The Correlation between Feed-Intake Cycle and Nutritional Zinc-Deficient Status in Rats

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Summary The characteristic cyclic variation in feed intake of rats fed a Zn-deficient diet (Mills et al, Am J Clin Nutr 22: 1240–1249 (1969)) followed a Cosinor curve, as determined by computer analysis (Tamaki et al, Br J Nutr 73: 711–722 (1995)). The values of amplitude for the feed-intake cycle had a positive correlation to their own day-to-day variations and to the correlation value of their own simulated cycles \( r^2 = 0.764, \text{ df } = 50, \ p < 0.001 \) and \( r^2 = 0.682, \text{ df } = 50, \ p < 0.001 \), respectively. The cyclic variation in feed intake was accompanied by a cyclic variation in body-weight change in rats fed the Zn-deficient diet, and cyclic variation in body-weight change occurred similarly in pair-fed control rats. There were no differences in the mesors of body-weight change cycles of Zn-deficient rats and pair-fed control rats (Zn-deficient rats: \( 2.5 \pm 1.0 \text{ g/d}, \text{ pair-fed rats: } 2.8 \pm 1.0 \text{ g/d}, \text{ mean } \pm \text{ SD, df } = 18, \ t = -0.674, \text{ ND} \)). Rats fed the Zn-deficient diet were given different amounts of Zn supplementation by daily subcutaneous injection. The amplitude of the feed-intake cycle was decreased with increasing Zn supplementation \( (r^2 = 0.919, \text{ df } = 5, \ p < 0.001) \). The concentration of Zn for the appearance of the feed-intake cycle was estimated to be \( 71.6 \pm 6.6 \mu \text{g/d} \) per rat. The Zn level in the serum showed a significant decrease in the Zn-deficient diet groups, but the supplement of Zn did not vary in the Zn-deficient rats injected with up to \( 47.3 \mu \text{g/d} \) per rat. From these results, an analysis of the feed-intake cycle allowed us to estimate the quantitative Zn-deficient status of rats.

Key Words zinc deficient, feed-intake cycle, body-weight change, zinc injection

It is well known that Zinc (Zn) is essential for growth in animals (1) as well as humans (2). Zn deficiency impairs growth by a combination of decreased feed intake, slowed anabolic response to the feed, and increased catabolic response (3).

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A cyclic pattern of feeding in rats given a Zn-deficient diet was proposed originally by Mills et al (4) and supported by others (1, 3, 5–10). Based on the criterion of anorexia, the standard deviation of feed intake of rats fed a Zn-deficient diet with respect to time is the basis of the estimate of the day-to-day variation in feed intake (1, 5, 7). A Zn-deficient, 5% protein diet affected feed-intake levels and reduced the variability of cycling observed in the deficient rats (5, 7). When the low-protein diet was supplemented with essential amino acids, the variability of intake was increased (5, 7). However, the most effective amino acid or amino acid metabolite could not be identified. The above method could not reveal a characteristic and periodical day-to-day feed-intake pattern for rats fed a Zn-deficient diet.

In previous reports (11, 12), feed-intake and body-weight change data were analyzed by the “Cosinor” method. In this study, we characterized the Zn-deficient status from the feed-intake cycle of rats fed a Zn-deficient diet in comparison with that of pair-fed control rats. Moreover, the feed-intake cycle disappeared with the subcutaneous injection of Zn to rats fed the Zn-deficient diet.

**MATERIALS AND METHODS**

*Animals.* Male albino rats (Crj:Wistar strain (weighing 90–100 g) were purchased from Charles River Japan (Atsugi, 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 7:00 a.m. to 7:00 p.m., local time). 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 acclimate them to the new environment. Feed intake and body weight were determined daily, sometime between 9:00 a.m. and 11:00 a.m. The rats were given the experimental diet for 4 wk, and sacrificed between 9:00 a.m. and 11:00 a.m. under anesthesia with diethyl ether. All procedures were in accordance with the Kobe Gakuin University Guidelines for the Care and Use of Laboratory Animals.

