Zinc’s Role in Rat Preference for a Low-Fat Diet in a Two-Choice Diet Program of Low- and High-Fat Diets

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Summary To investigate the change in preference for a low-fat diet (LFD) and a high-fat diet (HFD) under disorders induced by a zinc (Zn)-deficiency, two groups of 4-wk-old male rats were fed a two-choice diet of Zn-deficient (ZnD; 0.75 mg/kg) and Zn-adequate (ZnA; 30.75 mg/kg) LFD and HFD. After 21 d, 10 rats in each of the two groups were sacrificed. The remaining ZnD rats were switched to ZnA diets for 7 d. Intakes of the LFD and the HFD were measured to determine the diet preferences of the ZnD, the ZnA and the Zn-recovered groups. Energy intake of the ZnD group was significantly lower than that of the ZnA group and showed cyclical 3- to 4-d patterns of decrease. In the ZnD group, although the LFD intake decreased parallel to the reduction in the energy intake, the HFD intake did not show the cyclical pattern of decrease. The reduced intake of the LFD in the ZnD rats was accompanied by a low carbohydrate intake and a low plasma insulin concentration. When the ZnD group recovered sufficient amounts of Zn, the energy intake was restored to normal levels and the difference in the LFD intake and the plasma insulin concentration disappeared between the ZnD and the ZnA groups. It was supposed that the specific change in the LFD intake patterns during development and recovery from Zn-deficiency might be related to Zn-mediated changes in impaired synthesis and the release of insulin from the pancreas.

Key Words zinc-deficiency, self-selection, preference for a low-fat diet, two-choice diet program, high-fat diet

Zinc (Zn) is essential for animals and humans, and is required for many physiological functions. A Zn-deficiency in both animals and humans causes anorexia, a taste disorder, a poor appetite, impaired growth and dermatitis (1–3). Within 3 to 5 d after giving rats a Zn-deficient (ZnD) diet, food intake is suppressed (3, 4). This decrease in food intake is the first sign of Zn-deficiency, followed by other symptoms associated with Zn-deficiency (3). Therefore, Zn may play an important role in the regulation of food intake.

From a whole-body perspective, ZnD rats have significantly reduced body weight and carcass fat, and lower circulating leptin and insulin concentrations that play key roles in energy nutrient metabolism compared to the Zn-adequate (ZnA) rats (4–7). Leptin is produced by adipose tissue and has potent effects on body weight and food intake regulation. Another adiposity signal is insulin, which is the controller of blood glucose levels, and chronically high levels of insulin consequently lead to obesity (8). Arora and Anubhuti reported that leptin and insulin are ideal candidates for lipogenic function because their levels are closely related to adiposity, and their central administration decreases food intake (9). However, feeding is a complex behavior and the mechanisms by which Zn-deficiency induces anorexia are unclear. Zn-deficiency results in a reduction of food intake, suggesting that Zn status may affect the complex mechanisms underlying normal food intake.

In a previous paper, we reported that when rats were placed on a self-selection regimen of a low-fat diet (LFD) and one of three kinds of high-fat diets (HFD) containing lard, soybean-oil or fish-oil, preference for the lard HFD was higher than that for the soybean-oil HFD and the fish-oil HFD. In addition, the fish-oil HFD guarded against the overeating of fats (10). Although fish-oil is nutritionally less desirable than lard and soybean-oil, when rats were placed on a self-selection regimen of a lard diet and a fish-oil diet they had the ability to consume both diets to obtain an adequate ratio of essential fatty acids (11, 12). We also reported that the lard HFD had food properties preferable to those of the LFD because energy density (kcal/g) of the HFD is higher than that of the LFD (13). Furthermore, Cunnane and Yang reported that Zn-deficiency has been shown to alter metabolism of essential fatty acids (FAs) with increased β-oxidation and greater utilization of linoleic acid in the de novo lipid synthesis (14). This may affect the amount and distribution of FAs in fat stores. As Zn-deficiency alters FA metabolism, we were particularly interested in examining whether preference for the lard HFD, which is associated with higher calorie intake as well as increased body weight and fat tissue deposition, was affected by the Zn status in rats.

The objective of this study was to investigate the change of preference for the HFD and the LFD in rats under structural and functional disorders induced by a

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Zn-deficiency. By observing preference for HFD or LFD during development and recovery from Zn-deficiency, we aim to provide evidence that contributes to a better understanding of anorexia attributed to consumption of a ZnD diet.

**MATERIALS AND METHODS**

*Animals and diets.* Four-week-old male Fisher 344 rats were commercially obtained from CLEA Japan, Inc. (Tokyo, Japan). They were housed individually in stainless steel cages in a room kept at 23±1°C with 50% humidity and illuminated with a 12-h light (7:00–19:00)/12-h dark (19:00–7:00) cycle. They had free access to food and de-ionized water. They were weighed and food intake was measured every day to determine daily intake.

The composition of the powdered ZnD diet is shown in Table 1. Dietary components were obtained from Oriental Yeast Co., Ltd. (Tokyo, Japan). