Lipid Alterations in the Liver and Serum of Rats in Histidine-Excess and Copper Deficiency

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Summary To obtain further information on lipid metabolism in the histidine-excess and copper-deficiency, rats were fed basal, histidine-excess (the addition of 50 g L-histidine/kg diet) or copper-deficient diets for 0, 7, 21 and 42 d ad libitum. Liver triacylglycerol accumulated and the serum triacylglycerol level decreased after feeding of the histidine-excess diet for 21 or 42 d, but not after feeding of the copper-deficient diet. Serum cholesterol level increased in rats fed the histidine-excess diet for 7, 21 and 42 d, but not in rats fed the copper-deficient diet. Copper content in the liver and serum significantly decreased in rats fed the histidine-excess diet. Copper content in the liver and serum was markedly decreased in rats fed the copper-deficient diet. Liver zinc content was constant, but the serum zinc level decreased in rats fed the histidine-excess diet. Feeding of the copper-deficient diet hardly affected zinc content in the liver and serum. Urinary copper and zinc increased in rats fed the histidine-excess diet, and decreased or showed a decreasing tendency in rats fed the copper-deficient diet. Overall results indicated that feeding the histidine-excess diet caused copper deficiency, whereas hypercholesterolemia was not shown in rats fed the copper-deficient diet although the livers of rats fed the copper-deficient diet contained less copper than those of rats fed the histidine-excess diet. Thus, the responses on liver triacylglycerol and serum cholesterol to copper deficiency induced by the feeding of a histidine-excess diet are different from those to copper deficiency induced by feeding of a copper-deficient diet.

Key Words histidine, copper-deficiency, fatty liver, hypercholesterolemia, zincuria

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Copper deficiency causes hypercholesterolemia when rats are fed a copper-deficient diet for 4–26 weeks (1–6), whereas excess dietary histidine also produces hypercholesterolemia (7–12). Solomon and Geison (13) and Qureshi et al (14) have demonstrated that the mechanism for the hypercholesterolemia induced by excess histidine might be due to the stimulation of in vitro cholesterogenesis in the liver (the rate of incorporation of radioactive precursors into digitonin precipitable fraction using liver slices and 5,000 × g supernatant fraction of liver homogenates). Hitomi-Ohmura et al (15) indicated in vivo stimulation of hepatic cholesterogenesis, significant increase in total and active HMG-CoA reductase activities, and no significant changes in the activity of hepatic cholesterol 7α-hydroxylase in rats fed a histidine-excess diet.

Harvey et al (8) have demonstrated that the addition of excess dietary histidine causes copper deficiency, and the simultaneous addition of copper to the histidine-excess diet abolished hypercholesterolemia after feeding for 46 d. Thus, it is surmised that excess dietary histidine causes copper deficiency, and then copper deficiency induced hypercholesterolemia. However, Aoyama et al (16) have reported that the injection of copper to rats fed a histidine-excess diet or the dietary addition of copper to a histidine-excess diet for 7 d could not ameliorate hypercholesterolemia. We have reported that hypercholesterolemia occurred within 7 d in rats when fed a histidine-excess diet (16, 17). In separate experiments, the feeding of a copper-deficient diet did not cause hypercholesterolemia within 7 d after the initiation of feeding (16, 18), but the feeding period of 7 d is considerably short as compared with a feeding period of 4–26 wk for copper deficiency (1–6).

Therefore, the present study was undertaken to compare effects of excess dietary histidine and copper deficiency on lipid, copper and zinc profiles in the liver and serum of rats fed for 0, 7, 21 and 42 d.

MATERIALS AND METHODS

**Animals and diets.** Male rats of the Wistar strain (Japan SLC, Hamamatsu, Shizuoka, Japan), weighing about 122 g as the initial body weight, were housed individually in screened, stainless-steel, wire-bottomed cages. Lighting was regulated to provide equal periods of light (08:00–20:00 h) and dark (20:00–08:00 h). This study complied with the Animal Experimental Guides according to the Committee of Experimental Animal Care, Nagoya University.