The following three experiments were performed. Experiment 1: Fifty-two rats were given a Zn-deficient diet ad libitum for 28 d. Experiment 2: Twenty rats were assigned to two groups after the stabilization period. One group (10 rats) was given the Zn-deficient diet ad libitum. The other group (10 rats) was individually pair-fed an amount of control diet that equaled the amount of diet eaten by the paired Zn-deficient rat on the previous day. Experiment 3: Thirty-two rats were assigned to 8 groups and given the Zn-deficient diet ad libitum. ZnSO₄·7H₂O was dissolved in 0.1 mL physiological saline and subcutaneously injected into the rats at 9:00–11:00 a.m. The 8 groups received 0, 3.2, 15.8, 23.7, 31.6, 47.4, 63.1 or 286 μg of Zn/rat once daily for 27 consecutive days. The rats were sacrificed 24 h after the final injection.

Blood samples were taken from the carotid artery into plain glass tubes and stored at 4°C until centrifugation at 5,000 × g for 20 min. Serum samples were stored...
Table 1. Composition of the diets (g/kg).

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Zinc-deficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egg albumin</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Dextrin</td>
<td>637</td>
<td>637</td>
</tr>
<tr>
<td>Corn oil</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Vitamin mixture*</td>
<td>11.7</td>
<td>11.7</td>
</tr>
<tr>
<td>Salt mixture (+Zinc)**</td>
<td>31.3</td>
<td>31.3</td>
</tr>
<tr>
<td>Salt mixture (−Zinc)**</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose powder</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

* Vitamins (mg/kg of diet) were: retinyl acetate (2.01), cholecalciferol (0.03), α-tocopheryl acetate (58.5), menadione (60.8), thiamine 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 (+zinc) (g/kg of diet) were: CaHPO₄·2H₂O (4.557), KH₂PO₄ (8.050), NaH₂PO₄ (2.927), NaCl (1.459), Ca-lactate (10.983), Fe-citrate (0.995), MgSO₄ (2.244), ZnCO₃ (0.034), MnSO₄·4·6H₂O (0.038), CuSO₄·5H₂O (0.009), and KI (0.003).

*** ZnCO₃ was omitted from the above mixture.

Diets. The compositions of the Zn-deficient and control diets are shown in Table 1. The contents of Zn in the Zn-deficient and control diets were analyzed by the previously described methods (11), and were 0.95 and 20 mg/kg diet, respectively. All rats were given deionized water ad libitum.

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

Zn content. Serum was diluted 1:4 with 0.83 M HCl and incubated for 30 min at about 4°C. After brief centrifugation (3,000 rpm for 10 min), the supernatant was removed and used for Zn analysis. The concentrations of stock Zn and sample solutions were measured by atomic absorption spectroscopy with a Hitachi Z-53000 Polarized Zeeman Atomic Absorption Spectrophotometer (Hitachi Ltd., Tokyo, Japan) at 213.8 nm.

Evaluation of feed intake and body-weight change. Feed-intake and body-weight change data were analyzed by the "Cosinor" method (11, 12). Feed intake \( F \) and body-weight change \( \Delta B \) on day \( t \) were derived using the following equation:

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

where \( M \), \( A \), \( \tau \) and \( \phi \) represent the mesor (the rhythm-adjusted mean), amplitude (maximum and minimum value from the adjusted mean), period (length of one complete cycle) and acrophase (phase of minimum value), respectively.
The experimental data obtained were fitted to the above equation by a nonlinear least-squares method. The four parameters, $M$, $A$, $\tau$ and $\phi$, were calculated using subroutine analysis (13) with a personal computer (14).

Statistical analysis. Values for feed intake and body-weight change are expressed as mean $\pm$ SD. One-way analysis of variance (ANOVA) was used to compare the groups. When a significant difference ($p<0.05$) was found between groups, the statistical significance of the difference between values was assessed by using Duncan’s multiple comparison test and considered significant at $p<0.05$. Correlation and regression analyses were performed with CA-Cricket Graph (Computer Associates International, USA). The kinetic data were calculated using subroutine analysis software (14).

