaneous copper accumulation in LEC rats.

**Materials and Methods**

**Animals**

LEC rats (n = 7) were purchased from the Charles River Japan, Inc. (Yokahama). Long-Evans Agouti rats (LEA; n = 3), an inbred sibling line of LEC rat simultaneously isolated from Long-Evans strain, and Sprague-Dawley rats (SD; n = 8) were used as controls. LEA rats were a generous gift from professor Hiroshi Kobayashi (Laboratory of Pathology, Cancer Institute, Hokkaido University School of Medicine, Sapporo). SD rats were purchased from Japan SLC Inc. (Hamamatsu). All rats were male and 12 week-old when used in experiments. The 12 week-old LEC rats did not express jaundice, bilirubinuria or histological signs of hepatitis. Previous studies have also shown that LEC rats at this age neither have hepatitis biochemically nor histologically (Kasai et al. 1990), while the rats do develop severe hepatitis with jaundice four to five months after birth (Kasai et al. 1990). All rats were housed in the animal experiment facility with a room temperature of 23 ± 1.5°C, a humidity of 55 ± 5%, under a constant light-dark cycle with the light on from 6 a.m. to 8 p.m. The animals were allowed free access to a standard laboratory diet and water, but
were fasted for 12 hr prior to experiments.

**Bile collection**

A complete biliary fistula was created between 6 a.m. and 8 a.m., because diurnal variations of biliary copper and lysosomal excretion have been reported (Gross et al. 1989; Nakano et al. 1990). Under pentobarbital anesthesia (50 mg/kg i.p.), bile duct was cannulated with polyethylene tubing (PE-10; Clay Adams, Parsippany, NJ, USA). We tied the proximal end of the tube 5 mm below the hepatic duct bifurcation in order to avoid contamination by pancreatic juice. The distal end was positioned approximately 3 cm below the point of cannulation. Bile was collected on ice in pre-weighted, plastic vials hourly for three hours. The animals were placed in a constant temperature-humidity apparatus at 25°C and 60 per cent humidity. The rectal temperature was monitored by an electronic thermometer (CD 700; Chino Works, Co. Ltd., Tokyo) and maintained between 37 and 38°C throughout the experiments. We determined the volume of collected bile by weight assuming the density of bile to be 1.0 g/ml. Bile samples were frozen at −80°C until analyzed.

**Liver homogenization and fractionation**

Rats were sacrificed by exsanguination. The livers were quickly removed and immersed in ice-cold 250 mM sucrose. Approximately 4 grams of liver was minced and homogenized in the isotonic sucrose solution with three strokes of a Potter-Elvejhem homogenizer (Kontes Glass, Vineland, NJ, USA) using a digital homogenizer (Iuchi Seieido, Osaka) at 1,600 r.p.m. The homogenate was centrifuged at 1,000 g for 11 min (RL-101; Tomy Seiko, Tokyo) to separate nuclei and unbroken cells (N-fraction) from cell sap and other cellular organelles (E-fraction; postnuclear supernate) by the method of de Duve et al. (1955) as described by LaRusso and Fowler (1979). Intracellular distribution of copper was investigated in LEC and SD rats (n = 3, respectively). To separate cytosol from the non-cytosolic fractions, the E-fraction was further centrifuged at 100,000 g for one hour in a RP-65T rotor (Hitachi Koki, Tokyo) using an ultracentrifuge (65P; Hitachi Koki). The resultant supernatant and pellet were used as cytosol and non-cytosolic fractions, respectively. The non-cytosolic fraction should contain all membrane-bound intracellular organelles except nuclei (i.e., mitochondria, lysosomes, peroxisomes, microsomes). Hepatic lysosomes were isolated by the method of Yamada et al. (1984). Briefly, E-fraction was first incubated with 1 mM CaCl₂ at 37°C to selectively reduce the density of mitochondria, a major contaminant in lysosomal isolates. The E-fraction was then layered on a Percoll gradient (specific gravity 1.08; Pharmacia, Uppsala, Sweden) and centrifuged at 60,000 g for 15 min at 4°C with a fixed-angle rotor (RP50-2, Hitachi Koki). Lysosomal fraction was collected and washed in isotonic sucrose. The yield and purification fold of isolated lysosomes were 40.0±7.6% and 69.2±26.7 fold, respectively (mean±s.d.). All procedures of homogenization and subfractionation were performed on ice or at 4°C with all fractions being frozen and stored at −80°C until analyzed.