The compositions of the basal, histidine-excess and copper-deficient diets are shown in Table 1. The histidine-excess diet had the same composition as the basal diet except for the addition of 50 g of L-histidine (Wako Pure Chemical Industries, Osaka, Japan) per kilogram of the diet. The basal and histidine-excess diets were supplemented with cupric carbonate to provide a final concentration of 6 mg copper/kg diet. The copper-deficient diet was prepared without adding cupric carbonate. The basal (copper-adequate), histidine-excess (copper-adequate) and copper-deficient diets contained copper at 6.14, 6.44 and 0.36 mg/kg diet, re-
|                     | Basal diet (+Cu)\(^a\) | His-excess diet (+Cu)\(^a\) | Copper-deficient diet (−Cu)\(^b\) |
|---------------------|-------------------------|-----------------------------|----------------------------------|
| Vitamin free casein\(^c\) | 250                     | 250                         | 250                              |
| L-Histidine\(^d\)   | 50                      | 50                          |                                   |
| Vitamin mixture\(^e,f\) | 10                      | 10                          | 10                                |
| Choline chloride\(^e\) | 2                       | 2                           | 2                                 |
| Mineral mixture\(^e\) | 35                      | 35                          |                                   |
| Mineral mixture\(^e\) minus CuCO\(_3\) | 34.99                  |                              |                                   |
| Corn oil\(^b\)      | 50                      | 50                          | 50                                |
| Corn starch\(^i\)   | 653                     | 603                         | 653.01                            |

\(^a\) Cupric carbonate (10 mg/kg diet) was added.
\(^b\) Cupric carbonate was omitted.
\(^c\) Sigma Chemical, St. Louis, MO, USA.
\(^d\) Wako Pure Chemical Industries, Osaka, Japan.
\(^e\) 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.
\(^f\) American Institute of Nutrition. 1980. Second report of the Ad Hoc Committee on Standards for Nutritional Studies. *J Nutr* 110: 1726.
\(^g\) Katayama Chemical Industries, Osaka, Japan.
\(^h\) Nihon Syokuhin Kako, Fuji, Shizuoka, Japan.
\(^i\) Gelatinized, Chuo Syokuryo, Inazawa, Aichi, Japan.

Dietary changes in the content of L-histidine and cupric carbonate were compensated for by adjusting the amount of corn starch in the diet.

The rats were allowed free access to food and distilled deionized water for 0, 7, 21 and 42 d until being sacrificed by guillotine between 09:30–10:30 h.

Urine was collected (days 0–2, 5–7, 19–21 and 40–42) from rats fed for 42 d using metabolic cages.

**Measurement of lipids.** Liver lipids were extracted and purified by the method of Folch et al (19), the liver lipids being gravimetrically estimated after removing the solvent. Cholesterol (20) and triacylglycerol (21) in the liver were estimated by enzymatic methods, respectively. Phospholipids in the liver were calculated by the method of difference [total lipids — (cholesterol and triacylglycerol)]. Serum cholesterol (20), triacylglycerol (21) and phospholipids (phospholipids containing choline) (22) were measured by enzymatic methods, respectively. For the calculation of concentration of triacylglycerol and phospholipids, 885.4 for triolein and 786.1 for L-a-phosphatidylcholine, dioleoyl were used as the molecular weights of triacylglycerol and phospholipids, respectively.
Table 2. Food intake and body weight gain of rats fed basal, histidine-excess or copper-deficient diets for 7, 21 and 42 d.