RESULTS

Evaluation of feed intake and body-weight change of male rats fed the Zn-deficient diet

The quantity of the feed intake and body-weight change of rats fed the Zn-deficient diet varied greatly from day to day, and during a sufficiently long experimental period cyclic patterns of feed intake and body-weight change were detectable. The rats showed typical symptoms of Zn deficiency, such as alopecia, depigmentation of hair, dermatitis of paws, anorexia and growth retardation, which have been described by Wallwork et al (8).

The feed intake and body-weight change in the previous 24-h period of each rat fed the Zn-deficient diet fit well to a Cosinor curve ($r^2=0.570\pm0.165$ and $0.572\pm0.169$, respectively, $n=52$). The maximum and minimum values of the correlation coefficients ($r^2$) between the feed intake and their own Cosinor curve were 0.826 and 0.207, respectively, and those between the body-weight change and their own Cosinor curve were 0.814 and 0.162, respectively. Moreover, all of the feed intake and body-weight change from 52 rats fed the Zn-deficient diet had a very significant correlation to each cosine wave ($p<0.05$).

The parameters of each cycle were calculated from the experimental data. The mean values with standard deviations of $M$, $A$, $\tau$ and $\phi$ from the feed-intake cycles ($n=52$) were $10.2\pm1.5$ g/d, $4.7\pm1.3$ g/d, $3.5\pm0.3$ d and $2.3\pm1.6$ radians, respectively. The body-weight change reflected the feed intake, and the values of $M$, $A$, $\tau$ and $\phi$ were $2.0\pm1.0$ g/d, $8.3\pm2.4$ g/d, $3.4\pm0.3$ d and $3.5\pm1.6$ radians, respectively ($n=52$).

The values of the amplitude ($A$) of the feed-intake and body-weight change cycles revealed a high correlation ($r^2=0.764$, df=50, $p<0.001$ and $r^2=0.715$, df=50, $p<0.001$, respectively) with their own day-to-day variations (Fig. 1, a and b). The amplitude of the feed-intake cycle was also in good correlation ($r^2=0.682$, df=50, $p<0.001$) with the correlation value of its own simulated cycle (Fig. 1c). The values of $M$, $\tau$ and $\phi$ for the cycles of feed intake had a low negative correlation ($r^2=0.082$, 0.108, and 0.081, respectively) to the correlation value of their own cycles. The values of $M$, $\tau$ and $\phi$ of the
Fig. 1. Correlation between the amplitude of the feed-intake cycle and variability of feed intake (a), the amplitude of the weight change cycle and variability of weight change (b), and the amplitude of the feed-intake cycle and correlation value of their own feed-intake cycle (c). The variabilities of feed intake and body-weight change were standard deviations from the mean of 28 d data for each rat fed the Zn-deficient diet. The relationships of (a), (b) and (c) were $y = -1.35 + 1.39 x$ ($r^2 = 0.763$, df = 50, $p < 0.001$), $y = -1.47 + 1.20 x$ ($r^2 = 0.715$, df = 50, $p < 0.001$) and $y = -2.35 + 9.43 x$ ($r^2 = 0.682$, df = 50, $p < 0.001$), respectively.

Body-weight change cycles had no correlation with the correlation values of their own cycles. These results suggest that the value of the amplitude indicates the correlation of their own cycles among the parameters of the feed-intake and body-weight change cycles.
Table 2. Mean values of parameters in Cosinor analysis of feed intake and body-weight change in the previous 24-h period for Zn-deficient and pair-fed control rats.

<table>
<thead>
<tr>
<th></th>
<th>Mesor (M) (g/d)</th>
<th>Amplitude (A) (g/d)</th>
<th>Period (τ) (d)</th>
<th>Acrophase (ϕ) (radian)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake</td>
<td>10.9 ± 1.3</td>
<td>5.1 ± 1.1</td>
<td>3.5 ± 0.4</td>
<td>2.8 ± 1.6</td>
</tr>
<tr>
<td>Body-weight change Zn-deficient</td>
<td>2.5 ± 1.0</td>
<td>8.5 ± 2.4</td>
<td>3.5 ± 0.4</td>
<td>3.0 ± 1.8</td>
</tr>
<tr>
<td>Pair-fed</td>
<td>2.8 ± 1.0</td>
<td>7.9 ± 2.1</td>
<td>3.5 ± 0.4</td>
<td>3.1 ± 1.6</td>
</tr>
</tbody>
</table>

The Zn-deficient rats (n=10) were fed a Zn-deficient diet for 28 d. The feed-restricted pair-fed rats (n=10) were given a Zn-adequate diet in an amount equal to that consumed on the previous day by their respective paired-mate ad libitum-fed Zn-deficient rats for 27 d. The pair-fed rats ate the restricted diet throughout the experiment, starting the second day. All rats were given deionized water. Values are presented as the average ± SD from ten rats.

Body-weight change cycle of pair-fed rats

The data of feed intake and body-weight change in the previous 24-h period for the Zn-deficient (n=10) and pair-fed control (n=10) rats were analyzed. The serum Zn contents (mean ± standard deviation) in the pair-fed control and Zn-deficient groups of rats were 1.13 ± 0.04 and 0.39 ± 0.06 µg/mL, respectively (df=18, p<0.01). The pair-fed control rats were fed a restricted amount of diet starting from the second day of the pair-fed experiments. The body-weight changes in the previous 24-h period of Zn-adequate pair-fed control rats revealed the characteristic cyclic changes shown in the case of the Zn-deficient rats. The experimental data of the body-weight change of the pair-fed rats were fitted to a cosine wave with very high correlation. The mean values with standard errors of M, A, τ and ϕ are summarized in Table 2. The values of M, A, τ and ϕ of the body-weight change cycles were not significantly different in the Zn-deficient and pair-fed rats. The daily feed intake of the Zn-deficient diet and body-weight change from both groups were simulated for 4 d in Fig. 2. The body-weight change cycle of the Zn-deficient rats is synchronized with their own feed-intake cycle. The body-weight change cycle of the pair-fed rats follows that of the Zn-deficient rats with similar parameters except for acrophase. The maximum value of the feed-intake cycle of rats fed the Zn-deficient diet was similar to the mean value of the daily food consumption of the Zn-adequate control rats.

Effect of Zn injection on feed-intake and body-weight change cycles of rats fed the Zn-deficient diet

The effects of Zn supplementation on the feed intake and body-weight change of the Zn-deficient rats were analyzed for 28 d. To prevent or diminish the effects of Zn in the diet on the rate of digestion of the diet and absorption
Feed Intake Cycle and Zinc Injection

Fig. 2. Simulation of feed-intake and body-weight change cycles of rats fed the Zn-deficient or pair-fed control diet. (a) Body-weight change of rats fed the Zn-deficient diet, $\Delta B = 2.5 + 8.5 \cos(2\pi t/3.5 + 4.9)$; (b) body-weight change of pair-fed rats, $\Delta B = 2.8 + 7.9 \cos(2\pi t/3.5 + 4.9)$; (c) feed intake of rats fed the Zn-deficient diet, $F = 10.9 + 5.1 \cos(2\pi t/3.5 + 2.8)$. The parameters of each cycle are those listed in Table 2. The acrophase of the body-weight change cycle from pair-fed rats was increased by 1.8 ($2\pi t/3.5, t = 1$) radians. The dashed line is the mean value of feed intake of rats fed the control diet, with the standard deviation (15.4 ± 2.2 g/d, n = 24) from a previous paper utilized (12).

