L-Threonine Supplementation Increases the Amplitude of the Feed Intake Cycle of Rats Fed a Low-Protein Zinc-Deficient Diet

Kazuhiro YAMASAKI, Tomoko OHYAMA, Yoko HORIKAWA, Koichi MATSUDA, Shigeko FUJIMOTO SAKATA and Nanaya TAMAKI*

Faculty of Nutrition and High Technology Research Center, Kobe Gakuin University,
Nishi-ku, Kobe 251–2180, Japan
(Received November 27, 2001)

Summary Rats fed a Zn-deficient diet show characteristic variations in feed intake. These variations were followed by means of a personal computer. The specific feed intake patterns in rats fed a zinc-deficient diet before and after supplementation with protein and several essential amino acids were determined. The high-protein diet decreased the amplitude of feed intake under zinc deficiency, probably because of a decrease in sensitivity to the deficiency. Furthermore, the zinc-deficient diet was supplemented with essential amino acids, and of them L-threonine showed the most marked effect on the increased variability of feed intake.

Key Words zinc deficiency, feed intake cycle, body weight change cycle, essential amino acid, threonine

Zinc (Zn) deficiency causes several biochemical and physiological problems, including loss of appetite, in humans and experimental animals. In animals, anorexia induced by Zn deficiency has been well documented (1) and is followed by growth retardation (2, 3).

The most striking phenomena associated with experimental Zn deficiency are the reductions in feed intake and body weight with cyclic feeding behavior (4–11). Mills et al. (4) reported a characteristic cyclic variation in feed intake. These variations were fitted to a Cosinor curve, and the cyclic period was about 3.5 days (3, 10, 11). A cyclic pattern of feed intake was also found in female rats fed a Zn-deficient diet (2), and the cyclical periods of the intake cycles had the same values in both sexes (11).

A Cosinor analysis of the cyclic period of feed intake in rats fed a Zn-deficient diet revealed characteristic properties (3). The values of amplitude for the feed-intake cycle show positive correlations with their own day-to-day variations and with their own simulated cycles. The cyclic variations in feed intake are accompanied by cyclic variations in body weight change that occur similarly in pair-fed control rats. There are no differences in the measures of body weight change cycle between Zn-deficient rats and pair-fed control rats. The cyclic feed intake appears to be caused by a periodic reduction in appetite (13).

Supplementation with Zn by oral feeding (2, 11) and subcutaneous injection (3) decreased the day-to-day variations in feed intake of rats fed a Zn-deficient diet. The amplitude of the feed-intake cycle decreased with increasing Zn supplementation (3, 11). The requirement for normalizing the feed intake of Zn from the diet in rats is about 120 µg/d (3).

On the other hand, a reduction of the protein content of the Zn-deficient diet to 5%, from 20%, results in an increase in feed intake and a disappearance of the cyclical pattern of intake (5, 7). When the Zn-deficient diet containing a low level (5%) of protein was supplemented with essential and nonessential amino acids, the day-to-day variations in feed increased in a manner correlated with the essential amino acid content of the diet (7). When Zn-deficient rats were fed diets containing adequate Zn, protein intake increased more than 50% during days 2–4 of repletion from separate carbohydrate-, protein-, and fat-containing diets (12).

In previous studies (3, 10, 11), cyclic variations of feed intake and body weight changes were analyzed by the Cosinor method. In the present study, a protein concentration in the diet affected the parameters of feed intake and body weight change cycles of rats under conditions of Zn deficiency. The amplitude of the feed intake cycle of rats fed a Zn-deficient diet containing 5% protein was increased twofold by a supplementation of the diet with L-threonine.

MATERIALS AND METHODS

Animals Male albino rats (Wistar ST strain, weighing 90–100 g) were purchased from Japan SLC Co. Ltd. (Hamamatsu, Japan) and housed in individual screen-bottomed cages in a room maintained at 23±1°C with 50% humidity under controlled lighting conditions (lights on from 07:00 to 19:00). The animals were fed a commercial stock diet of Oriental MF (Oriental Yeast Ltd., Tokyo, Japan) and given tap water ad libitum for 1 wk before the experiment to allow acclimatization to the new environment. Feed intake and body weight
Table 1. Compositions of the Zn-deficient low- and high-protein diets (g/kg).

| Ingredients          | Protein concentration (%) |
|----------------------|---------------------------|
|                      | 0  | 5  | 20 | 40 | 60 |
| Egg albumin          | 780| 730| 580| 380| 180|
| Dextrin              | 60 | 60 | 60 | 60 | 60 |
| Corn oil             | 20 | 20 | 20 | 20 | 20 |
| Vitamin mixture*     | 60 | 60 | 60 | 60 | 60 |
| Salt mixture (−Zn)** | 80 | 80 | 80 | 80 | 80 |

* Vitamins (mg/kg of diet): retinyl acetate (2.01), cholecalciferol (0.03), α-tocopheryl acetate (58.3), menadione (60.8), thiamin hydrochloride (14.0), riboflavin (46.8), pyridoxine hydrochloride (9.4), cyanocobalamin (0.006), ascorbic acid (351), D-biotin (0.23), folic acid (2.34), calcium pantothenate (58.5), p-aminobenzoic acid (58.5), niacin (70.2), and choline chloride (2.340).

** Minerals (g/kg of diet): CaHPO₄·2H₂O (4.557), KH₂PO₄ (8.050), NaH₂PO₄ (2.927), NaCl (1.459), Ca-lactate (10.983), Fe-citrate (0.995), MgSO₄ (2.244), MnSO₄·4–6H₂O (0.038), CuSO₄·5H₂O (0.009), and KI (0.003).

Table 2. Composition of the Zn-deficient Thr-supplemented diets (g/kg).

| Ingredients          | Thr supplemented |
|----------------------|------------------|
|                      | None | +5% | +10% | +15% | +25% | +35% |
| Egg albumin          | 50   | 50  | 50   | 50   | 50   | 50   |
| l-Thr                | 0    | 2.26| 4.52 | 6.78 | 11.30| 15.82|
| Dextrin              | 730  | 727.74| 725.48| 723.22| 718.70| 714.18|
| Corn oil             | 60   | 60  | 60   | 60   | 60   | 60   |
| Vitamin mixture*     | 20   | 20  | 20   | 20   | 20   | 20   |
| Salt mixture (−Zn)** | 60   | 60  | 60   | 60   | 60   | 60   |
| Cellulose powder     | 80   | 80  | 80   | 80   | 80   | 80   |

+5%, +10%, +15%, +25%, and +35% indicate the level of Thr added to the Zn-deficient diet containing 5% protein. The levels of Thr supplementation were the molar equivalent of those in the diets containing the amounts of 5%, 10%, 15%, 25%, and 35% protein. Vitamin mixture* and salt mixture (−Zn)** are described in Table 1.

were determined daily from 09:00 to 11:00. The rats were given the experimental diet for 4 wk and sacrificed under anesthesia with diethyl ether from 09:00 to 11:00. All procedures were performed in accordance with the Kobe Gakuin University Guidelines for the Care and Use of Laboratory Animals.

Diet. The compositions of the low- and high-protein diets are shown in Table 1. The content of Zn in the Zn-deficient diet containing 20% protein was 0.95 mg/kg diet. The level of Zn contamination in egg albumin was 2.2 mg/kg. In regard to amino acid supplementation, amino acids equivalent to the amount contained in 15% protein were included in the 5% protein diet, and the same amount of dextrin was subtracted from the diet. The contents (g) of amino acids in 1 kg of egg albumin were Ile, 55.76; Leu, 89.42; Lys, 69.23; Met, 39.42; Cys, 30.76; Phe, 59.61; Tyr, 45.19; Trp, 15.38; Val, 73.07; His, 25.00; Arg, 58.65; Ala, 62.50; Asp+Asn, 105.76; Glu+Gln, 134.61; Gly, 36.53; Pro, 36.53; Ser, 64.42.

Amino acids used for dietary supplementation were the L-form except where indicated. The compositions of the l-Thr supplemented diets are shown in Table 2.

Chemicals. All chemicals used were of analytical grade and were purchased from Nacalai Tesque (Kyoto, Japan) unless otherwise stated. Animal feed was obtained from Oriental Yeast Ltd.

Zn content. A 1 g portion of each test diet was heated for 48–72 h in a muffle oven at 450°C. After the samples cooled, 2 mL of 1 M HCl was added, and the digestates were heated and diluted with double-distilled deionized water. The stock Zn and sample solution were analyzed by atomic absorption spectroscopy with a Hitachi Z-5300 Polarized Zeema Atomic Absorption Spectrophotometer (Hitachi Ltd., Tokyo, Japan) at 213.8 nm.

Evaluation of feed intake and body weight changes. Feed intake and body weight change data were analyzed by the Cosinor method (3, 10, 11). Feed intake (F) and body weight change (ΔB) on day t were determined with the following equation:

\[ F \text{ or } \Delta B = M + A \cos(2\pi t / \tau + \phi) \]

where M, A, τ, and φ represent the mesor (the rhythm-adjusted mean), amplitude (maximum and minimum value the adjusted mean), period (length of one complete cycle), and acrophase (phase of minimum value), respectively.
The experimental data were fitted to this equation by the nonlinear least-squares method, and the four parameters, \( M, A, \tau, \) and \( \phi \), were calculated through subroutine analysis (13) with a personal computer (14). On the other hand, when the data were not fitted to the above equation, comparisons among groups were performed between a mean variation of daily feed intake and body weight change. The variation was calculated for each rat with the standard deviation of the estimate of the day-to-day variation in feed intake and body weight change for 28 d, and the group means are presented.

**Statistical analysis.** Values for feed intake and body weight changes are expressed as means±SD, except where otherwise indicated. A one-way analysis of variance test (ANOVA) was used for the comparisons of groups. The statistical significance of differences between groups was assessed with a Duncan multiple comparison test, with \( p<0.05 \) considered significant.

**RESULTS**

**Effects of dietary protein on feed intake and body weight change cycles**

The feed intake and body weights of rats were estimated daily. The weight gains (g) of rats fed a Zn-deficient diet containing 5%, 10%, 20%, 40%, and 60% egg albumin were (mean±SD) 7.3±3.0\(a\), 79.3±32.2\(b\), 58.5±25.0\(c\), 65.2±21.2\(d\), and 72.1±26.6\(e\), respectively, for 4 wk. The number of rats in each group was different, as shown in Table 3.

The feed intake and body weight changes in the previous 24 h period of each rat fed a Zn-deficient diet containing various amounts of protein fit well to a Cosinor curve, and the mean values with a standard deviation of \( M, A, \tau, \) and \( \phi \) are summarized in Table 3.

The values of the mesor of the feed intake and body weight change cycles of rats fed a Zn-deficient diet containing 5% protein were less than those of rats fed a Zn-deficient diet containing 10% protein. Protein concentration in the Zn-deficient diet from 10% to 60% affected neither the values of \( M \) of feed intake nor of \( M \) of body weight change. With protein concentration in the Zn-deficient diet group, the \( A \) values of both cycles were increased to a content of 20%. The \( A \) values of the feed intake and body weight change cycles of rats fed a Zn-deficient diet containing 20% protein were significantly increased by 6.6- and 7.0-fold, respectively, in comparison with rats fed a Zn-deficient diet containing 5% protein. Protein concentrations greater than 20% in the Zn-deficient diet failed to affect the \( A \) values of either the feed intake or the body weight change cycles.

On the other hand, when the protein concentration in the Zn-deficient diet was varied from 5% to 60%, the \( \tau \) values of the feed intake cycle and the body weight change cycle were unaffected (Table 3). The \( \phi \) values of both cycles were markedly different among groups, but this may have been due merely to variations in the rate of initial onset of deficiency and of no biological significance as described previously (3, 10).