|                      | Basal diet | His-excess diet | Copper-deficient diet |
|----------------------|------------|-----------------|-----------------------|
| Food intake (g/d)    |            |                 |                       |
| (g/7 d)              | 99 ± 2.1   | 63 ± 1          | 96 ± 2                |
| (g/21 d)             | 303 ± 11   | 210 ± 7         | 311 ± 7               |
| (g/42 d)             | 642 ± 19   | 496 ± 14        | 671 ± 27              |
| Body weight gain (g) |            |                 |                       |
| (g/7 d)              | 36.8 ± 3.0 | 13.3 ± 2.1      | 34.1 ± 0.8            |
| (g/21 d)             | 101.5 ± 4.5 | 58.2 ± 2.4      | 103.6 ± 4.3           |
| (g/42 d)             | 172.2 ± 4.9 | 110.7 ± 5.6    | 170.8 ± 8.3           |

1 Means ± SE for five rats.

a, b Means within the same horizontal column that do not share a common superscript letter were significantly different, p < 0.05.

Measurement of copper and zinc. Aliquots of liver and diets were prepared by wet ashing with concentrated nitric acid and subsequent complete digestion with 30% hydrogen peroxide (23). Copper and zinc in the liver, serum, urine and diets were estimated by atomic absorption spectrophotometry (24) using certified reference standards.

Statistical analysis. Data were subjected to Duncan’s multiple range test (25) and Student’s t-test (26) to determine if the difference in means was significant.

RESULTS

Food intake and body weight gain for rats fed the basal, histidine-excess or copper-deficient diets for 7, 21 and 42 d are shown in Table 2. The excess dietary histidine lowered both food intake and body weight gain as compared with those of the basal diet, respectively. No changes in food intake and body weight gain were observed between rats fed the basal and copper-deficient diets.

Liver weight and liver lipid composition are shown in Table 3. Livers of rats fed the histidine-excess diet were enlarged as compared to those of rats fed either the basal or copper-deficient diets for 7, 21 and 42 d. There were no significant changes in liver cholesterol content among the three groups when fed for 7 d. Liver cholesterol contents of rats fed the histidine-excess diet for 21 and 42 d were significantly higher than those of rats fed either the basal or copper-deficient diet, respectively. Liver triacylglycerol content of rats fed the histidine-excess diet decreased after feeding for 7 d, and thereafter adversely increased markedly when fed for 21 and 42 d. Liver phospholipids of rats fed the histidine-excess diet also decreased when fed for 7 d as compared to the values for the basal and copper-
Table 3. Liver weight and liver lipid composition.

| Feeding period (d) | Basal diet | His-excess diet | Copper-deficient diet |
|-------------------|------------|-----------------|-----------------------|
| Liver weight (g/100 g body wt) | 4.68 ± 0.11<sup>a</sup> | 5.10 ± 0.13<sup>a</sup> | 4.44 ± 0.08<sup>b</sup> |
| 7 | 4.41 ± 0.15<sup>b</sup> | 4.36 ± 0.08<sup>b</sup> |
| 21 | 4.30 ± 0.06<sup>b</sup> | 4.36 ± 0.08<sup>b</sup> |
| 42 | 3.98 ± 0.09<sup>b</sup> | 4.08 ± 0.12<sup>b</sup> |
| Cholesterol (µmol/g) | 7.06 ± 0.23 | 5.90 ± 0.18<sup>a</sup> | 5.97 ± 0.25<sup>a</sup> |
| 7 | 5.85 ± 0.25<sup>a</sup> | 5.59 ± 0.16<sup>b</sup> |
| 21 | 6.13 ± 0.23<sup>b</sup> | 5.97 ± 0.16<sup>b</sup> |
| 42 | 6.03 ± 0.13<sup>b</sup> | 5.97 ± 0.16<sup>b</sup> |
| Triacylglycerol (µmol/g) | 22.7 ± 2.2 | 18.4 ± 1.2<sup>b</sup> | 27.4 ± 1.8<sup>a</sup> |
| 7 | 26.0 ± 1.6<sup>a</sup> | 29.0 ± 1.5<sup>b</sup> |
| 21 | 28.1 ± 2.0<sup>b</sup> | 40.1 ± 5.1<sup>b</sup> |
| 42 | 30.8 ± 1.7<sup>b</sup> | 40.1 ± 5.1<sup>b</sup> |
| Phospholipids (µmol/g) | 32.7 ± 1.7 | 26.2 ± 1.0<sup>b</sup> | 35.1 ± 3.1<sup>a</sup> |
| 7 | 35.4 ± 2.2<sup>a</sup> | 37.7 ± 2.2<sup>a</sup> |
| 21 | 35.0 ± 3.8<sup>a</sup> | 37.7 ± 2.2<sup>a</sup> |
| 42 | 31.2 ± 1.5<sup>b</sup> | 34.0 ± 2.0<sup>ab</sup> |

