Preference for Glucose in Zn-Deficient Rats Selecting from Glucose and Fructose Diets

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(Received March 19, 2004)

Summary Glucose and fructose selection patterns of rats were analyzed for 28 d by a 2-choice selection in either Zn-adequate or Zn-deficient status. In this paper, we describe the following serial studies: (1) For the first 24 h, rats fed a Zn-deficient diet preferred a fructose-diet compared with a glucose-diet. On and after the third day, rats fed both Zn-adequate and Zn-deficient diets preferred the glucose-diet. (2) Throughout the experimental period, many of the rats fed a Zn-adequate and Zn-deficient diet continuously selected one diet. (3) Some of the rats fed a Zn-adequate and Zn-deficient diet suddenly changed preference for the glucose-diet or the fructose-diet. (4) The sum of daily glucose- and fructose-diet intake in rats fed a Zn-deficient diet showed a characteristic variation with the cyclic period of 3.9±0.4 d.

Key Words zinc deficiency, food intake cycle, glucose, fructose

It has been well established that zinc (Zn) deficiency causes anorexia, growth retardation, reproductive dysfunction, alopecia, dermatitis, slow wound healing, delayed sexual development and function, and reduced immune competence (1, 2). Reduction in food intake was accompanied by cyclical patterns of food intake in rats fed a Zn-deficient diet (3–10). Mills et al. (3) suggested originally that rats fed a Zn-deficient diet develop a characteristic cyclical pattern in food intake, which has a frequency of 3.5–4 d. This cyclical variation in food intake in rats fed a Zn-deficient diet followed a Cosinor curve, as determined by computer analysis (11–13).

The values of amplitude for the food-intake cycle were positively correlated to their own day-to-day variations and to the correlation value of their simulated cycles (13). The cyclical variation in food intake in rats was accompanied by a cyclical variation in body-weight in rats fed a Zn-deficient diet, which also occurred in pair-fed control rats (13). As the maximum value of consumption in rats fed the Zn-deficient diet was the same as that of rats fed the Zn-adequate control diet, the cyclical pattern of food intake appeared not to be caused by excess food consumption, but rather by a periodic reduction in the rats’ appetite (13). The amplitude of food intake in rats fed a Zn-deficient diet increased with the protein content in their diet up to 20%, then decreased with further increases in protein level (14).

On the other hand, Rains and Shay (15) investigated differences in macronutrient preferences in Zn-deficient and Zn-adequate rats in a long-term (28-d) study using complete macronutrient selection by simultaneously providing each animal with three different diets, each consisting mainly of either fat, protein or carbohydrate. As carbohydrate intake decreased in Zn-deficient rats compared to the reduction in total intake, the intake of protein and fat on an energy percentage basis increased. When dextrin, maltose, sucrose, glucose, and fructose were used as carbohydrate sources in a Zn-deficient diet, the food intake of the dextrin group was the highest (16). Though the molecular formula of fructose is the same as that of glucose, the food intake of the fructose group was less than that of the glucose group. However, the preference between glucose- and fructose-diets could not be clarified from the previous paper (16).

This paper describes differences in monosaccharide preference in Zn-deficient and Zn-adequate rats provided two different glucose- and fructose-diets for 28 d. We found a characteristic cyclical variation in the sum of glucose- and fructose-diet intake in rats fed a Zn-deficient diet.

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 (Original Yeast Ltd., Tokyo, Japan) and given tap water ad libitum for 1 wk before the experiment to allow for acclimatization to the new environment. Food intake and body weight were determined daily between 09:00 and 11:00. The rats were given the experimental diet for 4 wk, then sacrificed between 09:00 and 11:00 under anesthesia with diethyl ether. All procedures were performed in accordance with the Kobe Gakuen University Guidelines for the Care and Use of Laboratory Animals.

Diet. The formulation of the Zn-deficient glucose-
and fructose-diets, and the Zn-adequate control glucose- and fructose-diets has been published previously (16). The contents of Zn in the Zn-deficient glucose and fructose diets from the mean values of the three separate experiments were 0.75 and 0.81 mg/kg, respectively, while those in the Zn-adequate glucose and fructose diets were 18.6 and 18.5 mg/kg, respectively.

Each rat was simultaneously and continuously provided with two diets, one containing glucose and the other containing fructose. Diets were provided as set of two Zn-adequate control diets or two Zn-deficient diets. Two diet glass jars were placed in a fixed position in the cage and were replaced daily. All rats were given the experimental diet and deionized water ad libitum.

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 Ltd. Glucose and fructose were from Nacalai Tesque, as was the d-form.

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, as shown in the previous paper (16).

Evaluation of food intake and body-weight change. Food intake and body weight change data from Zn-deficient rats were analyzed by the "Cosinor" method (11-13). Food intake (F) and body weight change (ΔW) on day t were determined using the following equation:

\[ F(t) + \Delta W = M + A \cos(2 \pi t / T + \alpha) \]

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 was fit to the above equation by the nonlinear least-squares method, and the four parameters, M, A, τ, and φ, were calculated using subroutine analysis (17, 18).

On the other hand, as the data from Zn-adequate rats was not fit to the above equation, comparison between the groups was evaluated between the mean variation in daily food intake.

Statistical analysis. Value for food intake, weight gain and body weight change are expressed as mean±SD, except where otherwise indicated. Data were analyzed by a paired Student's t test to evaluate the significance in the group. For comparison between the two groups, a Student's t test was used.

RESULTS

Weight gain of rats fed a Zn-adequate diet and a Zn-deficient diet

The initial average body weights of the Zn-adequate and Zn-deficient groups were 135±3 g and 137±5 g, respectively. The Zn-deficient rats showed typical symptoms of Zn deficiency such as alopecia, depigmentation of the hair, dermatitis of the paws and growth retardation. Figure 1 shows the average values of weight gain in each group. In the two-choice method for selecting separate glucose- and fructose-containing diets, the weight gain of the rats fed a Zn-deficient diet was retarded, as shown in rats fed a Zn-deficient dextrin-containing diet (11). The body weight change in rats fed a control and Zn-deficient diet was 3.7±0.4 and 0.6±0.5 g/d, respectively (p<0.01).

The mean Zn concentrations of the serum at 28 d in the control and the Zn-deficient rats were 2.30±0.33 and 0.62±0.21 mg/L, respectively (p<0.01).

Selection of glucose- and fructose-diets in Zn-adequate and Zn-deficient rats

Daily selected food intakes from glucose- and fructose-diets for 28 d by Zn-adequate control and Zn-deficient rats, respectively, are illustrated in Figs. 2 and 3. Rats fed a Zn-deficient preferred the fructose-diet on the first day and then changed their preference to the glucose diet (Table 1). On the other hand, the Zn-adequate control group ate equal amounts of the glucose- and fructose-diets. Most rats in both the Zn-adequate and Zn-deficient groups changed preference to the glucose diet at day 3 (Table 1). Though the two diet jars of the glucose- and fructose-diets were replaced daily, the rats fed a Zn-adequate and Zn-deficient diet continuously selected one diet (Figs. 2 and 3). Therefore, both rats fed a Zn-adequate and a Zn-deficient diet could discriminate between the glucose- and fructose-diets.

Rat 10, fed a Zn-adequate diet, continuously preferred the fructose-diet until day 9, then changed its preference to the glucose diet. Rat 14, fed a Zn-deficient diet, continuously preferred the glucose-diet until day 18, then changed its preference to the fructose diet. On the other hand, Rat 15, fed a Zn-deficient diet, preferred the fructose-diet until day 11, then changed its preference to the glucose-diet. Average daily intake of the glucose-diet, fructose-diet and total diet intake was calculated from the figures (Fig. 4). Rats fed both a Zn-adequate and a Zn-deficient diet preferred the glucose-diet to the fructose-diet. The selected glucose diets in rats fed a Zn-adequate and a Zn-deficient diet were 87% and 81%, respectively. There were significant differences in the values of selection of the glucose diet between Zn-adequate and Zn-deficient groups, but not
in the fructose diet. The total food intake of the glucose-
and fructose-diets in rats fed a Zn-deficient diet was sig-
nificantly lower than in rats fed a Zn-adequate control
diet.

Food intake and body-weight change cycles in rats selecting
glucose- and fructose-containing diets
The daily total food intake in Zn-deficient rats fed glu-
cose- and fructose-containing diets was well fit to a Cos-
inor curve (Table 2). The four parameters of the mean
values of cycle were calculated and illustrated (Table 2).
The daily body-weight change was also fitted to a Cos-
inor curve (not shown) and the values of the four
parameters as follows: M, 0.7±0.2 g/d; A, 5.3±0.4 g/
d; τ, 4.0±0.1 d; φ, 1.7±0.2 radian. But neither the
total food intake nor body-weight change in the Zn-ade-
quate rats were correlated to a Cosinor curve. The max-
imum and minimum values of the food intake cycle
were calculated as 12.3 and 5.1 g/d, respectively. The
maximum value of the food intake cycle did not over-
come the mean value (15.0±0.8 g/d) of food intake for
28 d in rats fed a Zn-adequate diet. The daily body-
weight changes varied from 6.0 g/d to −4.6 g/d. The
cyclical period of food intake was 3.9 d and was the
same as that of the body-weight change cycle. These
results suggest that body weight changes follow food
intake as shown in previous papers (11-13). The fruc-
tose-diet selected daily in Zn-deficient rats did not show
any signs of cyclical variation in food intake and the
selected glucose-diet diminished the correlation to the
Cosinor curves, compared to the sum of both the glu-
cose- and fructose-diets.

DISCUSSION
Weight gain in rats fed a Zn-adequate diet increased
progressively, whereas that in rats fed a Zn-deficient diet
was retarded with gross standard deviations on the
fourth day after feeding on the experimental diet (Fig.
1). The plasma Zn concentration in rats fell by approxi-
mately 40% after 1 d on the Zn-deficient diet (19). The
anorexia in rats fed the Zn-deficient diet may have been
cauased in the early stages after feeding on the experi-
mental diet. The consumption of food in rats fed a Zn-
deficient diet was less than that of Zn-adequate control
rats from day 1 (Table 1). Rats may consider the Zn-defi-
cient diet unsavory and thus their appetite may dimin-
ish. But the initiation and degree of anorexia may be
different among rats fed a Zn-deficient diet. Therefore,
the food intake in rats fed the Zn-deficient diet showed a
cyclical variation, but had different acrophase values
(Table 2), and the standard deviation of body weight

Fig. 2. Daily intake of Zn-adequate glucose- and fructose-diets over time. Rats 1 to 10 were simultaneously and continu-
ously provided with two Zn-adequate diets, one containing glucose (○) and the other fructose (△).
Gain in Zn-deficient rats was greater than that of the control rats, in the early stage (Fig. 1).

In this paper we found the cyclical retardation of food intake of rats fed Zn-deficient glucose- and fructose-diets selectively, as shown in the single diet method of glucose or fructose. Neuropeptide Y is a potent activator to stimulate feeding in the rats (20–22). Both hypothalamic concentrations of neuropeptide Y and neuropeptide mRNA are higher in Zn-deficient rats than in Zn-adequate rats (23, 24). The exogenous administration of neuropeptide Y to the paraventricular nucleus increases food intake of rats both Zn-deficient and Zn-adequate diets (24). No significant difference of hypothalamic neuropeptide Y and the neuropeptide mRNA concentration was observed between the Zn-deficient rats sacrificed at peak or though of food intake (24).
These results could not explain the function of neuropeptide Y on the cyclical variation of food intake of rats fed a Zn-deficient diet. In a previous paper (16) we found that food intake of a fructose-diet was less than that of a glucose-diet in Zn-deficient diet rats as well as Zn-adequate rats. However, the single diet intake method of glucose or fructose could not explain the preference between the two diets. We adapted the selecting method from separate glucose- and fructose-diets. Rats fed a Zn-adequate diet selected the same amount of glucose- and fructose-diets on the first day after changing to the experimental diet form the laboratory diet. More rats fed a Zn-deficient diet preferred the fructose diet than the glucose diet on the first day. However, beyond the third day, both groups of rats changed their preference to glucose over fructose (Table 1). Fructose feeding appears to interfere with glucose utilization in vivo, inducing an insulin resistant state (25). Overproduction of apolipoprotein is found after chronic fructose feeding for 3 wk, but not observed in short-term (2 d) fructose feeding (26). Zn deficiency increases serum total lipids, cholesterol, triglycerides and low density lipoprotein, and decreases serum high density lipoprotein (27). As glucose has the advantage of maintaining homeostasis in living cells, the zinc-deficient rats may change and select the glucose diet over the fructose diet. The exact explanation of these phenomena await further investigation.

