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Excess dietary histidine decreases the liver copper level and serum alanine aminotransferase activity in Long-Evans Cinnamon rats

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Long-Evans Cinnamon (LEC) rats spontaneously develop fulminant hepatitis, associated with excess Cu accumulation in the liver: thus, they are considered an animal model of Wilson's disease. In the present study, we investigated the ability of excess dietary histidine to reduce the excess accumulation of liver Cu in LEC rats by comparing them with Fischer rats. The results clearly showed that the excess-histidine diet markedly stimulated the Cu excretion in urine, and significantly decreased the liver Cu content in LEC rats by 47·5%. The serum Cu content in LEC rats was not influenced by excess dietary histidine. We also compared the effects of excess dietary histidine on some liver antioxidant enzyme activities, liver and serum lipid levels and serum alanine aminotransferase activity of LEC and Fischer rats. Dietary histidine decreased the activities of total and Cu,Zn-superoxide dismutase in the liver of both strains. In LEC rats, the liver cholesterol content decreased, and serum cholesterol and phospholipids levels increased on feeding the excess-histidine diet. When fed on the basal diet, the serum alanine aminotransferase activity was higher in LEC rats than in Fischer rats, but a significant decrease in serum alanine aminotransferase activity of LEC rats was observed on feeding the excess-histidine diet. These results suggest that excess dietary histidine is effective in removing Cu ions from the liver of LEC rats. Thus, it may be of benefit in the prevention or treatment of liver injury in LEC rats and in patients with Wilson's disease.

Copper: Histidine: Alanine aminotransferase: Long-Evans Cinnamon rats

Long-Evans rats with a cinnamon-like colour (LEC rats) are an animal model of Wilson's disease, a hereditary disease caused by a mutation in the Cu-transporting P-type ATPase gene (Wu *et al.* 1994). They show all the features of Wilson's disease, including excess Cu in the liver, reduced excretion of Cu into bile, reduced serum Cu and a remarkable decrease in serum caeruloplasmin activity (Li *et al.* 1991; Muramatsu *et al.* 1994). LEC rats spontaneously develop acute hepatitis at about 4 months of age and hepatocellular carcinoma from 1 year after birth. It has been demonstrated that excess hepatic Cu results in the development of liver injury in LEC rats (Masuda *et al.* 1988; Li *et al.* 1991; Sone *et al.* 1992; Mori *et al.* 1994). Chelation therapy, such as D-penicillamine, or feeding a Cu-deficient diet can ameliorate the symptoms of LEC rats and Wilson's disease (Walshe, 1956; Sugawara *et al.* 1991*a*; Togashi *et al.* 1992; Klein *et al.* 2000). D-Penicillamine is the first-choice therapy for Wilson's disease (Loudianos & Gitlin, 2000), but 25–30% of patients show sensitivity to D-penicillamine in the form of rashes, fever, lymphadenopathy, leucopaenia or thrombocytopaenia, and a smaller number develop serious penicillamine toxicity with arthalgias, nephrotic syndrome, lupoid-like reactions and pemphigus (Steen *et al.* 1986).

Aoyama *et al.* (1999) showed that when Wistar rats were fed an excess-histidine diet (addition of 50 g L-histidine/kg diet), urinary output of Cu markedly increased and the hepatic Cu content significantly decreased through the whole test period (42 d). This interesting observation prompted us to examine whether excess dietary histidine might reduce excess Cu accumulation in liver and thus to investigate other therapeutic approaches that might also be relevant to Wilson's disease. Thus, we supplied excess histidine in the diet of LEC rats to elucidate the effects of excess dietary histidine on Cu content in the liver, serum and urine, some liver antioxidant enzyme activities, liver and serum lipid levels, and serum alanine aminotransferase activity of LEC rats. We compared the results with those of Fischer rats.

Materials and methods

Animals and treatments

The present study complied with the Animal Experimental Guides according to the Committee of Experimental Animal Care, Hokkaido University. Two strains of male rats (LEC rats, Centre for Experimental Plants and

Abbreviation: LEC, Long-Evans Cinnamon.

