Decreased Preference for Sucrose in Rats Fed a Low Protein Diet and Its Intensification by Zinc-Deficiency

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Summary This study examined whether protein concentrations in the diet of rats fed adequate Zn or deficient Zn affect their preference for disaccharides of sucrose and maltose. Sucrose and maltose were used as a source of carbohydrate and the selection patterns of rats were studied for 28 d by a two-choice selection method. Diets provided as a set of two either Zn-adequate or Zn-deficient diets containing 10, 20 and 40% protein each were changed in position daily. For the first 24 h, both the control Zn-adequate and Zn-deficient rats preferred sucrose to maltose and then gradually selected the maltose diet. Protein in the diet affected the selection of the disaccharides for both the control and Zn-deficient rats. The decrease order of the ratio of consumed sucrose to maltose over 28 d was 10% > 20% > 40% protein diet group, and Zn-deficiency emphasized the decrease. These results suggest that sucrose has a stronger taste effect than maltose in rats, but that the selection of sucrose is decreased by the post-ingestive effect which is stimulated in low-protein and Zn-deficient diets.

Key Words Zn deficiency, sucrose, maltose, low protein diet

The indication of zinc (Zn) deficiency in animals was first reported by Todd et al. (1) and followed by Follis et al. (2). Highly purified diets and elaborate analytical tools such as atomic absorption spectrophotometry and radioactive and stable isotopes have developed the study of physiological functions of Zn in rats. Zn-deficiency results in sharply reduced food intake, severe dermatitis, slow wound healing, severe tetrageneric abnormalities, and abnormal metabolisms of carbohydrate, lipid, and protein (3).

Food intake of Zn-deficient rats falls to about 70% of that of control rats (4, 5). Rats consume most of their energy during the dark phase of the light/dark cycle. Higher intake generally occurs just before the end of the dark phase (6, 7). When the food intake of rats is examined for periods of 2-h throughout the day, Zn-deficient rats are found to eat on fewer occasions than control rats. However, in those periods when the Zn-deficient rats do eat, the quantities eaten in 2 h showed the same distribution of weights as those of Zn-adequate rats (8).

When Zn-deficient rats were allowed to freely select from simultaneously provided carbohydrate-, protein-, and fat-rich diets, carbohydrate intake decreased in parallel with the reduction in total intake (9). When dextrin, maltose, sucrose, glucose or fructose were used as a source of carbohydrate in a diet, the preference for sugars of rats fed a Zn-deficient diet was somewhat different from that of rats fed a Zn-adequate diet, with a single diet method (10). On the selection of dextrin, maltose and glucose diet in the 3-choice method, control rats selected widely from the three diets throughout 28 d, while Zn-deficient rats selected exclusively and continuously the dextrin or dextrin and glucose diets (11). In the choice between maltose and sucrose, Zn-adequate control rats preferred sucrose while Zn-deficient rats preferred maltose to sucrose (11).

In the macronutrient selection patterns of rats analyzed using a 3-choice macronutrient selection over 28 d, protein intakes were not affected by Zn-deficiency (9). But when rats were given a choice of two isocaloric diets containing either 10 or 50% protein, the Zn-deprived rats preferred a diet with a lower proportion of protein than those fed diets adequate in Zn (12). When the diets were low in Zn, rats fed 5% protein diets consumed more food than those fed 20% protein diets (8). These results suggest that protein concentrations in the diet affect the preference for sugars. In this paper, we describe how protein concentration in the diet changed a preference in selection between sucrose and maltose diets with a 2-choice method in Zn-adequate control and the Zn-deficient rats.

MATERIALS AND METHODS

Animals. Male albino rats (Wistar/ST, 4 wk) were purchased from Japan SLC, Inc. (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 Co., Ltd., Tokyo, Japan) and given tap water with free access for 3 d before the experiment to allow acclimatization to their new environment. Acclimatized rats showing progressive weight

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gain were selected and separated into groups. Sixty rats were divided into three groups and they could eat diets containing 10, 20 and 40% protein. Rats in each group were separated into two subgroups of 10 rats each: one received Zn adequate diets and the other received Zn-deficient diets. Rats in each subgroup were allowed to select sucrose- and maltose-diets. The two diets were placed at fixed positions in the cage and were replaced daily. All rats were given the experimental diet and denoized water with free access.

Food intake and body weight were determined daily between 09:00 and 11:00. The rats were given the experimental diet for 4 wk. All procedures were performed in accordance with the Kobe Gakuin University Guide-lines for the Care and Use of Laboratory Animals.

