《論文》

Undernutrition due to a low-protein diet affects iron, zinc, and copper metabolism

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The effects of undernutrition due to a low-protein diet were examined. Despite otherwise similar diets, a protein-deficient diet resulted in low growth and hypoalbuminosis, and the addition of amino acids improved growth. Furthermore, according to the model of the "two-hit theory", iron overload occurs in the liver. However, in this study, the liver iron levels decreased with undernutrition. In addition, the zinc and copper level decreases in the liver may be the cause of hypoalbuminosis. Zinc and copper are essential for the functions of enzymes, such as superoxide dismutase (SOD). Degradation of the antioxidant effect may promote hepatic injury.

キーワード：iron、zinc、copper

Introduction

Undernutrition with a low-protein diet is an important nutritional problem, because a low-protein diet increases the risk for non-alcoholic steatohepatitis (NASH), fatty liver, and hypoalbuminosis[1]. NASH can turn into cirrhosis and ultimately cancer. Thus, it is important to clarify the detailed mechanism of NASH.

According to the model of the "two-hit theory", the "first hit" involves lipid accumulation in the hepatocytes[2]. The "first hit" increases the vulnerability of the liver to many factors that constitute the "second hit" and promote hepatic injury, inflammation, and fibrosis. It is important to clarify the mechanism that constitutes the "second hit", but it is still incompletely understood.

The aim of this study was to investigate mineral metabolism and growth with a protein-deficient diet.

Materials and Methods

Animals and Diets

Four-week-old male Wistar rats were obtained from Japan SLC Inc. (Hamamatsu, Japan). Rats were housed in individual plastic cages. The animal room was kept at a temperature of 22±2℃ and a relative humidity of 65%±5%. Room lighting consisted of 12-h periods of light and dark (dark period: 08:00-20:00). The rats were randomly divided into 5 groups of 6 animals each. Their food and body weight were measured every day. All animal studies were carried out under the guidelines for animal experiments of the Hagoromo

University of International Studies.

Five different diets were prepared. The diets given to each group had similar lipid, carbohydrate, vitamin, mineral, and energy contents, and all rats were fed the experimental diets for 4 weeks. Their composition is shown in Table 1. The Control diet was prepared based on AIN-93.

Then, 28 days from the start of the experiment, each rat's abdomen was opened under isoflurane anesthesia (Wako, Osaka, Japan).

At the end of the feeding trial, blood, kidneys, spleen, and liver samples were collected and used for analyses. Concentrations of various minerals were determined.

Table 1. Composition of each experimental diet

Contents	Control	Gluten	Zein	Gluten + AA	Zein + AA
Casein	20.0	−	−	−	−
Gluten	−	20.0	−	20.0	−
Zein	−	−	20.0	−	20.0
L-lysine	−	−	−	1.5	1.5
L-threonine	−	−	−	0.4	0.3
L-tryptophan	−	−	−	0.1	0.2
L-cysteine	0.3	0.3	0.3	0.3	0.3
Corn starch	39.7	39.7	39.7	37.7	37.7
α-Corn starch	13.2	13.2	13.2	13.2	13.2
Sucrose	10.0	10.0	10.0	10.0	10.0
Soybean oil	7.0	7.0	7.0	7.0	7.0
Cellulose	5.0	5.0	5.0	5.0	5.0
Mixed minerals [1)]	3.5	3.5	3.5	3.5	3.5
Mixed vitamins [2)]	1.0	1.0	1.0	1.0	1.0
Choline bitartrate	0.25	0.25	0.25	0.25	0.25
Tertiary butyl hydroquinone	0.0014	0.0014	0.0014	0.0014	0.0014
Total	100.0	100.0	100.0	100.0	100.0

(g/100 g)

According to the AIN-93 formula.

1) Mixture of minerals purchased from Oriental Yeast Co., Ltd.

2) Mixture of vitamins purchased from Oriental Yeast Co., Ltd.

Biochemical parameters

Serum samples were analyzed by the A/B test kit Wako (Wako) and the ALT/AST test kit Wako (Wako).