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Animals, Hokkaido University, Japan; Fischer rats, Japan SLC Inc., Hamamatsu, Shizuoka, Japan), with an initial body weight 108 g, were housed individually in stainless-steel wire-bottomed cages in an air-conditioned room kept at approximately 23°C and with a 12 h light–dark cycle (lights on at 08.00 hours). The initial ages of LEC and Fischer rats were about 54 and 49 d respectively. The rats in each strain were divided into two groups of six rats each and were provided *ad libitum* access to either the basal or excess-histidine diet and deionized–distilled water for 28 d. The composition of the experimental diets is shown in Table 1. The excess-histidine diet was similar to the basal diet, except for the addition of 50 g L-histidine (Katayama Chemical Industries Co. Ltd, Osaka, Japan)/kg diet replacing an equal weight of maize starch. Body weight and food intake were measured each day during the whole experimental period.

After being fed for 28 d, rats were killed by decapitation and blood samples were collected. The livers were immediately removed, weighed and frozen by dropping into liquid N_2. Serum was prepared from blood samples by centrifuging at 3000 ***g*** for 10 min. Samples of the liver and serum were stored at −80°C until analysis. During days 13–14 and 27–28, urine was collected using metabolism cages.

Measurements

A sample of each liver was prepared by wet-ashing with concentrated HNO_3 and subsequent complete digestion with H_2O_2 (300 ml/l) (Forbes *et al.* 1983). Cu and Zn contents in the liver, serum and urine were estimated by atomic absorption spectrophotometer (Hitachi Ltd, 1978) using certified reference standards.

Superoxide dismutase activity was measured by the inhibition of Nitroblue Tetrazolium reduction mediated by the xanthine–xanthine oxidase-generated superoxide anions and monitored spectrophotometrically at 560 nm (McCord & Fridovich, 1969). Samples of liver were homogenized using a Potter-Elvehjem type Teflon homogenizer (Tokyo Rikakikai Co. Ltd, Tokyo, Japan). The homogenizing buffer consisted of 0·25 M-sucrose, 0·5 mM-EDTA and Triton X-100 (5 ml/l; pH 8·0). The homogenates were centrifuged at 5000 ***g*** for 20 min. Mn-superoxide dismutase activity was inhibited by the addition of 0·2 mM-KCN to the tissue homogenate. Total superoxide dismutase activity was measured without KCN solution. Cu,Zn-superoxide dismutase activity was calculated by the method of difference (total superoxide dismutase activity – Mn-superoxide dismutase activity). One unit of activity was defined as the amount of enzyme required to inhibit the rate of Nitroblue Tetrazolium reduction by 50%.

Catalase activity was determined following H_2O_2 reduction as described by Aebi (1974). Samples of liver were homogenized using a Potter-Elvehjem type Teflon homogenizer. The homogenizing buffer consisted of 320 mM-sucrose, 1 mM-EDTA and 10 mM-Tris-HCl (pH 7·4). Homogenates were centrifuged at 12 000 ***g*** for 30 min. Using the supernatant fraction, catalase activity was measured spectrophotometrically at 240 nm and one unit of activity was defined as the amount of enzyme that liberated half the peroxide oxygen from an H_2O_2 solution per min.

Glutathione peroxidase activity was measured as the reduction of NADPH at 340 nm by the method of Paglia & Valentine (1967). Liver samples were homogenized using the Potter-Elvehjem type Teflon homogenizer. The homogenizing buffer consisted of 50 mM-Tris-HCl, 5 mM-EDTA, and 1 mM-2-mercaptoethanol. Homogenates were centrifuged at 12 000 ***g*** for 30 min at 4°C. The supernatant fraction was used for measuring glutathione peroxidase activity. One unit of activity was defined as the amount of enzyme necessary to reduce 1 mol NADPH per min.

The protein content was determined using bicinchoninic acid (Smith *et al.* 1985) with bovine serum albumin as the standard.