In this paper, the decrease in total food intake in Zn-deficient rats selecting from separate glucose- and fructose-diets followed the decrease in the glucose-diet (Table 2 and Fig. 4). The ratios of the fructose-diet intake to the total diet intake in rats fed Zn-adequate and Zn-deficient diets were calculated to be 13% and 19%, respectively. These results suggest that the preference for glucose in rats fed a Zn-adequate diet did not change with Zn-deficient status. Moreover, with selection from glucose- and fructose-diets appeared the cyclical variation in food intake in rats fed a Zn-deficient diet.

The position of the glucose- and fructose-diet glass jars changed. However, rats fed a Zn-deficient diet as well as those fed a Zn-adequate diet could make their own selection from glucose- and fructose-diets. But a few of the rats fed Zn-adequate and Zn-deficient diets suddenly changed preference for glucose or fructose, as shown in rats 10, 14 and 15 (Figs. 2 and 3). These phenomena could not be merely explained by differences in the metabolism of glucose and fructose, but as shown by Ruxton (28), further study of sugars could resolve these problems.

**Table 2. Rhythmetronic summary of Cosinor analysis of food intake of rats selectively fed Zn-deficient glucose- and fructose-diets.**

<table>
<thead>
<tr>
<th>Rat</th>
<th>$M$ (g/d)</th>
<th>$A$ (g/d)</th>
<th>$\tau$ (d)</th>
<th>$\phi$ (radian)</th>
<th>$r^2$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>8.2±0.4</td>
<td>3.0±0.5</td>
<td>3.7±0.05</td>
<td>1.7±0.4</td>
<td>0.612</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>12</td>
<td>8.0±0.4</td>
<td>4.4±0.5</td>
<td>3.9±0.04</td>
<td>1.9±0.3</td>
<td>0.745</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>13</td>
<td>8.1±0.3</td>
<td>2.9±0.4</td>
<td>3.5±0.04</td>
<td>0.7±0.3</td>
<td>0.612</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>14</td>
<td>7.2±0.3</td>
<td>4.0±0.5</td>
<td>4.2±0.04</td>
<td>2.6±0.3</td>
<td>0.733</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>15</td>
<td>9.5±0.4</td>
<td>5.8±0.5</td>
<td>3.9±0.03</td>
<td>2.5±0.2</td>
<td>0.812</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>16</td>
<td>9.7±0.3</td>
<td>3.3±0.4</td>
<td>4.0±0.04</td>
<td>0.7±0.2</td>
<td>0.731</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>17</td>
<td>9.4±0.4</td>
<td>2.8±0.5</td>
<td>3.9±0.06</td>
<td>1.6±0.4</td>
<td>0.545</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>18</td>
<td>9.3±0.4</td>
<td>3.2±0.5</td>
<td>4.7±0.07</td>
<td>0.7±0.3</td>
<td>0.608</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>19</td>
<td>8.9±0.6</td>
<td>3.5±0.8</td>
<td>3.6±0.06</td>
<td>0.5±0.5</td>
<td>0.437</td>
<td>&lt;0.0002</td>
</tr>
<tr>
<td>20</td>
<td>8.4±0.3</td>
<td>3.4±0.4</td>
<td>3.8±0.04</td>
<td>1.0±0.3</td>
<td>0.668</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Average 8.7±0.8 3.6±0.9 3.9±0.4 1.4±0.8

*Food intake $(F)$ in the previous 24-h period at day $t$: $F=M \cos(2 \pi t/\tau+\phi)$. Each value is the mean ± SD.*
REFERENCES