<sup>a, b</sup> Means within the same horizontal column that do not share a common superscript letter were significantly different, \( p < 0.05 \).

<sup>1</sup> Means ± SE for five rats.

deficient diets, respectively. Excess dietary histidine had no effect on liver phospholipids when fed for 21 d. When fed for 42 d, liver phospholipids of rats fed the histidine-excess diet showed a significant increase as compared with rats fed the basal diet. Only slight changes in all parameters between rats fed the basal and copper-deficient diets were observed.

Serum cholesterol of rats fed the histidine-excess diet for 7 and 21 d was significantly higher than those fed the basal and copper-deficient diets. Feeding the histidine-excess diet for 42 d increased serum cholesterol as compared with that of the basal diet by Student’s \( t \)-test (26), but there was no difference from the copper-deficient diet. Serum triacylglycerol of rats fed the histidine-excess diet for 21 and 42 d was lower as compared with those values of rats fed the basal and copper-deficient diets. Serum phospholipids increased when fed the histidine-excess diet for 7 d as compared with those values of rats fed either the basal or copper-deficient diet, but there was no difference when fed for 42 d. When fed for 21 d, serum phospholipid content of the histidine-excess group was higher than that of the basal group, but there was no difference from the copper-deficient
Table 4. Serum lipids.

| Feeding period (d) | Basal diet | His-excess diet | Copper-deficient diet |
|--------------------|------------|-----------------|-----------------------|
| Cholesterol (mmol/L) |
| 0                  | 2.59 ± 0.16<sup>a</sup> | 4.01 ± 0.10<sup>a</sup> | 2.82 ± 0.08<sup>b</sup> |
| 7                  | 2.90 ± 0.10<sup>b</sup> | 4.29 ± 0.08<sup>a</sup> | 3.05 ± 0.10<sup>b</sup> |
| 21                 | 3.05 ± 0.13<sup>b</sup> | 3.78 ± 0.16<sup>a</sup> | 3.41 ± 0.23<sup>a</sup> |
| 42                 | 3.21 ± 0.16<sup>a</sup> |                     |                       |
| Triacylglycerol (mmol/L) |
| 0                  | 1.31 ± 0.10 | 1.56 ± 0.18<sup>a</sup> | 1.66 ± 0.11<sup>a</sup> |
| 7                  | 1.56 ± 0.09<sup>a</sup> | 1.46 ± 0.07<sup>b</sup> | 2.10 ± 0.10<sup>a</sup> |
| 21                 | 2.08 ± 0.08<sup>a</sup> | 1.39 ± 0.26<sup>b</sup> | 2.38 ± 0.29<sup>a</sup> |
| 42                 | 2.87 ± 0.26<sup>a</sup> |                     |                       |
| Phospholipids (mmol/L) |
| 0                  | 2.58 ± 0.08 | 3.38 ± 0.10<sup>a</sup> | 2.40 ± 0.05<sup>b</sup> |
| 7                  | 2.49 ± 0.11<sup>b</sup> | 3.10 ± 0.08<sup>a</sup> | 2.81 ± 0.09<sup>a</sup>|<sup>b</sup> |
| 21                 | 2.68 ± 0.13<sup>b</sup> | 2.87 ± 0.15<sup>a</sup> | 3.15 ± 0.15<sup>a</sup> |
| 42                 | 3.14 ± 0.17<sup>a</sup> |                     |                       |

<sup>1</sup> Means ± SE for five rats.
<sup>a,b</sup> Means within the same horizontal column that do not share a common superscript letter were significantly different, p < 0.05.

group. But, serum phospholipids of the rats fed the histidine-excess diet were higher than those of the copper-deficient diet by Student’s t-test (26). The copper-deficient diet hardly affected the serum lipids (Table 4).