of nutrients in the digestive tract, various fixed quantities of Zn were injected subcutaneously daily. Rats fed the Zn-deficient diet supplemented subcutaneously with Zn at up to 31.6 µg/d showed significant cyclical feed intake and body-weight change. In the group injected with Zn 47.4 and 63.1 µg/d/rat, the feed intake and body-weight change of three and two rats, respectively, were significantly correlated to a cosine curve, but those of the other one and two rats, respectively, were less correlated with the curve (0.05 < p < 0.20). The average value with standard errors of the four parameters of the feed-intake and body-weight change cycles for the different amounts of subcutaneously injected Zn are summarized in Table 3. The values of the mesor of the feed-intake and body-weight change cycles were increased with the Zn contents in the injection, but the amplitudes of the two cycles decreased. The values of period and acrophase of both cycles did not differ grossly among the groups.

We evaluated the parameters of both cycles with daily Zn injection. In rats injected daily with Zn at up to 63.1 µg/rat, the amplitudes of both cycles were inversely related to the amount of Zn injected, with pseudo first-order profiles (Fig. 3). From the intercept of the horizontal axis, the cut-off concentrations of Zn for the appearance of feed-intake and body-weight change cycles were estimated to be 71.6 ± 6.6 and 80.4 ± 7.5 µg/d/rat (mean ± SD), respectively. The feed intake and body-weight change of rats fed the Zn-deficient diet and given

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Table 3. Effect of Zn injection to rats fed a Zn-deficient diet on the parameters of feed-intake and body-weight change cycles.

<table>
<thead>
<tr>
<th>Zn injection (µg/d/rat)</th>
<th>0</th>
<th>3.2</th>
<th>15.8</th>
<th>23.7</th>
<th>31.6</th>
<th>47.4</th>
<th>63.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Feed intake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ (g/d)</td>
<td>$9.9 \pm 1.0^a$</td>
<td>$10.6 \pm 1.2^{a,b}$</td>
<td>$11.9 \pm 0.3^{b,c}$</td>
<td>$12.6 \pm 0.2^c$</td>
<td>$12.6 \pm 0.6^c$</td>
<td>$15.0 \pm 1.1^d$</td>
<td>$15.4 \pm 0.5^d$</td>
</tr>
<tr>
<td>$A$ (g/d)</td>
<td>$5.9 \pm 0.7^a$</td>
<td>$5.3 \pm 0.9^{a,b}$</td>
<td>$5.3 \pm 0.8^{a,b}$</td>
<td>$3.9 \pm 1.4^{b,c}$</td>
<td>$3.2 \pm 1.1^c$</td>
<td>$1.1 \pm 0.3^d$</td>
<td>$1.3 \pm 0.3^d$</td>
</tr>
<tr>
<td>$\tau$ (d)</td>
<td>$3.4 \pm 0.3$</td>
<td>$3.5 \pm 0.2$</td>
<td>$3.6 \pm 0.2$</td>
<td>$3.8 \pm 0.3$</td>
<td>$3.6 \pm 0.2$</td>
<td>$3.8 \pm 0.5$</td>
<td>$3.1 \pm 0.2$</td>
</tr>
<tr>
<td>$\phi$ (radian)</td>
<td>$2.9 \pm 0.7$</td>
<td>$3.2 \pm 1.0$</td>
<td>$3.1 \pm 0.5$</td>
<td>$3.5 \pm 1.5$</td>
<td>$2.3 \pm 1.9$</td>
<td>$3.0 \pm 2.0$</td>
<td>$3.5 \pm 2.5$</td>
</tr>
<tr>
<td>Body-weight change</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$M$ (g/d)</td>
<td>$2.2 \pm 1.0^a$</td>
<td>$2.4 \pm 0.6^a$</td>
<td>$2.8 \pm 0.3^{a,b}$</td>
<td>$3.4 \pm 0.3^b$</td>
<td>$3.4 \pm 0.3^{b,h}$</td>
<td>$5.1 \pm 0.4^e$</td>
<td>$5.2 \pm 0.2^c$</td>
</tr>
<tr>
<td>$A$ (g/d)</td>
<td>$10.2 \pm 0.8^a$</td>
<td>$8.8 \pm 1.8^{a,b}$</td>
<td>$7.3 \pm 1.8^{b,c}$</td>
<td>$7.0 \pm 1.5^{b,c}$</td>
<td>$4.8 \pm 2.5^{c,d}$</td>
<td>$3.0 \pm 0.8^d$</td>
<td>$3.1 \pm 1.2^d$</td>
</tr>
<tr>
<td>$\tau$ (g/d)</td>
<td>$3.4 \pm 0.2$</td>
<td>$3.5 \pm 0.2$</td>
<td>$3.6 \pm 0.2$</td>
<td>$3.7 \pm 0.3$</td>
<td>$3.5 \pm 0.3$</td>
<td>$3.5 \pm 0.3$</td>
<td>$3.1 \pm 0.3$</td>
</tr>
<tr>
<td>$\phi$ (radian)</td>
<td>$3.2 \pm 0.7$</td>
<td>$3.7 \pm 1.1$</td>
<td>$3.5 \pm 0.5$</td>
<td>$3.6 \pm 1.6$</td>
<td>$3.8 \pm 2.1$</td>
<td>$1.7 \pm 0.6$</td>
<td>$1.5 \pm 1.1$</td>
</tr>
</tbody>
</table>