Hepatic lipids, extracted and purified by the method of Folch *et al.* (1957), were gravimetrically estimated after removing the solvent. Triacylglycerol (Nagele *et al.* 1985) and cholesterol (Siedel *et al.* 1983) in the liver were estimated by enzymatic methods. Phospholipids in the liver were calculated by the method of difference (total lipids − (triacylglycerol + cholesterol)). Serum triacylglycerol (Nagele *et al.* 1985), cholesterol (Siedel *et al.* 1983) and phospholipids (Takayama *et al.* 1977) were measured by enzymatic methods. For the calculation of concentration of triacylglycerol and phospholipids, molecular masses of 885·4 (triolein) and 786·1 (L-α-phosphatidylcholine dioleoyl) were used for tricylglycerol and phospholipids respectively.

The activity of alanine aminotransferase was determined by the method of Wroblewski & LaDue (1956).

Table 1. Composition of experimental diets (g/kg diet)

Ingredients	Basal diet*	Excess-histidine diet†
Casein‡	250·0	250·0
L-Histidine§	–	50·0
Vitamin mixture (AIN-93G-VX)‖	10·0	10·0
Choline bitartrate¶	2·5	2·5
Mineral mixture (AIN-93G-MX)‖	35·0	35·0
Soyabean oil¶	70·0	70·0
Maize starch**	632·5	582·5

* The diet was calculated to contain 17·4 MJ digestible energy/kg from the main ingredients (casein 16·7, soyabean oil 37·6, maize starch 16·8 kJ/g).
† The diet was calculated to contain 17·4 MJ digestible energy/kg from the main ingredients (casein 16·7, L-histidine 16·7, soyabean oil 37·6, maize starch 16·8 kJ/g).
‡ New Zealand Dairy Board, Wellington, New Zealand.
§ Katayama Chemical Industries Co. Ltd, Osaka, Japan.
‖ Formulated according to Reeves *et al.* (1993).
¶ Wako Pure Chemical Industries Ltd, Osaka, Japan.
** Gelatinized; Chuo Shokuryo Co. Ltd, Inazawa, Aichi, Japan.

Statistical analysis

Data were analysed by two-way ANOVA (Snedecor & Cochran, 1989) with diet and strain as the independent variables, and significance of the differences between mean values was determined by Duncan's multiple range test (Duncan, 1955). A probability of $<0·05$ was considered significant.

Table 2. Food intake (g/28 d), body-weight gain (g/28 d) and final liver weight (g/kg body weight) of rats fed the basal or excess-histidine diet*

(Mean values with their standard errors for six rats per group)

	Initial body weight		Food intake		Body-weight gain		Liver weight	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
LEC rats								
Basal	108·3	2·5	351[a]	7	87·3[b]	5·9	31·7[c]	0·7
Excess-histidine	108·1	1·9	280[b]	10	44·0[c]	7·1	39·5[b]	0·7
Fischer rats								
Basal	108·1	0·7	366[a]	9	127·9[a]	3·4	40·5[b]	0·5
Excess-histidine	108·1	0·7	286[b]	8	87·7[b]	3·0	47·0[a]	0·6
Statistical significance of effect (ANOVA, *P*)								
Diet			$<0·001$		$<0·001$		$<0·001$	
Strain			NS		$<0·001$		$<0·001$	
Diet×strain			NS		NS		NS	

LEC, Long-Evans Cinnamon.
[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 574.

Results

Food intake, body-weight gain and liver weight

Food intake, body-weight gain and liver weight of rats are shown in Table 2. The food intake of both strains of rats fed on the excess-histidine diet significantly decreased, but there was no significant difference between the two strains. The body-weight gains of both strains of rats was significantly decreased by feeding the excess-histidine diet. Body-weight gain was lower in LEC than in Fischer rats when fed on either the basal or excess-histidine diet. The liver weight of LEC rats was significantly lower than that of Fischer rats when fed on the excess-histidine diet. Both strains of rats had significantly increased liver weights compared with those fed the basal diet.

Liver copper and zinc concentrations

Cu and Zn concentrations in the livers of rats are shown in Table 3. Those in the liver of LEC rats fed the basal diet were about 62·90 and 2·85 times greater than those of Fischer rats respectively. In the livers of LEC rats fed the excess-histidine diet, the Cu and Zn concentrations were significantly reduced to 52·5 and 67·5% of those fed the basal diet, although they were still higher than those of Fischer rats.