Diets. The compositions of the Zn-adequate and Zn-deficient sucrose- and maltose-diets, containing 10, 20 and 40% protein are shown in Table 1. The composition of the Zn-deficient diet was the same as that of the Zn-adequate control diets with the exception that ZnCO₃ was omitted from the salt mixture. The content of Zn in each diet was evaluated from the mean values of three separate experiments. The contents of Zn in the Zn-adequate sucrose diets containing 10, 20, and 40% protein were 31, 31, and 32 mg/kg diet, respectively, while those in the Zn-deficient diets were 0.6, 1.2, and 2.0 mg/kg diet, respectively. The contents of Zn in the Zn-adequate maltose diets containing 10, 20, 40% protein were 30, 31, 32 mg/kg diet, respectively, while those in the Zn-deficient diets were 0.5, 0.9 and 2.0 mg/kg diet, respectively.

The diet was in powder form and contained in a 9 cm-diameter glass jar covered with a stainless steel lid containing nine 1.2 cm-diameter holes.

Zn content. A sample preparation for estimation of Zn content in the diets was performed as shown in a previous paper (11). The contents of Zn were estimated with a Hitachi Z-5300 Polarized Zeema Atomic Absorption Spectrophotometer (Hitachi Ltd., Tokyo, Japan).

Chemicals. All chemicals used were of analytical grade and were purchased from Nacalai Tesque, Inc. (Kyoto, Japan) unless otherwise stated. Animal feed was obtained from Oriental Yeast Co., Ltd. Sucrose and maltose used for dietary ingredients were in D-form and from Nacalai Tesque, Inc.

Evaluation of food intake and body-weight changes. The daily food intake and body-weight change of rats fed a Zn-deficient diet showed a cyclic variation. The data food intake and body-weight change were fitted to a cosine curve (14, 15). Food intake (F) and body weight change (ΔB) on day t were determined using the following equation:

\[
F (t, ΔB) = M + A \cos(2πt / τ + φ)
\]

where M, A, τ and φ represent the mesor (the rhythm-adjusted mean), amplitude (maximum and minimum values of the adjusted mean), period (length of one complete cycle) and acrophase (phase of minimum value), respectively. The experimental data were fitted to the above equation by the nonlinear least-squares method (16), and the four parameters, M, A, τ and φ, were calculated using subroutine analysis (17).

As the data from Zn-adequate rats were not fitted to the above equation, comparisons among groups were evaluated between mean values.

Statistical analysis. Values for food intake and body-weight change are expressed as mean±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 means was assessed using Tukey’s multiple comparison test and considered significant at p<0.05. Data were analyzed by the paired Student’s t-test to evaluate the significance in each group. For comparison between the two groups, the non-paired Student’s t-test was used.

RESULTS

Effect of protein on the food intake and body-weight change of rats fed either Zn-adequate or Zn-deficient diet

The protein used as a macronutrient was egg albu-

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>10% Protein</th>
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<th>20% Protein</th>
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<th>40% Protein</th>
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<tr>
<td></td>
<td>Zn-adequate</td>
<td>Zn-deficient</td>
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<td>Zn-deficient</td>
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<tr>
<td>Egg albumin</td>
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<td>200</td>
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<td>200</td>
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<td>432.486</td>
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<td>Soybean oil</td>
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<td>70</td>
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<td>70</td>
<td>70</td>
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<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
<td>Salt mixture (+Zn)³</td>
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<tr>
<td>Salt mixture (−Zn)³</td>
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<td>35</td>
<td>—</td>
<td>35</td>
<td>—</td>
<td>35</td>
</tr>
<tr>
<td>Cellulose powder</td>
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<td>50</td>
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<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
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<tr>
<td>Choline hydrogen tartrate</td>
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<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
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</tr>
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<td>tert-Butylhydroquinone</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
<td>0.014</td>
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</tbody>
</table>

¹ Carbohydrates used were maltose and sucrose.
² AIN-93G vitamin mixture (13) was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan).
³ AIN-93G mineral mixture (13) was purchased from Oriental Yeast Co., Ltd.
⁴ Minerals (−Zn) (g/kg diet): ZnCO₃ was omitted from AIN-93G mineral mixture (13).
min. and the food intake and body weight were estimated daily. The initial average body weights of the Zn-adequate rats fed diets containing 10, 20, and 40% protein were 144±7, 147±4, 146±9 g respectively, while those of the Zn-deficient rats were 145±7, 147±4, 149±8 g, respectively. The Zn-deficient rats showed typical symptoms of Zn deficiency such as alopecia, dermatitis of the paws, and anorexia with growth retardation, throughout 28 d.