The mean feed intake and mean body weight change were estimated daily for each rat for 4 wk: The mean feed intakes of rats fed a Zn-deficient diet including 5%, 10%, 20%, 40%, and 60% protein were 8.9±1.0\(a\), 12.8±2.8\(b\), 10.2±1.5\(c\), 9.7±1.1\(d\), and 9.7±1.7\(e\)/d, respectively. The mean values of body-weight gain were 0.3±0.1\(a\), 2.8±1.1\(b\), 2.1±0.8\(c\), 2.4±0.7\(d\), and 2.5±0.9\(e\)/d, respectively. The values of variabilities from the mean feed intake of rats fed a Zn-deficient diet including 5%, 10%, 20%, 40%, and 60% protein were 1.4±0.2\(a\), 2.8±1.0\(b\), 4.7±0.7\(c\), 3.4±0.6\(d\), and 3.3±0.8\(e\)/d, respectively, and the values of variability from mean body-weight change were 2.2±0.7\(a\), 4.4±1.7\(b\), 7.6±1.3\(c\), 6.8±1.3\(d\), and 7.2±2.4\(e\)/d, respectively. The values of variability of the feed-intake and body-weight change well reflected the amplitudes of both cycles (Table 3).

| Protein concentration in the diet (%) (number of rats) | 5 (10) | 10 (10) | 20 (13) | 40 (6) | 60 (7) |
|------------------------------------------------------|--------|---------|--------|-------|-------|
| **Feed intake**                                       |        |         |        |       |       |
| \( M \) (g/d)                                        | 9.0±1.1\(a\) | 12.8±2.8\(b\) | 10.1±1.5\(c\) | 9.6±1.1\(d\) | 9.6±1.7\(e\) |
| \( A \) (g/d)                                        | 0.8±0.2\(a\) | 2.3±1.4\(b\) | 5.3±0.8\(c\) | 3.7±0.6\(d\) | 3.5±1.3\(e\) |
| \( \tau \) (d)                                       | 3.6±0.3 | 3.7±0.4 | 3.4±0.3 | 3.3±0.2 | 3.5±0.2 |
| \( \phi \) (radian)                                  | 2.2±1.7 | 2.2±1.5 | 2.5±0.8 | 2.8±1.4 | 3.5±1.1 |
| **Body-weight change**                                |        |         |        |       |       |
| \( M \) (g/d)                                        | 0.3±0.1\(a\) | 2.8±1.2\(b\) | 2.0±0.8\(c\) | 2.4±0.8\(d\) | 2.4±1.0\(e\) |
| \( A \) (g/d)                                        | 1.2±0.4\(a\) | 3.9±2.0\(b\) | 8.4±1.6\(c\) | 6.6±1.2\(d\) | 7.2±2.4\(e\) |
| \( \tau \) (d)                                       | 3.6±0.5 | 3.5±0.3 | 3.4±0.3 | 3.2±0.1 | 3.5±0.2 |
| \( \phi \) (radian)                                  | 2.1±1.7 | 1.9±1.9 | 3.0±0.8 | 2.1±1.1 | 2.9±1.6 |

Each rat was fed the experimental diet for 28 d under the conditions described in Materials and Methods. Feed intake \( (F) \) or body-weight change \( (\Delta B) \) in the previous 24-h period at day \( t \): \( F (or \Delta B) = M + A \cos(2\pi t/\tau + \phi) \). Each value is the mean±SD. Values in each horizontal row not sharing a common superscript letter are significantly different \( (p<0.05) \).
Effects of supplementation with amino acids or amino acid mixtures on the feed intake and body weight change cycles

The rats fed a Zn-deficient diet containing 5% protein supplemented with essential amino acids (EAA) or a mixture of Met, Phe, Trp, and Thr (MFWT) showed significant cyclical feed intake and body weight changes. The values of the amplitude of the feed intake cycle of EAA and MFWT groups were 4.0±1.1 and 1.9±0.3