Concentrations of various minerals

A sample of 1 g of tissue weight of each test diet was heated for 48-72 h in a muffle oven at 450℃. After the sample was cooled, 2 mL of 1 M HCL were added. After brief

centrifugation (600×g for 10 min), the ash was removed and used for analysis. The mineral sample solution was analyzed by atomic absorption spectroscopy with an AA-7000 (Shimazu Ltd., Kyoto, Japan)

Statistical analysis

All values are expressed as means±SE. Statistical analysis was performed using two-way analysis of variance and Student's *t*-test. $P<0.05$ was considered significant.

Results and Discussion

Body weight gain, feed efficiency, and the characteristics of the subjects are shown in Table 2. Despite the similar diets given to each group, the final body weight was different, and the body weight gain was significantly lower in the Gluten and Zein groups than in the control group. Total food intake did not differ among the four groups except for the Zein group. The feed efficiency ratio in the Zein group was less than in the control group. On the other hand, Gluten + AA was improved by the addition of amino acid.

It is known that dietary proteins serve as the source of 20 amino acids commonly found in tissues. Nine of these amino acids (tryptophan, lysine, leucine, isoleucine, valine, threonine, phenylalanine, methionine, and histidine) are essential and cannot be synthesized in the body. In this study, compared with the Gluten and Zein groups, the groups with added amino acid showed improved growth.

Table 2. Body weight gain, feed intake, and feed efficiency in rats fed each experimental diet for 28 days

	Control	Gluten	Zein	Gluten + AA	Zein + AA
Initial body weight (g)	71.0±4.90	69.0±2.54	69.8±2.03	70.1±1.76	70.3±3.71
Final body weight (g)	221.9±11.3	109.6±8.3*	65.5±3.2*	196.3±7.0*	106.4±49.7*
Body mass gain (g/28 days)	150.9±7.9	40.5±6.3*	−4.3±2.6*	126.2±8.0*	36.0±47.3*
Total food intake (g/28 days)	356.2±56.5	297.4±61.6	234.9±43.1*	324.4±94.0	361.5±208.5
Food intake per day (g/day)	12.7±2.0	10.6±2.2	8.4±1.5*	11.6±3.4	14.7±4.3
Feed efficiency ratio (%)	43.3±7.3	13.8±1.3*	−1.8±1.2*	44.4±22.9	8.1±10.9*

Values are means±SE.

* $p<0.05$, significantly different from control.

The organ weights are shown in Table 3. Liver and kidney weights were significantly lower in the Gluten and Zein groups than in the control group, but spleen weight was similar among the groups.

Table 3. Organs and tissues

	Control	Gluten	Zein	Gluten + AA	Zein + AA
Liver	7.31±0.7	3.21±0.2*	2.11±0.5*	5.77±0.4*	3.65±2.0*
Kidneys	1.53±0.2	0.84±0.1*	0.73±0.2*	1.78±0.2	0.83±0.3*
Spleen	0.60±0.1	0.58±0.4	0.49±0.3	0.46±0.2	0.41±0.2

Values are means±SE.

* $p<0.05$, significantly different from control.

The effects of diet on the serum protein levels in rats were observed (Table 4). Differences in diet showed no effect on total protein concentration. However, albumin concentration was significantly lower in the Zein and Zein + AA groups than in the control group. Therefore, A/G was significantly lower in the Zein and Zein + AA groups than in the control group.

Undernutrition with a low-protein diet causes hypoalbuminosis. In this study, albumin globulin ratio (A/G) was decreased.

Table 4. Effects of diet on serum protein levels in rats

	Control	Gluten	Zein	Gluten + AA	Zein + AA
Total protein (g/dl)	6.8±0.5	8.4±3.6	7.6±3.1	7.4±0.7	7.9±2.8
Albumin (g/dl)	4.7±0.3	4.5±0.4	3.8±0.3*	4.4±0.3	3.6±1.1*
A/G	2.3±0.7	1.2±7.6	1.0±1.5	1.5±0.7	0.8±0.3*

Values are means±SE.

* $p<0.05$, significantly different from control.

Serum ALT and AST have been used to screen for liver injury. ALT and AST levels were measured in rats fed each experimental diet (Table 5). There were no significant differences among the five groups in ALT and AST levels. There results suggest that hepatic injury, inflammation, and fibrosis were not promoted at this stage.