From the average daily intake of diets by selection from the sucrose and maltose diets, the daily total diet intake was calculated and illustrated in Fig. 1A. The average total food intake of rats fed a Zn-deficient diet was significantly less than for the Zn-adequate diet, over broad protein concentrations from 10 to 40% in the diet. The increasing order of the total food intake of rats fed a Zn-adequate diet was 10% protein, 20% protein, and 40% protein each, progressively increased, while those from the Zn-deficient rats were 10% protein group was less than those of 20 and 40% protein groups of rats fed both Zn-adequate and Zn-deficient diets. Then the Zn-deficient rats or Zn-adequate rats as well as Zn-adequate and Zn-deficient rats are illustrated in Fig. 2. The statistical evaluations for percentage of the consumed sucrose diet at 1 d, 2 d and over 28 d are shown in Fig. 3. The preference for sucrose decreased with time in the Zn-adequate control and Zn-deficient rats. Compared with the 10% protein group, the 20 and 40% protein groups showed less sucrose preference to maltose with time for Zn-deficient as well as Zn-adequate rats (Figs. 2 and 3).

On the first day, the 10, 20, and 40% protein groups of rats fed Zn-deficient as well as Zn-adequate diets showed a remarkable preference for the sucrose diet. The percentage of consumed sucrose diet was not different between the Zn-adequate and Zn-deficient rats in the 10, 20 or 40% protein groups at the onset of the first day (Fig. 3). However, the ratio of the consumed sucrose diet in the 10% protein group was less than those of 20 and 40% protein groups of rats fed both Zn-adequate and Zn-deficient diets. Then the Zn-deficient rats as well as Zn-adequate rats changed their sucrose preference to maltose with time (Figs. 2 and 3). An exponential decrease in the selection of the sucrose diet was found in the 10% protein group of rats fed both the Zn-adequate and Zn-deficient diets (Fig. 2). Over 28 d, a high concentration of protein in the diet prevented a decreasing sucrose preference. Moreover, the Zn-deficiency decreased the preference for sucrose to maltose with time (Figs. 2 and 3). The ratio of the consumed sucrose to maltose in the Zn-deficient rats was decreased more than that of the Zn-adequate rats in the 20 and 40% groups (Fig. 3C).
Food intake

The day-to-day variations in the total food intake of rats fed a Zn-adequate diet containing 10, 20 and 40% protein were 1.2±0.3, 1.3±0.3 and 1.4±0.2 g/d, respectively, and those of rats fed a Zn-deficient diet were 4.5±0.8b, 4.3±0.5a and 3.4±0.8a g/d, respectively (p<0.05). The variations in the food intake of Zn-deficient rats were significantly greater than those of Zn-adequate rats in the corresponding protein groups. The high value of the day-to-day variation results on a Cosinor curve of the daily food intake of the Zn-deficient rats was consistent with the previous paper (18). The four parameters of the mean values of the cycle of food intake in the Zn-deficient rats were calculated and are illustrated in Table 2. The body-weight changes in the previous 24-h period for each rat were also fitted to a Cosinor curve (Table 2). The increased order in mean food intake value of the cycle was 10%>40%>20% protein groups. But the mean values of the body-weight change cycle were not different among the three protein groups. The amplitude (A) values of the food intake and body-weight change cycles in the 40% protein group were the least among the groups. The period of the body-weight change cycles followed that of the food intake cycles.

**DISCUSSION**

Rodents have separate taste channels, one for polysaccharides and one for sucrose and other sugars (19, 20). Moreover, Harper and Spivey (21), and Weller et al. (22) found an inverse relationship between the osmotic pressure exerted by a carbohydrate and food intake. When sucrose and maltose was used as a source of carbohydrate in a diet, the food intake of rats fed a Zn-adequate diet was not different between sucrose and maltose groups, but that of rats fed a Zn-deficient diet was higher in the maltose group than in the sucrose group (10). On the other hand, Mori et al. (23) proposed that the animals’ primary concern was energy intake and their secondary concern was protein nutrition, regardless of flavoring. In the present study, we found that Zn-deficient rats as well as Zn-adequate control rats remarkably changed their preference from sucrose to maltose when fed a low protein diet.

Recently, the coupled proteins of T1R2 and T1R3 were isolated and found to function as a sweet-responsive receptor (24–28). Sucrose is a more potent stimulus than maltose for the responses from the chorda tymai nerve (28–30). Therefore, Zn-deficient rats as

**Table 2. Effect of dietary proteins on parameters of food intake and body-weight change-cycles of Zn-deficient rats selectively fed Zn-deficient sucrose- and maltose-diets.**