Fig. 1. Effects of amino acid supplementation of the Zn-deficient diet containing 5% protein on the feed intake and body-weight change of rats. +Essential amino acids, +(Met, Phe, Trp, Thr), +(Lys, Arg, His), and +(Val, Leu, Ile) represent the Zn-deficient diet containing 5% protein plus indicated essential amino acids. The amounts of essential amino acids, (Met, Phe, Trp, Thr), (Lys, Arg, His), and (Val, Leu, Ile) added were described in Materials and Methods. The experimental diets were given for 28 d. The mean values of feed intake (a) and body weight change (c) were determined from 10 rats for the 5% protein group and from 4 rats for each of the +Essential amino acids, +(Met, Phe, Trp, Thr), +(Lys, Arg, His), and +(Val, Leu, Ile) groups. The variabilities of feed intake (b) and body weight change (d) are given as standard deviations from the mean of 28 d data for groups. The values are shown as means with standard errors indicated by the vertical bars. Values not sharing a common superscript letter are significantly different (p<0.05).
g/d, respectively, and those of the body weight change cycle were 6.2±1.7 and 2.5±0.2 g/d, respectively. The feed intake and body weight changes in groups fed the Zn-deficient diet containing 5% protein supplemented with a mixture of Lys, Arg, and His (KRH) or Val, Lys, and Ile (VLI) did not fit a Cosinor curve. Therefore the criteria of the day-to-day variations of feed intake and body weight changes were evaluated by their variability with standard deviations from the means of 28 d data.

A supplementation of the Zn-deficient diet containing 5% protein with EAA, MFWT, KRH, and VLI had no effect on the mean feed intake during the experimental period of 28 d (Fig. 1a). A supplementation of the Zn-deficient diet with EAA and MFWT increased the variabilities of feed intake and body weight change (Fig. 1, b and c).

The Zn-deficient diet containing 5% protein was supplemented with Met, Phe, Thr, D-Thr, or Gly, and the Thr group showed significantly increased variabilities of feed intake and body weight change (Fig. 2, b and d). A supplementation with neither D-Thr nor Gly increased the variabilities of feed intake or body weight change. Increases in the amplitude of feed intake and body weight change cycles by L-Thr supplementation

Data for rats fed a Zn-deficient diet containing 5% protein supplemented with L-Thr up to 35% did not fit to a Cosinor curve. The day-to-day variations of feed intake and body weight changes were evaluated with

---

Fig. 2. Effects of Met, Phe, Trp, Thr, D-Thr, and Gly supplementation on the feed intake and body weight change of rats. +Met, +Phe, +Trp, +Thr, +D-Thr, and +Gly indicate supplementation of the Zn-deficient diet containing 5% protein with each amino acid. The amounts of Met, Phe, Trp, Thr, and Gly added are described in Materials and Methods. The amount of D-Thr supplemented in the diet was the same as that of L-Thr supplemented in the +Thr-group. The mean values of feed intake (a) and body weight change (c), and the variabilities of feed intake (b) and body-weight change (d) were estimated as described in the legend of Fig. 1. Each value is the mean±SE for 4 rats. Values not sharing a common superscript letter are significantly different (p<0.05).
Threonine Supplementation and Zinc-Deficient

variability as described above. The mean values of daily feed intake and body weight changes were increased by a supplementation with Thr, and the highest value was observed when 5% Thr was supplemented (Fig. 3, a and c). The day-to-day variation of feed intake was increased with Thr supplementation up to 10%, then decreased with further increases in the level of Thr (Fig. 3b). The variation of body weight change was correlated with feed intake.

The data of feed intake and body weight change in the previous 24 h period of rats fed a Zn-deficient diet containing 10% Thr for 28 d were fitted to a Cosinor curve. The mean values with standard deviations of $M$, $A$, $\tau$, and $\phi$ are summarized in Table 4, and we compared them with those of the EAA and MFWT groups. The $M$ values of the feed intake and body weight change cycles were not significantly different among groups. The $A$ values of the feed intake and body weight change cycles of the MFWT- and 10%-Thr groups were not restored to the level of that of the EAA group (Table 4).

