Protein Utilization in Rats during Early Stages of Zinc Deficiency

Takafumi Norii 1, Hiroo Suzuki 2

13 Ube College, Ube, Yamaguchi, 755-8550, Japan.
2 Department of Biochemistry and Food Science, Kagawa University, Kita-gun, Kagawa, 761-0795, Japan

Abstract
The effects of zinc deficiency on protein utilization in rats during gastric tube feeding were investigated. Rats fed a zinc-deficient diet ad libitum ceased growing on the 4th day. Thereafter, rats were fed equal amounts of the zinc-deficient diet or a control diet via gastric tube. Apparent absorption of diet and protein were not reduced, but urinary excretion of nitrogen and urea were increased in zinc-deficient rats. These results show that utilization of absorbed amino acids was reduced in zinc-deficient rats.

Keywords: protein utilization, zinc deficiency, tube feeding, rats

Introduction
It is well known that rats reduce food intake and stop growing after a few days on a zinc-deficient diet [1]. Zinc-deficient rats have an aversion to protein intake [2]. A large decrease in food intake occurs when zinc-deficient rats are supplied diet with an adequate level of protein, but this is not observed when they are fed a diet with low levels of protein [3]. When given a choice, zinc-deficient rats select a protein-free diet instead of a protein-adequate diet, and as a result, survive longer [4]. This indicates that rats are able to choose protein intakes that are favorable for survival. Furthermore, growth of zinc-deficient rats is not maintained by feeding with a gastric tube [1,5,6]. These observations show that zinc-deficient rats may have impaired protein utilization.

In this study, the effects of zinc deficiency on protein utilization in rats during gastric tube feeding were investigated.

Materials and Methods
Experimental procedure: Isolated soy protein (Fujiyoko-R, Fuji Oil, Osaka, Japan) was suspended in water and adjusted to pH 4.4 with 6 N HCl. Precipitated protein was washed and lyophilized. The zinc content of this demineralized soy protein (DP) was reduced to less than 1 mg/kg. The composition of the 20%DP zinc-deficient diet was: DP(13.6%N), 23.5%; salt mixture [7] (zn was omitted from this salt mixture), 4%; CrK(SO4)·12H2O, 19.25 mg/kg; Na2SeO3, 0.35 mg/kg; vitamin mixture [7], 1%; cellulose powder, 4%; corn oil, 5%; choline chloride, 0.15%; and sucrose, 62.35%. Zinc content in the zinc-deficient (ZnD) diet was less than 0.4 mg/kg. Zinc sulfate was then added to the ZnD diet to bring the zinc content to 25 mg/kg (control diet). Male Wistar rats aged 4 weeks (Nippon SLC, Hamamatsu, Japan) were housed individually in hanging stainless steel cages in a room at 23±2°C with a fixed light-dark cycle (light: 08:00-20:00). Rats were fed a 20% casein diet and deionized water for acclimatization to the laboratory conditions until body weight reached to 120 g. For the first stage of the experiment, all rats had free access to the ZnD diet and deionized water until their growth stopped. For the second stage of the experiment, rats were fed a liquid diet via gastric tube every 6 hours (beginning at 12:00 on the day rats stopped growing) for a total of 12 times. The liquid diet (0.60 g/ml) was prepared by mixing 100 g of the control or ZnD diet with 100 ml of deionized-distilled water immediately before use. The

Correspondence: Takafumi Norii
Ube College, Ube, Yamaguchi, 755-8550, Japan
Fax: +81-0836-35-9505,
E-mail: norii@pub.ube-c.ac.jp
則井孝文
〒755-8550 宇部市文京町5-40 宇部短期大学

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gastroduodenal tube having an 18-gauge stainless steel needle was
connected to a syringe. Approximately 5.0-5.5 ml of the
liquid diet was fed to each rat per feeding. In a
preliminary experiment, rats weighing 120 g were fed with
free access to a control diet. They ate over 13 g per day.
Therefore, the amount of diet delivered by gastroduodenal tube
was decided to be 12 to 13 g/day in the present experiment.
Body weight and food intake were measured daily at 09:00.
Six hours after the last feeding (in practice, 11:50-13:29),
the control rats and the ZnD rats were alternately
anesthetized with ether and bled by heart puncture. All
procedures were conducted in accordance with the
guidelines for the care and use of laboratory animals of the
Faculty of Agriculture at Kagawa University.