Serum copper and zinc concentrations

Cu and Zn concentrations in serum of rats are shown in Table 4. Those in LEC rats fed on the basal diet were significantly lower than those of Fischer rats fed on the basal diet. The excess-histidine diet decreased serum Cu and Zn concentrations in Fischer rats, but did not influence those in LEC rats.

Urine copper and zinc concentrations

Cu and Zn concentrations in the urine of rats are shown in Table 5. The excess-histidine diet markedly stimulated the urinary output of Cu and Zn in both strains of rats. During the first period, Cu and Zn concentrations in the urine of

Table 3. Copper and zinc concentrations (μmol/g liver) in the livers of rats fed the basal or excess-histidine diet*

(Mean values with their standard errors for six rats per group)

	Copper		Zinc	
	Mean	SE	Mean	SE
LEC rats				
Basal	3·56[a]	0·27	1·03[a]	0·05
Excess-histidine	1·87[b]	0·16	0·695[b]	0·039
Fischer rats				
Basal	0·0566[c]	0·0017	0·362[c]	0·011
Excess-histidine	0·0349[c]	0·0012	0·374[c]	0·006
Statistical significance of effect (ANOVA, *P*)				
Diet	$<0·001$		$<0·001$	
Strain	$<0·001$		$<0·001$	
Diet × strain	$<0·001$		$<0·001$	

LEC, Long-Evans Cinnamon.
[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 574.

Table 4. Serum copper and zinc concentrations (nmol/ml) in rats fed the basal or excess-histidine diet*

(Mean values with their standard errors for six rats per group)

	Copper		Zinc	
	Mean	SE	Mean	SE
LEC rats				
Basal	1·91[c]	0·19	26·5[b]	1·1
Excess-histidine	2·78[c]	0·26	27·9[b]	2·8
Fischer rats				
Basal	17·5[a]	0·4	36·3[a]	1·5
Excess-histidine	14·7[b]	1·4	25·0[b]	0·5
Statistical significance of effect (ANOVA, *P*)				
Diet	NS		$<0·01$	
Strain	$<0·001$		NS	
Diet × strain	$<0·05$		$<0·01$	

LEC, Long-Evans Cinnamon.
[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 574.

LEC rats were similar to those of Fischer rats when fed on the basal diet, but when fed on the excess-histidine diet, the urinary excretion of Cu and Zn in LEC rats was significantly higher than that of Fischer rats. During the second period, Cu and Zn concentrations in the urine of LEC and Fischer rats were similar when fed on either the basal or excess-histidine diet.

Liver superoxide dismutase, catalase and glutathione peroxidase activities

Superoxide dismutase, catalase and glutathione peroxidase activities in the liver are shown in Table 6. The activities of total superoxide dismutase and Cu,Zn-superoxide dismutase in the liver of rats fed on the excess-histidine diet were significantly decreased in both LEC and Fischer rats. The Mn-superoxide dismutase activity in the liver of Fischer rats fed on the excess-histidine diet significantly decreased. When fed on the basal diet, the activities of total, Mn- and Cu,Zn-superoxide dismutases were lower in LEC rats than in Fischer rats. When fed on the excess-histidine diet, total superoxide dismutase activity of LEC rats was lower than that of Fischer rats, but Mn- and Cu,Zn-superoxide dismutase activities were similar in both strains.

The activity of catalase in the liver was not significantly different between rats fed the basal and excess-histidine diets, but that of Fischer rats was markedly higher than that of LEC rats. The activities of glutathione peroxidase in the liver of both strains were similar between those fed on the basal and excess-histidine diet. When fed on the basal diet, the activity of glutathione peroxidase in the liver of LEC rats was lower than that of Fischer rats. However, the activity of glutathione peroxidase was

Table 5. Urine copper and zinc concentrations (μmol/kg body weight per 2 d) in rats fed the basal or excess-histidine diet*

(Mean values with their standard errors for six rats per group)