Fig. 3. Effects of the level of Thr added to the Zn-deficient diet containing 5% protein on the feed-intake and body-weight change of rats. +5% Thr, +10% Thr, +15% Thr, +25% Thr, and +35% Thr indicate the level of Thr added to the Zn-deficient diet containing 5% protein. The level of supplemented Thr was the molar equivalent that in the diet containing the amount of indicated protein. The mean values of feed intake (a) and body-weight change (c), and the variabilities of feed intake (b) and body-weight change (d) were estimated as described in Fig. 1. Each value is the mean ± SE from 10 rats in the 5% protein group and from 4 rats in the other groups. Values not sharing a common superscript letter are significantly different ($p<0.05$).
Table 4. Effects of dietary amino acid supplementation on the parameters of feed-intake and body-weight-change cycles of rats fed a Zn-deficient diet.

| Amino acids | Essential amino acid | Met, Phe, Trp, Thr | Thr |
|-------------|----------------------|--------------------|-----|
| Feed intake |                      |                    |     |
| M (g/d)     | 9.2±0.3              | 9.5±0.8            | 10.4±1.0 |
| A (g/d)     | 4.0±1.1b             | 1.9±0.3a           | 2.9±0.7nb |
| τ (d)       | 3.6±0.2a             | 4.3±0.1c           | 4.0±0.1b |
| φ (radian)  | 2.6±1.9              | 3.3±0.2            | 3.5±1.3 |
| Body-weight change |            |                    |     |
| M (g/d)     | 1.5±0.3              | 0.9±0.1            | 1.0±0.4 |
| A (g/d)     | 6.2±1.7b             | 2.5±0.2a           | 3.8±1.0a |
| τ (d)       | 3.5±0.2a             | 4.4±0.3c           | 4.0±0.1b |
| φ (radian)  | 1.3±0.8              | 1.5±1.3            | 4.4±2.2 |

Each rat was fed the experimental diet for 28 d under the conditions described in Materials and Methods. Feed intake (F) or body weight change (ΔB) in the previous 24 h period at day t: F or (ΔB) = M+A cos(2πt/τ+φ). Each value is the mean±SD. Values in each horizontal row not sharing a common superscript letter are significantly different (p<0.05). The essential amino acid group was fed the Zn-deficient diet containing 5% protein plus an essential amino acid. The amount of supplemented essential amino acid in the diet was the molar equivalent of that in the 15% protein diet, and the same amount of dextrin was omitted from the diet. The amounts of essential amino acids (g/kg diet) supplemented were Met 5.91, Phe 8.94, Trp 2.31, Thr 6.78, Lys 10.38, Arg 8.80, His 3.75, Val 10.95, Leu 13.41, and Ile 8.36. The mixture of the Met, Phe, Trp, and Thr group was supplemented with Met, Phe, Trp, and Thr at a molar equivalent to the contents in the 15% protein diet, and the same amount of dextrin was omitted. The amounts of Met, Phe, Trp, and Thr supplemented were 5.91, 8.94, 2.31, and 6.78 g/kg diet, respectively. The Thr group received a diet supplemented with Thr at a molar equivalent to the contents in the 10% protein diet, and the same amount of dextrin was omitted. The amount of Thr supplemented was 4.52 g/kg diet.

**DISCUSSION**

Rats fed a Zn-deficient diet show decreased feed intake with marked growth retardation (2, 3). A cyclic pattern of feeding in rats given a Zn-deficient diet was proposed originally by Mills et al. (4) and supported by others, and we have evaluated the daily feed intake and body weight changes by Cosinor analysis (3, 10, 11). The values of the parameters M, A, τ, and φ of feed intake and body weight change cycles could be used to clarify the nutritional Zn-deficient status in rats.