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. Means in each horizontal row not sharing a common superscript letter are significantly different ($p < 0.05$).
Feed Intake Cycle and Zinc Injection

Fig. 3. Correlation between the amplitude of the feed-intake and body-weight change cycles and Zn injection. The amplitudes of the feed-intake and body-weight cycles were determined for rats fed the Zn-deficient diet and injected subcutaneously with different quantities of Zn. Values are means, with their standard errors indicated by the vertical bars. (a) The relationship for the amplitude of the feed-intake cycles with Zn injection was $y = 5.88 - 0.082x$ ($r^2 = 0.919$, $df = 5$, $p < 0.001$). (b) The relationship for the body-weight change cycles with Zn injection was $y = 9.39 - 0.012x$ ($r^2 = -0.961$, $df = 5$, $p < 0.001$).

Zn injections of 286 µg/d/rat had no sign of cyclic variation during the experimental period of 4 wk. In the group of rats injected with Zn at 286 µg/d/rat, the daily feed intake and body-weight change were 16.8 ± 1.7 and 6.3 ± 2.9 g/d (mean ± SD), respectively.

The Zn level in the serum showed a significant decrease during the course of Zn depletion in the Zn-deficient diet groups. However, the Zn content in rats fed the Zn-deficient diet with Zn supplementation did not vary in the rats injected with Zn at up to 47.3 µg/d (Fig. 4). A daily Zn injection of 63.1 µg/rat increased the serum level of Zn significantly. The group injected with Zn 286 µg/d had 2.35 ± 0.15 µg/mL (mean ± SD) of Zn in the serum (not shown).

DISCUSSION

It is well established that male rats fed a Zn-deficient diet show typical signs of Zn deficiency, including anorexia with marked growth retardation. The associated reduction in daily feed intake follows a cosine curve, as shown in previous reports (11, 12). The cosine curve of the feed intake was accompanied by a slightly retarded cosine wave of body-weight change in the Zn-deficient rats. Among 4 parameters, $M$, $A$, $\tau$ and $\phi$, of the cycles of feed intake and body-weight change, the value of $A$ shows a relationship to their own cosine
Fig. 4. Zn concentration in serum versus amount of Zn injected. The rats were injected subcutaneously with different quantities of Zn. Values are means, with their standard errors indicated by the vertical bars. Values not sharing a common superscript letter are significantly different ($p<0.05$).

curves. In preliminary reports (5, 7), the day-to-day variation of feed intake was measured as the standard deviation from the regression to evaluate the cyclic patterns of the feed intake of the rats fed the Zn-deficient diet. The value of $A$ was found to be correlated with the variability of the day-to-day feed intake of each rat. However, the value of this variability did not show the typical periodical feed-intake phenomenon of the rats fed the Zn-deficient diet. Therefore, we would like to propose the Cosinor method for the analysis of a cyclical pattern in the change of both feed intake and body weight. The value of $A$ for both cycles may provide a good characteristic index for anorexia.