<table>
<thead>
<tr>
<th>Diet</th>
<th>M (g/d)</th>
<th>A (g/d)</th>
<th>τ (d)</th>
<th>φ (radian)</th>
</tr>
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<tbody>
<tr>
<td>Food intake</td>
<td></td>
<td></td>
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<tr>
<td>10% Protein</td>
<td>11.2±0.7a</td>
<td>5.2±1.0b</td>
<td>3.9±0.2b</td>
<td>1.9±0.6a</td>
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<tr>
<td>20% Protein</td>
<td>9.1±0.7a</td>
<td>4.6±0.7b</td>
<td>3.6±0.2b</td>
<td>2.3±1.1a</td>
</tr>
<tr>
<td>40% Protein</td>
<td>10.4±0.8b</td>
<td>2.8±0.6a</td>
<td>3.8±0.3b</td>
<td>3.3±1.2b</td>
</tr>
<tr>
<td>Body-weight change</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>10% Protein</td>
<td>0.6±0.3</td>
<td>6.9±1.4b</td>
<td>3.9±0.2b</td>
<td>2.2±0.5a</td>
</tr>
<tr>
<td>20% Protein</td>
<td>0.8±0.3</td>
<td>6.6±0.9b</td>
<td>3.6±0.2a</td>
<td>2.3±1.2a</td>
</tr>
<tr>
<td>40% Protein</td>
<td>0.8±0.5</td>
<td>5.5±1.0a</td>
<td>3.8±0.3b</td>
<td>4.0±1.4b</td>
</tr>
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Each rat was fed the experimental diet for 28 d under the conditions described in "Materials and Methods." Food intake (F) or body-weight change (ΔB) in the previous 24-h period at day t: F (or ΔB)=M+Acos(2πt/τ+φ). Each value is the mean±SD. Values in column not sharing a common superscript letter are significantly different (p<0.05).

**Fig. 3.** Percentage of sucrose diet selection in rats given a choice between sucrose and maltose Zn-adequate (open column) or Zn-deficient (closed column) diets for a short and long term. (A), (B) and (C) mean the value at the 1st day, at the 2nd day and over 28 d, respectively. The values are shown as mean with standard deviation. The mean value was significantly different from that of the Zn-adequate group, p<0.01. The mean value was significantly different from that of the percentage of consumed maltose diet, p<0.05 and p<0.01, respectively. Values in each column not sharing a common superscript letter are significantly different, p<0.05, 10% protein, 20% protein and 40% protein mean protein concentration in the diet.
well as Zn-adequate rats may have exclusively preferred sucrose to maltose at day 1 after the beginning of the selection of the sucrose and maltose, regardless of the protein concentration in the diet, and thereafter their selection of maltose increased. These results mean that the taste effect induces a preference for sucrose over maltose in both Zn-adequate and Zn-deficient rats and that the post-ingestive effect changes their preference for sucrose. The post-ingestive effect may be effective in a low protein diet from day 1, so the percentage of the consumed sucrose diet in 10% protein group was less than in the 20 and 40% protein groups.

The decreasing degree of sucrose preference to maltose with time in control rats was 10%−20%−40% protein groups in increasing order (Figs. 2 and 3C). It is well known that glucose is a source of non-essential amino acid-related glycolysis (31, 32). Fructose is catabolized by ketohexokinase, induces hepatic insulin resistance (33), increases intrahepato cellular lipid (34) and stimulates hepatic lipogenesis (34). Therefore, rats fed a low protein diet may select maltose earlier than rats fed a 20 or 40% protein diet and may show sustained weight gain (Fig. 1B). The increased selection of the maltose diet in the 10% protein group may reduce the disadvantage of sucrose as a macronutrient by the post-ingestive effect.

The selection of the sucrose diet increased with an increased protein concentration in the diet for the Zn-adequate as well as the Zn-deficient rats (Fig. 3C). The weight gain in the Zn-adequate and Zn-deficient rats showed no significant difference among 10, 20 and 40% protein groups (Fig. 1B). Therefore, the selection of sucrose or maltose diet might not affect the weight gain over 28 d. The increasing order of the daily total food intake from sucrose and maltose diets was 10% 40% 20% protein groups in both the Zn-adequate and Zn-deficient rats (Fig. 1A), but the weight gain was not different among the groups (Fig. 1B). These results show that 20% protein in the diet is more efficient than 10 or 40% proteins, for weight gain of weanling rats.

The total daily food intake and body-weight change in the Zn-deficient rats showed a cyclic variation. Although the preference for sucrose changed with time, the food intake of Zn-deficient rats showed a fixed cycle. The cycles of the food intake and body-weight change may be determined by factors other than preference for sugars. The values of amplitude of the food intake and the body-weight change cycles of rats fed a Zn-deficient diet containing 40% protein were less than those of the rats fed a Zn-deficient diet containing 10 or 20% proteins. When dextrin was used as a source of carbohydrate in Zn-deficient diets, a high-protein diet decreased the decreased the amplitude of food intake cycle (35). Therefore, not the preference for sugars but an amino acid pool or metabolites of amino acids may affect the cycles of food intake and body-weight change cycles. Analyses of the parameters of the food intake and body-weight change cycles of Zn-deficient rats may be a useful tool for the study of anorexia induced by Zn-deficiency.

REFERENCES