Here we found that the level of protein in the Zn-deficient diet affected the feed-intake and body-weight change cycles. The amplitudes of these cycles of rats fed a Zn-deficient diet increased with the protein content in the diet, to 20%, then decreased with further increases in diet protein level (Table 3). These results suggested that feeding with the Zn-deficient diet containing a high concentration of protein led to a large amino acid pool or high concentrations of amino acid metabolites, which may have decreased the cycle amplitude. The rats fed a Zn-deficient diet containing a low protein content may have overcome the cyclical retardation of appetite to maintain their body weight and to secure amino acids for their physiological demand. Therefore the amplitude of the feed-intake cycle of rats fed a Zn-deficient diet containing 10% protein would be about half of that of the 20% protein group, but the mean value of feed intake was not significantly different between the two groups (Table 3). The amino acid pool and amino acid metabolites could not affect the cyclic period of the feed intake or body weight changes (Table 3).

In this study, we used egg albumin as a protein source in the Zn-deficient diet, but the protein was contaminated with Zn at 2.2 mg/kg. This contamination of the high-protein Zn-deficient diet with Zn decreased the amplitude of the feed intake cycles. The contents of Zn in the Zn-deficient diets containing 20%, 40%, and 60% protein diets were 0.95, 1.26, and 1.58 mg/kg diet, respectively. From the mean values of daily feed intake, the amount of Zn intake of rats fed the Zn-deficient diets containing 20%, 40%, and 60% protein were calculated to be 9.7, 12.1, and 15.2 μg/d, respectively. Previously (11), we found that a supplementation of a Zn-deficient diet with Zn decreased the amplitude of the feed intake cycle in rats with a negative correlation to a Cosinor curve. When the Zn-free diet was supplemented with 12.1 or 15.2 μg/d of Zn, the amplitudes of the feed intake cycle were calculated to be 2.2 and 5.1%, respectively, less than that of the group fed the diet supplemented with 9.7 μg/d of Zn. The amplitude of the feed-intake cycle of rats fed Zn-deficient diets containing 40% and 60% protein were 3.7 and 3.5 g/d, respectively (Table 3), 31.2 and 34.0% less than that of the 20% protein group, respectively. Therefore a high-protein diet may decrease the amplitude of the feed intake cycle under conditions of Zn-deficiency in rats, but the decrease in amplitude in rats fed a high-protein diet may not be due to contamination by Zn in the dietary protein.

Chesters and Will (5) found that the supplementation of a Zn-deficient diet containing 5% protein with nonessential amino acids did not increase the variability of daily feed intake. We also found that a supplementation of the Zn-deficient diet containing 5% protein with 15% β-alanine did not increase the variability of daily feed intake (data not shown). Therefore the nitro-
The addition of 5% Thr to a Zn-deficient diet increased the variability of daily feed intake up to 10% (Fig. 3). On the other hand, the value of feed intake was highest with the addition of 5% Thr. This discrepancy may have been due to the differences in the optimum dose of Thr for appetite and the day-to-day variation of feed intake. The same phenomena were observed in the M and A values on the effects of dietary protein on the parameters of feed intake cycles of rats fed a Zn-deficient diet (Table 3).

The feed intake and body weight changes of rats fed a Zn-deficient diet containing both 5% protein and 10% Thr showed good fits to a Cosinor curve, and the values of the amplitude of both cycles were significantly greater than those of rats fed a Zn-deficient diet containing only 5% protein. The addition of Gly had no effect on the variability of the feed intake. The effect of Thr on the amplitude of the feed intake cycle could not be mediated through metabolites such as C1 units, which are products of Thr, Gly, and Ser. The addition of ω-Thr had no effect on the level of variation of daily feed intake. Therefore the increase in the amplitude of the feed-intake cycle of rats fed a Zn-deficient diet may be dependent on the physiological function of ω-Thr.

The period of feed intake and body weight change cycles of rats fed a Zn-deficient diet containing 20% protein was about 3.5 d. However, the addition of a mixture of Met, Phe, Trp, and Thr (MFWT) or Thr to the Zn-deficient diet containing 5% protein increased the period of the feed intake cycle to 4.3 and 4.0 d, respectively. The amino acid imbalance may affect the period of the cycle.