**Biochemical analyses**

**Measurement of copper concentration**

Copper concentration was determined by an atomic absorption spectrophotometer equipped with a flameless atomizer (model FLA-100; Nippon Jarrel Ash, Kyoto) and a graphite furnace (Mitsubishi Pencil, Tokyo) as we have described (Takikawa 1990). Liver fractions were diluted with deionized water following digestion with nitric acid, while bile was diluted appropriately with deionized water and Triton X-100 for copper measurements. Intra- and interassay coefficients of variation for the determination of copper concentration were both less than 10%.

**Measurement of lysosomal enzyme activities**

The activities of three lysosomal enzymes, namely, N-acetyl-β-glucosaminidase (EC
3.2.1.30; NAG), β-galactosidase (EC 3.2.1.23; GAL), and β-glucuronidase (EC 3.2.1.31; GLU) were measured fluorometrically using 4-methylumbelliferyl substrates (Sigma Chemical, St. Louis, MO, USA) with a fluorometer (F-3000; Hitachi Koki) as described by LaRusso and Fowler (1979).

Assessment of structural integrity of hepatic lysosomes

Structural integrity of the hepatic lysosomes was assessed by latency measurement of NAG in E-fraction (Gross et al. 1989). The latency was calculated using the following equation: latency (%) = (total activity - free activity)/total activity x 100. The free activity represents the activity in the absence of 0.1% Triton-X in the assay mix, while the total activity was determined in the presence of 0.1% Triton-X. Therefore, if all lysosomes were intact, the latency would be 100%.

Other biochemical measurements

The total protein concentration was spectrophotometrically measured using the Coomassie Brilliant Blue method (Bradford 1976) with a commercially available kit (Bio-Rad Protein Assay; Bio-Rad Laboratories, Richmond, CA, USA) and a U-2000 spectrophotometer (Hitachi Koki). The use of this protein assay for bile has been extensively validated by us (Yamazaki et al. 1988). The total bile acid concentration was determined by the method of Mashige et al. (1976).

Statistical analyses

All data were presented as the mean±S.D. Statistical analysis was performed using Student's t-test and one-way analysis of variance with p values less than 0.05 considered to be statistically significant.

Results

Body weight and liver-to-body weight ratio

Body weight was significantly smaller in LEC rats (178±11 g, p<0.001) than in LEA (330±27 g) or SD rats (340±12 g). Liver-to-body weight ratio was also significantly less in LEC rats (2.68±0.10%, p<0.01) than in LEA (3.12±0.20%)

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<td>(n=7)</td>
<td>(n=3)</td>
<td>(n=8)</td>
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<td>Copper (μg/g liver)</td>
<td>340±10</td>
<td>7.9±0.5</td>
<td>8.3±2.2</td>
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<td>Protein (mg/g liver)</td>
<td>223±13</td>
<td>198±15†</td>
<td>247±6</td>
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<tr>
<td>NAG</td>
<td>1.36±0.06**</td>
<td>0.68±0.07</td>
<td>0.84±0.14</td>
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<td>Enzyme activity (Ug/liver)</td>
<td>0.71±0.10**</td>
<td>0.26±0.02</td>
<td>0.42±0.07</td>
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<td>GLU</td>
<td>1.84±0.24**</td>
<td>1.01±0.09</td>
<td>1.03±0.13</td>
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<tr>
<td>NAG</td>
<td>6.11±0.49**</td>
<td>3.44±0.10</td>
<td>3.41±0.52</td>
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<td>Specific activity (Ug/protein)</td>
<td>3.17±0.48**</td>
<td>1.33±0.06</td>
<td>1.70±0.27</td>
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<tr>
<td>GLU</td>
<td>8.29±1.29**</td>
<td>5.10±0.10</td>
<td>4.19±0.56</td>
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<td>Lysosomal latency (%)</td>
<td>82.5±1.9*</td>
<td>not tested</td>
<td>86.2±2.8</td>
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The data are expressed as mean±s.d.
NAG, N-acetyl-β-glucosaminidase; GAL, β-galactosidase; GLU, β-glucuronidase.
*p<0.05 vs. SD; **p<0.001 vs. LEA and SD; †p<0.01 vs. LEC, p<0.001 vs. SD.
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Biochemical analyses of the liver

Table 1 summarizes the results of the biochemical analyses of the liver. The hepatic copper concentration in LEC rat was more than 40 times greater in LEC rats compared with LEA or SD rats ($p < 0.001$). The total activity of the lysosomal enzyme, NAG, was significantly increased in LEC rats ($p < 0.001$). Specific activity of NAG was also significantly higher in LEC rats, ($p < 0.001$). Similar results were obtained for the two other lysosomal enzymes, GAL and GLU.