	1st period (days 13–14)				2nd period (days 27–28)			
	Copper		Zinc		Copper		Zinc	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
LEC rats								
Basal	2·50[c]	0·18	0·71[c]	0·10	2·45[b]	0·53	0·70[b]	0·08
Excess-histidine	13·0[a]	1·3	81·0[a]	3·4	9·59[a]	1·86	56·7[a]	11·1
Fischer rats								
Basal	1·81[c]	0·08	0·92[c]	0·11	1·82[b]	0·09	0·72[b]	0·08
Excess-histidine	7·82[b]	0·25	64·3[b]	2·9	7·34[a]	0·57	58·2[a]	1·6
Statistical significance of effect (ANOVA, *P*)								
Diet	<0·001		<0·001		<0·001		<0·001	
Strain	<0·001		<0·01		NS		NS	
Diet×strain	<0·01		<0·01		NS		NS	

LEC, Long-Evans Cinnamon.
[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 574.

Table 6. Liver superoxide dismutase, catalase and glutathione peroxidase activities (U/mg protein) in rats fed the basal or excess-histidine diet*

(Mean values with their standard errors for six rats per group)

	Superoxide dismutase									
	Total		Mn-		Cu, Zn-		Catalase		Glutathione peroxidase	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
LEC rats										
Basal	9·31[b]	0·25	2·44[b]	0·08	6·87[b]	0·25	112[b]	5	0·862[b]	0·032
Excess-histidine	8·41[c]	0·24	2·41[b]	0·08	6·00[c]	0·20	102[b]	5	0·970[ab]	0·049
Fischer rats										
Basal	10·4[a]	0·3	2·86[a]	0·14	7·56[a]	0·23	285[a]	8	1·09[a]	0·031
Excess-histidine	8·91[b]	0·11	2·51[b]	0·08	6·40[bc]	0·13	286[a]	14	1·09[a]	0·067
Statistical significance of effect (ANOVA, *P*)										
Diet	<0·001		NS		<0·001		NS		NS	
Strain	<0·01		<0·05		<0·05		<0·001		<0·01	
Diet × strain	NS		NS		NS		NS		NS	

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[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 574.

similar between LEC and Fischer rats when fed on the excess-histidine diet.

Liver total lipids, triacylglycerol, cholesterol and phospholipids concentrations

Lipid concentrations in the liver of rats are shown in Table 7. The concentrations of total lipids, triacylglycerol and phospholipids in the liver of LEC rats were not significantly different from those fed on the basal and excess-histidine diets. The cholesterol concentration in the liver of LEC rats fed on the excess-histidine diet was significantly lower. In Fischer rats, feeding the excess-histidine diet significantly increased the concentrations of total lipids, triacylglycerol and cholesterol in the liver. The content of phospholipids in the liver of Fischer rats fed on the excess-histidine diet was similar to that of the basal diet. When fed on the basal diet, the concentrations of total lipids, triacylglycerol, cholesterol and phospholipids were not different between the two strains. However, when fed on the excess-histidine diet, the concentrations of total lipids, triacylglycerol and cholesterol in the livers of LEC rats were significantly lower than those of Fischer rats, and the concentrations of phospholipids in the livers were similar in both strains.

Serum triacylglycerol, cholesterol and phospholipids concentrations

The levels of triacylglycerol, cholesterol and phospholipids in the serum are shown in Table 8. The levels of cholesterol and phospholipids in the serum of LEC and Fischer rats fed on the excess-histidine diet significantly increased. Triacylglycerol level in the serum of Fischer rats significantly decreased on feeding the excess-histidine diet, but serum triacylglycerol level was not affected in LEC rats. When fed on the basal diet, the levels of triacylglycerol and phospholipids in the serum of LEC rats were significantly lower than those of Fischer rats, and cholesterol level was similar in both strains. On the excess-histidine diet, the levels of cholesterol and phospholipids in the serum of LEC rats were lower than those of Fischer rats, and triacylglycerol level was similar in both strains.

Serum alanine aminotransferase activity

Alanine aminotransferase activity in the serum is shown in Table 9. As compared with Fischer rats, serum alanine aminotransferase activity of LEC rats was higher when fed on the basal diet, and there was no difference between the strains when fed on the excess-histidine diet. Feeding of the excess-histidine diet to LEC rats, but not to Fischer rats, decreased the activity of alanine aminotransferase in the serum.