From simulation of the feed-intake cycle (Fig. 2), the level of the daily feed consumption of rats fed the Zn-deficient diet was not greater than that of the Zn-adequate control rats. The maximum value of consumption in the cycle of rats fed the Zn-deficient diet was the same as the average feed intake of rats fed the Zn-adequate control diet. Therefore, the cyclical feed intake appears not to be caused by excess feed consumption, but rather by a periodic reduction in the rat's appetite.

The cyclic feeding behavior of rats fed the Zn-deficient diet was analyzed for both Zn-deficient and pair-fed animals (Fig. 2). The values of the parameters $M$, $A$, $\tau$ and $\phi$ of the body-weight change cycles did not differ between the two groups. Consumption of the Zn-adequate control diet by the pair-fed group neither diminished nor abolished the cyclicity of the body-weight change of rats fed the Zn-deficient diet. The cyclic variation of the body weight is likely the cause of the feed intake of rats fed Zn-deficient and pair-fed diets. As the difference of body weight between the peak and trough of rats fed the
Zn-deficient diet is about 17 g, we would like to propose the mesor value of the cycle for the evaluation of the weight gain of pair-fed rats, since there is a significant difference between the weight change of Zn-deficient and pair-fed rats (3, 8, 9, 15–17) and no significant difference under other conditions (5, 18).

The amplitude of the feed-intake cycle reflects the Zn deficiency and decreases with the amount of Zn injected (Fig. 3). However, the injection of Zn up to 47.4 µg/d into rats fed the Zn-deficient diet did not effect the serum level of Zn. These results suggest that the level of Zn in the serum reveals only the qualitative, and not quantitative deficiency of Zn in rats. Recently, Zhang and Allen (19) determined, by the dialysis method, that the free Zn concentration in plasma is 9.22 pg/mL. The ratio of free to total Zn in plasma was calculated to be $8.2 \times 10^{-6}$ in the control pair-fed rats (19). Free Zn$^{2+}$ may be important for mediating constant appetite or food consumption.

The daily Zn requirement for normalizing the feed intake could be calculated from the reduction in the amplitude of the feed-intake cycle and is as follows. The slope of the amplitude value in the feed-intake cycle against Zn injection was $-0.082$ g/µg (Fig. 3). From a previous report (12), the slope of the amplitude against Zn intake supplemented in a Zn-deficient diet is $-0.055$ g/µg. The presumable absorption of Zn could be roughly calculated from both slopes to be 67%. Mean Zn absorption in rats from wheat and chicken meat fed separately is 18.5 and 68.2%, respectively, and from a mixture of the two containing the same level is 50.1% (20). These results suggest that there is no gross difference of Zn absorption between severely-moderately Zn-deficient and normal-adequate Zn diets. If the value of the amplitude of the feed-intake cycle is 0, the content of Zn at the disappearance of the feed-intake cycle is assumed to be 71.6 ± 6.6 µg/d. The mean daily feed intake of the control diet was 15.4 g/d. The Zn content in the 15.4 g Zn-deficient diet was 14.6 µg. Therefore, the requirement for normalizing the feed intake of Zn from the diet in rats is calculated to be 121.6 ($=71.6/0.67 + 14.6$) µg/d.

From the above results, the amplitude values of the feed-intake and body-weight change cycles reveal the quantitative Zn-deficient status of rats. Thus, these values may facilitate the study of the physiological function of Zn.

REFERENCES

5) Chesters JK, Quarterman J. 1970. Effects of zinc deficiency on food intake and