Structural integrity of the hepatic lysosomes

The latency of NAG in E-fractions was determined in LEC and SD rats (Table 1). The latency in LEC rats was significantly lower than that of SD rats ($82.5 \pm 1.9\%$ vs. $86.2 \pm 2.8\%$, $p < 0.05$), suggesting that the structural integrity of lysosomes in the liver of LEC rats was impaired.

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**Fig. 1.** Bile flow and biochemical changes in the bile. 1a, hourly bile flow; 1b, hourly bile acid output into bile; 1c, hourly copper output into bile; 1d, hourly output of NAG activity into bile. NAG: N-acetyl-$\beta$-glucosaminidase. The abscissa indicates hours of bile collection. The data are expressed as mean with standard deviation.

- LEC rat; □ LEA rat; △ SD rat. *$p < 0.05$ vs. LEC; **$p < 0.01$ vs. LEC; ***$p < 0.001$ vs. LEC.
**Bile flow**

As shown in Fig. 1a, stable bile flow was maintained during bile collection for three hours in each rat. When expressed in μl per gram liver weight, bile flow was significantly higher in LEC rats (149.4 ± 21.7 μl/g liver/hr for the first one hour [(p < 0.05)]) compared with SD or LEA rats (112.0 ± 13.4 μl/g liver/hr and 86.0 ± 10.2 μl/g liver/hr, respectively).

**Biochemical analyses of the bile**

As shown in Fig. 1b, total bile acid output into bile decreased over the three hours, presumably due to the interruption of the enterohepatic circulation of bile acids by the complete biliary fistula. However, bile acid output itself did not differ among the three strains. The protein output into bile was all stable during

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![Graphs showing correlations among specific activities of three lysosomal enzymes](image)

**Fig. 2.** Correlations among the specific activities of three lysosomal enzymes in bile. NAG, N-acetyl-β-glucosaminidase; GAL, β-galactosidase; GLU, β-glucuronidase.

- LEC rat; ○ LEA rat; △ SD rat.
the experiment and did not significantly differ among the three groups (for the first one hour: LEC rats; 142 ± 65 μg/g liver/hr, LEA rats: 95 ± 34 μg/g liver/hr, SD rats; 120 ± 24 μg/g liver/hr). Fig. 1c demonstrates the hourly copper output into bile, which was stable throughout the experiment for three hours in each strain. The copper output was significantly reduced in LEC rats (for the first one hour: 43.1 ± 12.0 ng/g liver/hr in LEC rats (p < 0.05 vs. SD rats, p < 0.01 vs. LEA rats), 83.0 ± 27.0 ng/g liver/hr in LEA rats, and 68.7 ± 16.4 ng/g liver/hr in SD rats). As shown in Fig. 1d, hourly biliary excretion of NAG activity was constant throughout the experiments. The biliary excretion of NAG activity in LEC rats was one-third of those of LEA and SD rats (for the first one hour: LEC rats, 0.28 ± 0.15 mU/g liver/hr; LEA rats, 0.86 ± 0.49 mU/g liver/hr; SD rats, 0.90 ± 0.38 mU/g liver/hr) and the differences were statistically significant (p < 0.01 vs. SD rats, p < 0.05 vs. LEA rats). The specific activities of NAG in bile were also significantly lower (p < 0.01) in LEC rats (for the first one hour: 2.2 ± 1.5 U/g protein) than in LEA rats (8.7 ± 2.9 U/g protein) and SD rats (7.8 ± 3.6 U/g protein). Similar significant reduction in enzyme activities was found for two other lysosomal glycosidases, GAL and GLU (data not shown). The strong positive correlations (r-value = 0.854 ~ 0.910) among the specific activities of the three lysosomal enzymes in bile (Fig. 2) indicated a coupled, coordinated

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Fig. 3. Correlation between biliary output of copper and lysosomal enzymes. 3a, all rats; 3b, LEC rats; 3c, LEA rats; 3d, SD rats. NAG, N-Acetyl-β-glucosaminidase.