Discussion

The large amount of Cu accumulated in the livers of the LEC rats, an animal model of Wilson's disease, was also observed by Li *et al.* (1991) and Muramatsu *et al.* (1994) (Table 3); this also occurs in Wilson's disease. The key therapeutic strategy of Wilson's disease is to reduce the amount of Cu in the liver and other tissues by administering Cu-chelating agents (Rakela *et al.* 2002). In addition to accumulation in the liver, Cu also accumulates in the brain and cornea of patients with Wilson's disease; however, this does not occur in LEC rats (Mori *et al.* 1994). Our aim was, therefore, to reduce the Cu in the liver of LEC rats with histidine as a chelator of metal ions. Similarly to D-penicillamine, histidine can chelate Cu to form stable complexes, which are readily excreted by the kidneys (Aoyama *et al.* 1992). As shown in Tables 3 and 5, after LEC rats were fed on the excess-histidine diet for 28 d, the excess Cu accumulation in the liver was markedly reduced and urinary Cu excretion increased. These effects were similar to the report of Togashi *et al.* (1992), who administered D-penicillamine (100 mg/kg body weight per d) to LEC rats for 12 weeks. However, the administration of D-penicillamine also decreased serum Cu concentration of LEC rats (Togashi *et al.* 1992). In contrast, no significant change of serum Cu level was found in LEC rats fed on the excess-histidine diet (Table 4).

Table 7. Liver total lipids (mg/g liver), triacylglycerol (μmol/g liver), cholesterol (μmol/g liver) and phospholipids (μmol/g liver) in rats fed the basal or excess-histidine diet*

(Mean values with their standard errors for six rats per group)

	Total lipids		Triacylglycerol		Cholesterol		Phospholipids	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
LEC rats								
Basal	50·2[b]	3·6	13·1[b]	1·3	5·09[b]	0·26	46·6	4·3
Excess-histidine	44·2[b]	3·3	5·64[b]	0·93	3·91[c]	0·15	48·0	4·1
Fischer rats								
Basal	52·1[b]	3·1	18·1[b]	1·0	5·55[b]	0·20	43·1	3·6
Excess-histidine	78·2[a]	8·7	37·3[a]	9·6	6·64[a]	0·31	54·2	3·5
Statistical significance of effect (ANOVA, *P*)								
Diet	NS		NS		NS		NS	
Strain	$<0·01$		$<0·01$		$<0·001$		NS	
Diet × strain	$<0·01$		$<0·05$		$<0·001$		NS	

LEC, Long-Evans Cinnamon.
[a,b,c] Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 574.

Table 8. Serum triacylglycerol (mmol/l), cholesterol (mmol/l) and phospholipids (mmol/l) in rats fed the basal or excess-histidine diet*

(Mean values with their standard errors for six rats per group)

	Triacyl-glycerol		Cholesterol		Phospho-lipids	
	Mean	SE	Mean	SE	Mean	SE
LEC rats						
Basal	1·10[b]	0·05	1·83[c]	0·08	2·28[c]	0·04
Excess-histidine	1·31[b]	0·15	2·57[b]	0·15	2·71[b]	0·11
Fischer rats						
Basal	3·08[a]	0·32	2·21[bc]	0·21	3·02[b]	0·17
Excess-histidine	1·58[b]	0·12	3·71[a]	0·11	3·47[a]	0·10
Statistical significance of effect (ANOVA, *P*)						
Diet	<0·01		<0·001		<0·001	
Strain	<0·001		<0·001		<0·001	
Diet × strain	<0·001		<0·05		NS	

LEC, Long-Evans Cinnamon.
[a,b,c]Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 574.