We previously (3) found that cyclic variation in feed intake is accompanied by a cyclic variation in body weight change in rats fed a Zn-deficient diet, and that the cyclic variation in this change occurs similarly in pair-fed control rats. The maximum value of the feed intake cycle of rats fed the Zn-deficient diet was similar to the mean value of the daily feed consumption of the Zn-adequate control rats (3). The cyclical feed intake appeared not to be caused by excess feed consumption, but by a periodic reduction in the rat's appetite. In this study we found that Thr acts as an appetite control factor. The addition of Thr, however, could not restore the amplitude of the feed intake cycle to the level seen with the addition of essential amino acids or of the Zn-deficient diet containing 20% protein. Protein deficiency or amino acid imbalance and Zn-deficient status may confuse the analysis of the mechanism of the cyclicity of feed intake and body weight change in rats.

REFERENCES

1) Todd WR, Elvehjem CH, Hart EB. 1934. Zinc in the nutrition of the rat. Am J Physiol 107: 146–156.
2) Williams RB, Mills CF. 1970. The experimental production of zinc deficiency in the rat. Br J Nutr 24: 989–1003.
3) Yamasaki K, Kaneko M, Matsuda K, Fujimoto Sakata S, Tamaki N. 1999. The correlation between feed-intake cycle and nutritional zinc-deficient status in rats. J Nutr Sci Vitaminol 45: 621–632.
4) Mills CF, Quarterman J, Chesters JK, Williams RB, Dalgarno AC. 1969. Metabolic role of zinc. Am J Clin Nutr 22: 1240–1249.
5) Chesters JK, Will M. 1973. Some factors controlling food intake by zinc-deficient rats. Br J Nutr 30: 555–566.
6) Wallwork JC, Fosmire GJ, Sandsted HH. 1981. Effect of zinc deficiency on appetite and plasma amino acid concentrations in the rat. Br J Nutr 30: 127–136.
7) Giugliano R, Millward DJ. 1984. Growth and zinc homeostasis in the severely Zn-deficient rat. Br J Nutr 52: 545–560.
8) Kramer TR, Briske-Anderson M, Johnson SB, Holman RT. 1984. Influence of reduced food intake on polyunsaturated fatty acid metabolism in zinc-deficient rats. J Nutr 114: 1224–1230.
9) Quinn PB, Cremin FM, O’Sullivan VR, Hewedi FM, Bond RJ. 1990. The influence of dietary folate supplementation on the incidence of tetratogenesis in zinc-deficient rats. Br J Nutr 64: 233–243.
10) Tamaki N, Fujimoto-Sakata S, Kikugawa M, Kaneko M, Onosaka S, Takagi T. 1995. Analysis of cyclic feed intake in rats fed on a zinc-deficient diet and the level of dihydropyrimidinidase (EC 3.5.2.2). Br J Nutr 73: 711–722.
11) Aiba K, Kimura M, Sakata S, Matsuda K, Kaneko M, Onosaka S, Yamaoka Y, Tamaki N. 1997. Cosinor analysis of feed intake cycle of rats fed a zinc-deficient diet and the effect of zinc supplementation. J Nutr Sci Vitaminol 43: 327–343.
12) Raits TM, Shad NF. 1995. Zinc status specifically changes preferences for carbohydrate and protein in rats selecting from separate carbohydrate-, protein-, and fat-containing diets. J Nutr 125: 2874–2879.
13) Marquardt DW. 1963. An algorithm for least-squared estimation of non linear parameters. J Soc Indust Appl Math 11: 431–441.
14) Yamaoka K, Tanigawara Y, Nakagawa T, Ueno T. 1981. A pharmacokinetic analysis program (MULTI) for microcomputer. J Pharmacobiodyn 4: 879–885.
15) Wallwork JC, Fosmire GJ, Sandstead HHL. 1981. Effect of zinc deficiency on appetite and plasma amino acid concentration in the rat. Br J Nutr 45: 127–136.