● LEC rat; ○ LEA rats; △ SD rats.
Correlation between biliary output of copper and lysosomal enzyme

As demonstrated in Fig. 3a, a significant correlation between biliary output of copper and NAG activity was observed when all the data of LEC, LEA and SD rats were combined ($r = 0.443$, $p < 0.001$). No apparent correlation was found when the bile of each rat was independently investigated (LEC rats, $r = 0.333$, $p = 0.141$ (Fig. 3b); LEA rats, $r = 0.229$, $p = 0.553$ (Fig. 3c); SD rats, $r = 0.079$, $p = 0.713$ (Fig. 3d)), although the $p$ value was smallest in LEC rats.

Intracellular distribution of copper

Table 2 summarizes copper distribution in the subfractions of the liver of LEC and SD rats. In SD rats, 83% of copper was found in E fraction (cytosol plus cellular organelles other than nuclei), of which 48% resided in cytosolic fraction. In contrast, in LEC rats, 95% copper was found in E fraction, of which 90% resided in cytosol fraction. Thus, in LEC rats, copper was more abundant in E fraction than in N fraction ($p < 0.05$) and cytosolic fraction than in non-cytosolic fraction ($p < 0.001$).

Intralysosomal concentration of copper

The direct measurement of copper concentration in purified lysosomal fraction revealed that the copper concentration was four times higher in hepatic lysosomes of LEC rats than in SD rats (3.82 ± 1.26 ng Cu/μg lysosomal protein vs. 0.98 ± 0.45 ng Cu/μg lysosomal protein, $p < 0.05$), suggesting significant accumulation of copper in hepatic lysosomes in LEC rats.

Discussion

Lysosomes play a pivotal role in intracellular digestion and storage of various macromolecules (LaRusso 1989; Yamazaki and LaRusso 1989) and, in he-
patocytes, lysosomes can excrete their contents into bile, presumably, by bulk exocytosis (LaRusso and Fowler 1979). This process has been termed the "lysosome-to-bile hepatic excretory pathway" (LaRusso 1989; Yamazaki and LaRusso 1989) and the alterations of this pathway have been extensively studied in experimental copper overload (Gross et al. 1989; Harada et al. 1993). In contrast, the studies on lysosomal exocytosis into bile during spontaneous copper accumulation have been limited mainly due to the rarity of the disease, the difficulty in obtaining bile samples from patients, and the limited access to animal model. The present study is the first systematic investigation, to our knowledge, of the alteration of the lysosome-to-bile excretory pathway in spontaneous copper accumulation.

We have demonstrated a coupled alteration between copper and lysosomal enzymes in the liver and bile from spontaneous copper accumulation in LEC rats (i.e., increase in the liver and decrease in bile). Table 3 summarizes the copper metabolism and lysosomal alterations in our study of endogenous copper overload in LEC rats as well as in previous studies on exogenous/experimental copper overload by Gross et al. (1989) and Harada et al. (1993). Two points should be emphasized. First, biochemical changes in the liver were similar between the endogenous and exogenous copper overload. The liver expressed, in both cases: i) marked copper accumulation, ii) increased activites of lysosomal enzymes, and iii) reduced structural integrity of lysosomes. These results indicate that, irrespective of the way of copper overload, hepatic copper accumulation can induce lysosomal activation in the liver, finally leading to the structural instability of lysosomal membrane. Second, despite the aforementioned similarities in the

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<th>Table 3. Comparison of copper metabolism and lysosomal alteration between exogenous and endogenous copper overload.</th>
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↑ increase, ↓ decrease, * histological demonstration without biochemical measurement
liver, the dynamics of biliary copper excretion and lysosomal exocytosis was apparently different between the two. In exogenous copper overload, biliary outputs of both copper and lysosomal enzymes are significantly increased (Gross et al. 1989; Harada et al. 1993), suggesting that the increased biliary outputs could be a simple reflection of the overflow of increased lysosomal contents into bile. In contrast, biliary outputs of copper and lysosomal enzymes are significantly decreased in intrinsic copper accumulation in LEC rats. From these data, it seems reasonable to assume that increased biliary output in exogenous copper overload is a consequence of increased hepatic copper accumulation, while decreased biliary excretion of copper by impaired lysosomal exocytosis per se is responsible, at least in part, for the endogenous hepatic copper accumulation in LEC rats. Similarly, increased activities of hepatic lysosomal enzymes and decreased biliary excretion of copper have been found in patients with Wilson's disease (Goldfischer 1963; Frommer 1974) and impaired biliary excretion of copper into bile via hepatocyte lysosomes has been hypothesized as a pathogenesis of Wilson's disease (Sternlieb et al. 1973). Taken together, LEC rats may serve as an excellent model to investigate the copper metabolism in spontaneous copper overload including Wilson's disease. It should be born in mind, however, that the findings in LEC rats may not be always extrapolated to Wilson's disease, because the clinical features are not completely identical between the two (e.g., high incidence of hepatocellular carcinoma in LEC rats (Masuda et al. 1988).