In addition to Cu, the contents of other metals such as Zn and Fe in the liver of LEC rats were also much higher than those in Fischer rats (Sugawara *et al.* 1991*b*). The high concentration of Zn in the liver of LEC rats is considered to have a protective effect though the induction of metallothionein, which sequesters Cu in cells (Sugawara *et al.* 1991*b*). Zn therapy is another treatment for Wilson's disease (Gitlin, 1998). Unfortunately, the Zn content in the liver of LEC rats was reduced by the excess-histidine diet (Table 3). This decrease in liver Zn content was also observed in another study, when we fed a diet containing D-penicillamine to LEC rats (H Xu and Y Aoyama, unpublished results). We did not determine Fe concentrations in the present study. The excess Cu and Fe levels may facilitate the Fenton reaction to produce hydroxyl radicals in LEC rats (Suzuki *et al.* 1993) and Obata *et al.* (1999) reported that histidine has a protective effect on iron(II)-induced hydroxyl radical generation in rat tissue.

Table 9. Serum alanine aminotransferase activity (U/l) in rats fed the basal or excess-histidine diet*

(Mean values with their standard errors for six rats per group)

	Alanine aminotransferase	
	Mean	SE
LEC rats		
Basal	80·0[a]	15·1
Excess-histidine	44·8[b]	3·7
Fischer rats		
Basal	33·1[b]	3·6
Excess-histidine	46·8[b]	6·2
Statistical significance of effect (ANOVA, *P*)		
Diet	NS	
Strain	<0·05	
Diet × strain	<0·05	

LEC, Long-Evans Cinnamon.
[a,b]Mean values within a column with unlike superscript letters were significantly different ($P<0·05$).
* For details of diets and procedures, see Table 1 and p. 574.

In LEC rats, excess Cu in the cell is considered to generate reactive oxygen radicals in the liver by catalysis via the Haber–Weiss reaction (Gutteridge & Wilkins, 1983; Ding & Chan, 1984; Mello Filho & Meneghini, 1984). Among enzymatic defences, superoxide dismutase, catalase and glutathione peroxidase have been considered essential to the cell in removing oxygen radicals from tissues exposed to oxidative stress (Fridovich, 1978; Leibovitz & Siegel, 1980). Yamamoto *et al.* (1999) have reported that the activities of catalase and glutathione peroxidase in the liver of LEC rats were markedly depressed. In the present study, the activities of superoxide dismutase (total, Mn- and Cu,Zn-), catalase and glutathione peroxidase in the liver of LEC rats were also significantly lower than those of Fischer rats when fed on the basal diet (Table 6). Significant declines in the activities of liver total and Cu,Zn-superoxide dismutase were observed in LEC rats fed on the excess-histidine diet (Table 6). It is possible that this might be due to the decreased concentrations of liver Cu and Zn.

Abnormalities of lipid metabolism also have been observed in LEC rats (Tables 7 and 8). Taniguchi *et al.* (1991) reported that the lipid patterns of LEC rats are similar to those of choline-deficient rats, in which the liver may fail to transfer the newly formed triacylglycerol and cholesterol into the plasma with a resultant increase in liver triacylglycerol content and a decrease in serum lipid levels. However, the contents of liver lipids were not increased when LEC rats were fed on the excess-histidine diet (Table 7). Furthermore, the excess-histidine diet increased the levels of serum cholesterol and phospholipids in LEC rats (Table 8). It seems that excess dietary histidine also has positive effects on the abnormal lipid metabolism in LEC rats, but we have no adequate explanation as yet.

An increase in the activity of serum alanine aminotransferase is one of the signs of liver damage (Wroblewski & LaDue, 1956). When fed on the basal diet, the activity of this enzyme in LEC rats was significantly higher than that in Fischer rats. However, when fed on the excess-histidine diet, the activity of serum alanine aminotransferase in LEC rats significantly decreased to values similar to those of Fischer rats fed on the basal diet (Table 9). This result was consonant with the reduction in excess liver Cu in LEC rats by the excess-histidine diet.

In summary, the present study indicates that excess dietary histidine is effective in reducing the excess Cu accumulation in the liver and stimulates the urinary excretion of Cu in LEC rats. This result offers new possibilities for additional approaches to chelation therapy in patients with Wilson's disease. However, caution must be exercised as together with the reduction in liver Cu came a massive increase in urinary Zn loss and a reduction in liver Zn. The preliminary results have also shown that excess dietary histidine may have a beneficial effect on the abnormal lipid metabolism in LEC rats.

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