**Assays:** The activities of alkaline phosphatase (EC
3.1.3.1) in the serum [8], pepsin (EC 3.4.23.1) in the
stomach contents [9], and trypsin (EC 3.4.21.4) and
chymotrypsin (EC 3.4.21.1) in the small intestine contents
[10] were measured. Leucine amino peptidase (EC
3.4.11.1) activity in the small intestinal mucosa was measured
using a LAP C-Test Wako kit (Wako Pure
Chemical, Osaka, Japan). Zinc was quantified by flame
atomic absorption spectrophotometry. Urinary urea was
measured using the Urea NB-Test Wako kit (Wako Pure
Chemical, Osaka, Japan). Nitrogen in diet and urine was
determined by the micro-Kjeldahl method.

**Statistics:** All values are expressed as means ± SE.
Analysis of variance and Student's t-test were performed
according to standard procedures.

**Results and Discussion**

ZnD rats stopped growing on day 4. Subsequently,
rats were fed a liquid diet via gastroduodenal tube. Rat growth was
restored in those receiving the control diet (12±1.2 g/3 day),
but was depressed (4±2.1 g/3 day, p<0.01) in those
receiving the ZnD diet. None of the rats exhibited
diarrhea. Lower values for serum alkaline phosphatase
activity (52.4±3.6 vs. 77.3±4.2 K-A unit, p=0.001) and
zinc concentration in serum (0.33±0.01 vs. 1.48±0.03
µg/ml, p>0.001) and femur (113±2.9 vs. 140±1.4 µg/g dry,
p<0.001) were observed in the ZnD rats when compared to
the control rats. These lower values in the ZnD rats
confirmed the zinc-deficient status of the body. Various
investigators [5,6,11,12] have reported that rats fed a zinc-
deficient diet via gastroduodenal tube stopped growing after a few
days. Thereafter, rats showed zinc deficiency symptoms,
making it difficult to continue tube feeding, and rats were
unable to survive beyond 8-12 days [6,11]. This suggests that
*ad libitum*-fed zinc-deficient rats reduce their diet

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<tr>
<th>Table 1. Body weight gain and apparent absorption of diet, protein and ash in zinc-deficient rats for 2 days prior to sacrifice</th>
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<td><strong>Contents</strong></td>
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<td>Body weight gain (g)</td>
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<td>Dietary intake (g)</td>
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<td>Fecal ash (g)</td>
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<td>Ash absorption (%)</td>
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Values are mean ± SEM for 2 days prior to sacrifice.

*ns*: not significant

Absorption: apparent absorption.

Protein absorption = (N intake - Fecal N) × 100 / N intake.

N balance = (N intake - Fecal N - Urinary N).

intake in an attempt to remain alive.

Apparent absorption of diet, protein and ash (minerals)
are shown in Table 1. Several novel observations are included. The apparent absorption did not decrease in ZnD rats when compared to control rats. Protein concentration and digestive protease activity in the gastrointestinal tract (pepsin, trypsin, chymotrypsin and leucine amino peptidase) did not change in the ZnD rats (data not shown). Digestion and absorption of protein were thus normal in the small intestine of ZnD rats.

<table>
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<th>Table 2. Gastrointestinal contents of zinc-deficient rats</th>
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<td>Small intestine</td>
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<td>Large intestine</td>
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Values are mean±SEM. ns: not significant (p>0.05).
As shown in Table 2, the amount of remaining digesta in the stomach of ZnD rats was greater than that of control rats. However, the apparent absorption of diet did not decrease in the ZnD rats (Table 1). It is possible that an increase in remaining stomach digesta occurred with the progress of zinc deficiency. Moreover, this is probably because zinc deficiency affects the apparent absorption of nutrients more slowly than in the stomach digesta.

The large amounts of digesta remaining in the stomach of ZnD rats suggest delayed movement from the stomach to the intestine. Taneja & Arya reported that zinc deficiency caused slower movement of stomach contents into the intestine in mice [13]. Delayed movement of digesta could be the reason for the lower body weight of zinc-deficient rats during ad libitum feeding. Remaining stomach digesta prevent further eating, and thus zinc-deficient rats are unable to eat further.

Urinary nitrogen (urea) excretion was increased, nitrogen balance was decreased (Table 1), and accordingly protein utilization was decreased in ZnD rats. These findings are novel and clearly demonstrated. Growth of ZnD rats was depressed by tube feeding despite the same amounts of diet being given and similar apparent absorption of diet and protein when compared with control rats. Absorbed amino acids were decomposed because protein biosynthesis was probably reduced in ZnD rats. Hicks & Wallwork reported that the protein synthetic ability of liver isolated from zinc-deficient rats was depressed [14]. Therefore, utilization of absorbed amino acids was probably reduced in ZnD rats.

References