Our data indicate copper concentration in purified hepatic lysosomes exhibits only a fourfold increase as opposed to a 43-fold increase in copper concentration in the liver. Ninety per cent of hepatic copper is located in the cytosolic (e.g., non-lysosomal) fraction and copper staining and x-ray microanalysis both failed to demonstrate marked copper accumulation in hepatocyte lysosomes in LEC rats (Yamazaki K and Masuda T: unpublished observations). It has been well established that copper shifts from a cytosolic localization to a lysosomal localization as Wilsonian patients become symptomatic (Goldfischer and Sternlieb 1968). Copper detection in the pre-symptomatic patients is often difficult due to diffuse cytosolic localization. Twelve week-old LEC rats used in our experiments are comparable with young, pre-symptomatic patients with Wilson's disease, since LEC rats spontaneously develop hepatitis about four to five months after birth (Kasai et al. 1990). Therefore, it is not surprising that a marked copper accumulation in hepatocyte lysosomes could not be observed.

An important question to be addressed here is whether decreased biliary excretion of copper is solely explained by the decreased lysosomal exocytosis into bile. While our data indicate that significant, but not marked, increase in copper accumulation does take place in hepatocyte lysosomes in 12-week-old LEC rat, another dysfunction(s) leading to decreased biliary copper excretion may be operative in addition to impaired lysosomal exocytosis in LEC rat. First, impaired copper transport from cytosol to lysosomes may result in reduced biliary copper
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excretion via the lysosomal pathway. Yamada et al. (1993) have recently shown, in an elaborative fashion, defective copper incorporation into ceruloplasmin and reduced copper transfer from cytosolic to non-cytosolic fraction in the hepatocytes of LEC rat. They speculated that the abnormality in copper delivery mechanism is the fundamental defect in LEC rats. In light of our data also indicating marked cytosolic accumulation of copper in the liver of LEC rats, the defective transport of cytosolic copper into lysosomes may have paramount importance in reduced biliary copper excretion. Second, decreased biliary copper excretion could be also explained by a impaired egress of copper from hepatocytes into bile via a non-lysosomal pathway. We have shown a strong correlation among the specific activities of three lysosomal enzymes in bile, suggesting a bulk, coordinat-ed exocytosis of lysosomal contents into bile. However, the correlation between biliary output of copper and lysosomal enzyme did not reach statistical significance in LEC rats ($p=0.14$). While this may be either due to small sample number or the narrow range of copper concentration and NAG activity in bile of LEC rats, a possibility could not be excluded that a portion of hepatocyte copper is excreted into bile via a non-lysosomal pathway. Previous studies suggest that the origin of biliary copper is heterogeneous (Kressner et al. 1984; Houwen et al. 1990) and Kuipers et al. (1993) have recently demonstrated the potential presence of a non-lysosomal pathway for biliary copper excretion. Current information regarding the non-lysosomal pathway of biliary copper excretion, however, is extremely limited and the quantitative implication of a non-lysosomal pathway in defective biliary excretion of copper in LEC rats remains to be elucidated.

The increased bile flow in LEC rat was an unexpected finding. Although actual bile flow per hour did not differ among the rats investigated, LEC rat showed a significantly higher rate of bile production when the bile flow was normalized by liver weight, since the liver weight of LEC rats was much smaller. Bile acids have been known as a major driving force of bile production (Erlinger 1988). Total bile acid concentration in the bile of LEC rats, however, was not different from that of the control. Therefore, increased bile flow in LEC rats may be caused by augmentation of bile acid-independent fraction of bile production.

In conclusion, we have shown, for the first time, a coupled alteration between copper and lysosomal enzymes in the liver and bile during spontaneous copper accumulation in LEC rats (i.e., increase in the liver and decrease in bile). Our data indicate that impaired copper and lysosomal excretion into bile plays a role in the pathogenesis of the hepatic copper accumulation. Further study is needed to elucidate the quantitative importance of the lysosome-to-bile hepatic excretory pathway in the impaired biliary copper excretion in LEC rats.

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References


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