Liver copper levels were decreased by either the excess dietary histidine or copper deficiency. Liver copper levels of rats fed the histidine-excess diet were significantly higher than those of rats fed the copper-deficient diet for 21 and 42 d, while, the excess dietary histidine increased liver zinc levels after feeding for 7 and 21 d. Liver zinc levels of rats fed the copper-deficient diet were lower than those of rats fed the histidine-excess diet. Copper and zinc levels in the serum decreased with the addition of excess histidine. The deficiency of copper in the diet also markedly lowered the serum copper levels. Zinc level in the serum was increased by feeding the copper-deficient diet for 21 d, but not by feeding for 7 or 42 d. Serum zinc levels of rats fed the histidine-excess diet were significantly lower than those values of rats fed the copper-deficient diet (Table 5).

Urinary copper and zinc excretion are shown in Table 6. Excess dietary histidine caused a significant increase in urinary copper and zinc throughout the entire experimental period, while copper deficiency tended to decrease the urinary excretion of copper. Urinary copper decreased significantly by feeding of the copper-deficient diet by Student’s t-test (26). Copper deficiency caused a significant decrease in the urinary excretion of zinc during days 5–7 and 19–21 by Student’s t-test (26). Urinary...
Table 5. Copper and zinc content in liver and serum.

| Feeding period (d) | Basal diet | His-excess diet | Copper-deficient diet |
|--------------------|------------|-----------------|-----------------------|
| Liver copper (μmol/g) |            |                 |                       |
| 0                  | 0.074 ± 0.003<sup>1</sup> | 0.050 ± 0.002<sup>b</sup> | 0.046 ± 0.002<sup>b</sup> |
| 7                  | 0.074 ± 0.002<sup>a</sup> | 0.042 ± 0.002<sup>b</sup> | 0.030 ± 0.002<sup>*</sup> |
| 21                 | 0.074 ± 0.002<sup>a</sup> | 0.046 ± 0.002<sup>b</sup> | 0.036 ± 0.004<sup>c</sup> |
| 42                 | 0.072 ± 0.002<sup>a</sup> |                 |                       |
| Liver zinc (μmol/g)  |            |                 |                       |
| 0                  | 0.442 ± 0.023            | 0.427 ± 0.014<sup>a</sup> | 0.372 ± 0.005<sup>b</sup> |
| 7                  | 0.387 ± 0.005<sup>b</sup> | 0.431 ± 0.014<sup>a</sup> | 0.388 ± 0.006<sup>b</sup> |
| 21                 | 0.399 ± 0.008<sup>b</sup> | 0.419 ± 0.015<sup>a</sup> | 0.385 ± 0.009<sup>b</sup> |
| 42                 | 0.442 ± 0.006<sup>a</sup> |                 |                       |
| Serum copper (μmol/L) |          |                 |                       |
| 0                  | 25.0 ± 0.6                |                 |                       |
| 7                  | 28.5 ± 0.5<sup>a</sup>    | 25.8 ± 0.5<sup>b</sup> | 8.81 ± 0.5<sup>c</sup>  |
| 21                 | 26.0 ± 0.8<sup>a</sup>    | 21.4 ± 0.3<sup>b</sup> | 8.03 ± 0.3<sup>c</sup>  |
| 42                 | 27.4 ± 0.6<sup>a</sup>    | 23.3 ± 1.1<sup>b</sup> | 6.77 ± 0.2<sup>c</sup>  |
| Serum zinc (μmol/L) |            |                 |                       |
| 0                  | 36.4 ± 1.0                |                 |                       |
| 7                  | 33.8 ± 1.5<sup>a</sup>    | 24.8 ± 0.6<sup>b</sup> | 34.4 ± 1.2<sup>a</sup>  |
| 21                 | 30.1 ± 1.0<sup>a</sup>    | 19.7 ± 0.9<sup>c</sup> | 34.7 ± 1.0<sup>a</sup>  |
| 42                 | 31.0 ± 0.6<sup>a</sup>    | 25.7 ± 0.8<sup>b</sup> | 34.4 ± 1.8<sup>a</sup>  |