Table 5. ALT and AST levels in rats fed each experimental diet

	Control	Gluten	Zein	Gluten + AA	Zein + AA
ALT (Karmen unit)	53.2±24.0	65.5±31.2	64.0±30.0	67.3±12.4	79.8±19.4
AST (Karmen unit)	175.0±72.7	164.4±27.9	181.0±93.6	202.6±33.4	209.4±58.3

Values are means±SE.

* $p<0.05$, significantly different from control.

The effects of diet on iron levels in various tissues were also observed (Table 6). Liver iron levels were lower in the Gluten, Zein, and Zein + AA groups than in the control group. The addition of amino acid in the Gluten + AA group improved the liver iron level. On the other hand, iron levels in the kidneys and spleen in the Gluten, Zein, and Zein + AA groups were greater than in the control group, and the addition of amino acid in the Gluten + AA group improved the levels.

Table 6. Iron levels of various tissues (μg/g)

	Control	Gluten	Zein	Gluten + AA	Zein + AA
Liver	15.5±9.1	9.2±5.9*	6.7±5.6*	16.4±15.1	10.5±9.1*
Kidneys	54.2±20.9	78.8±26.8*	215.2±111.8*	61.1±44.4	140.0±70.5*
Spleen	4.0±1.1	5.7±0.8*	6.2±2.5*	4.5±1.4	6.5±0.9*

Values are means±SE.

* $p<0.05$, significantly different from control.

The effects of diet on zinc levels of various tissues were examined (Table 7). Liver zinc levels were lower in the Zein and Zein + AA groups than in the control group. On the other hand, the Gluten and Gluten + AA groups showed no differences. Zinc levels in the kidneys and spleen were greater in the Gluten, Zein, and Zein + AA groups than in the control group, and the addition of amino acid improved levels in the Gluten + AA group.

Zinc is particularly essential for rapidly growing tissues. It is known that, after absorption, zinc circulates bound to albumin[3-8]. In addition, zinc is initially concentrated in the liver and then distributed to the tissues. In this study, the decreased zinc levels in the liver may be the cause of hypoalbuminosis.

Table 7. Zinc levels of various tissues (μg/g)

	Control	Gluten	Zein	Gluten + AA	Zein + AA
Liver	2.4±1.2	2.5±0.5	1.6±0.4*	2.7±1.7	1.9±1.0*
Kidneys	5.4±1.8	7.1±2.1*	15.6±8.2*	5.1±5.2	8.6±4.6*
Spleen	4.0±1.1	5.7±0.8*	6.2±2.5*	4.5±1.4	6.5±0.9*

Values are means ±SE.

* $p<0.05$, significantly different from control.

The effects of diet on copper levels of various tissues were examined (Table 8). Liver copper levels were lower in the Zein and Zein + AA groups than in the control group. On the other hand, copper levels of the kidneys and spleen did not differ among the five groups. It is known that copper plays a key role in iron absorption and mobilization. However, in this study, changes in copper levels appeared unrelated to the changes in iron levels.

Table 8. Copper levels of various tissues (μg/g)

	Control	Gluten	Zein	Gluten + AA	Zein + AA
Liver	1.5±0.7	1.5±0.4	0.8±0.2*	1.1±0.7	0.8±0.5*
Kidneys	3.2±1.4	3.8±1.5	6.9±5.6	2.0±2.7	3.1±1.7
Spleen	0.1±0.6	1.4±1.3	3.7±1.7	1.5±0.6	3.2±1.3

Values are means ±SE.

* $p<0.05$, significantly different from control.

In conclusion, the effects of undernutrition with a low-protein diet were examined. Despite similar diets being given, a protein-deficient diet induced low growth and hypoalbuminosis, and the addition of amino acid improved growth.

In addition, the zinc and copper levels decreases in the liver may be the cause of hypoalbuminosis. Zinc and copper are essential for the function of enzymes, such as superoxide dismutase (SOD)[9-10]. Degradation of the antioxidant effect may promote hepatic injury.

According to the model of the "two-hit theory", iron overload occurs in the liver. However, in this study, liver iron levels were decreased in the Gluten, Zein, and Zein + AA groups.

It is necessary to clarify how undernutrition promotes hepatic injury.

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