<sup>1</sup> Means ± SE for five rats.
<sup>a,b,c</sup> Means within the same horizontal column that do not share a common superscript letter were significantly different, p < 0.05.

Table 6. Urinary copper and zinc.

| Periods collected (d) | Basal diet | His-excess diet | Copper-deficient diet |
|-----------------------|------------|-----------------|-----------------------|
| Copper (μmol/100 g body wt) |            |                 |                       |
| 0–2                   | 0.526 ± 0.013<sup>1,b</sup> | 0.999 ± 0.074<sup>a</sup> | 0.442 ± 0.017<sup>b</sup> |
| 5–7                   | 0.376 ± 0.047<sup>b</sup> | 0.922 ± 0.079<sup>a</sup> | 0.231 ± 0.035<sup>b</sup> |
| 19–21                 | 0.304 ± 0.014<sup>b</sup> | 0.686 ± 0.115<sup>a</sup> | 0.149 ± 0.009<sup>b</sup> |
| 40–42                 | 0.225 ± 0.013<sup>b</sup> | 0.590 ± 0.058<sup>a</sup> | 0.098 ± 0.006<sup>c</sup> |
| Zinc (μmol/100 g body wt) |           |                 |                       |
| 0–2                   | 0.268 ± 0.018<sup>b</sup> | 1.73 ± 0.35<sup>a</sup> | 0.258 ± 0.043<sup>b</sup> |
| 5–7                   | 0.164 ± 0.008<sup>b</sup> | 4.60 ± 0.23<sup>a</sup> | 0.104 ± 0.003<sup>b</sup> |
| 19–21                 | 0.214 ± 0.008<sup>b</sup> | 4.47 ± 0.75<sup>a</sup> | 0.092 ± 0.014<sup>b</sup> |
| 40–42                 | 0.075 ± 0.005<sup>b</sup> | 3.55 ± 0.20<sup>a</sup> | 0.064 ± 0.005<sup>b</sup> |

<sup>1</sup> Means ± SE for five rats.
<sup>a,b,c</sup> Means within the same horizontal column that do not share a common superscript letter were significantly different, p < 0.05.
excretion of copper and zinc from rats fed the histidine-excess diet was significantly higher than that of rats fed the copper-deficient diet.

**DISCUSSION**

Hypercholesterolemia was reported when rats were fed a copper-deficient diet for 4–16 wk (1–6), and when fed a histidine-excess diet for 4–46 d (7–18). Excess dietary histidine produced copper deficiency (8). In fact, feeding of either a histidine-excess or a copper-deficient diet decreased serum copper by 9–18% and 69–75% of the value of the basal group, respectively (Table 5). Excess dietary histidine caused a decrease in liver copper content (Table 5). Furthermore, it was found that the copper content in the liver of rats fed the copper-deficient diet were significantly lower than those in rats fed the histidine-excess diet, suggesting that copper deficiency is more severe in rats fed a copper-deficient diet than in rats fed a histidine-excess diet. The serum cholesterol level in rats fed the copper-deficient diet for 7 and 21 d did not increase, but hypercholesterolemia was observed in rats fed the histidine-excess diet for 7 and 21 d (Table 4). Thus, hypercholesterolemia induced by excess dietary histidine when fed for 7 d might not be related to the state of copper deficiency. Although Lei (1) and others (2–6) have reported hypercholesterolemia in rats fed a copper-deficient diet for 4–26 wk, they had not reported hypercholesterolemia after feeding of a copper-deficient diet for 7 d. We have no adequate explanation for the reasons that the results concerning the response on serum cholesterol in copper deficiency shown in Table 4 are not consistent with those reported by Lei (1) and others (2–6).

Hypercholesterolemia was also shown after feeding of a histidine-excess diet for 7, 21 and 42 d (Table 4). Liver cholesterol when expressed as μmol/g liver also accumulated when rats consumed the histidine-excess diet for 21 and 42 d, respectively (Table 3). Cholesterol content in the liver when expressed as μmol per whole liver per 100 g body weight was increased after feeding for 7 d (not shown in table). These results might be due to enhanced cholesterogenesis in the liver as previously reported (13–15).

We found that the development of fatty liver in rats has been demonstrated to occur on histidine-excess diet for 21 and 42 d. The main fraction responsible for lipid accumulation in the liver was triacylglycerol from an analysis of liver lipids (Table 3). As shown in Table 3, the time course of changes in liver lipid content (cholesterol + triacylglycerol + phospholipids) revealed that liver lipid content decreased after feeding of the histidine-excess diet for 7 d, and thereafter increased after feeding for 21 d. This is the first report concerning nutritional fatty liver that liver lipids were decreased followed by liver lipid accumulation. From other results, the rate of triacylglycerol accumulation in the serum of rats after injection of Triton WR 1339 was significantly lower in a histidine-excess group than in a basal group (unpublished data by Aoyama et al). Therefore, one of the candidates for the accumulation of triacylglycerol in the liver might be due to the decreased transport.
of triacylglycerol from the liver into the serum. We are attempting to elucidate the mechanism for the accumulation of triacylglycerol in the liver induced by the addition of excess histidine.

Triacylglycerol in the serum is mainly located in very-low-density lipoproteins (27), while cholesterol in the serum of rats exists more in high-density lipoproteins than in very-low-density lipoproteins and low-density lipoproteins (28). Triacylglycerol from the liver is transported into blood as very-low-density lipoproteins. Therefore, it is assumed that a decreased content of triacylglycerol in very-low-density lipoproteins might be one of the factors responsible for the accumulation of lipids in the liver as described previously. The cholesterol in high-density lipoproteins increased in the serum of rats fed a histidine-excess diet as compared with the serum of rats fed a basal diet (unpublished data by Aoyama et al). Hypercholesterolemia induced by excess histidine might be due to the enhanced cholesterogenesis (i.e., the increased activity of hepatic HMG-CoA reductase) but no changes in the activity of hepatic cholesterol 7α-hydroxylase as previously reported (15). Thus, decreased triacylglycerol and increased cholesterol in the serum of rats fed the histidine-excess diet are shown in Table 4.

Copper content in the liver and serum of rats fed either the histidine-excess or copper-deficient diet was decreased (Table 5). When rats were fed a copper-deficient diet for 21 d, no changes in cholesterol, triacylglycerol or phospholipids in the liver were observed (Table 3). Thus, the accumulation of triacylglycerol in the liver of rats fed the histidine-excess diet might be related to histidine itself, but not the copper deficiency induced by excess dietary histidine. In fact, histidine administration led to an increase in the corticosterone concentration in the serum (17). Therefore, this hormone might be due to the changes in lipid profiles of the serum and liver.

Urinary output of copper and zinc increased in rats fed the histidine-excess diet (Table 6). Such phenomena might be due to the chemical nature of histidine, which has a chelating action (29, 30). Thus, the content of copper in the liver, and concentrations of copper and zinc in the serum were lower in rats fed the histidine-excess diet as compared to rats fed the basal diet. We have no adequate explanation why the liver zinc content between rats fed the basal and histidine-excess diets was maintained although a marked urinary output of zinc from rats fed the histidine-excess diet was observed (Table 6). It cannot be explained by the binding capacity with metallothionein because of stronger affinity of metallothionein to copper as compared with that of this protein to zinc (31).

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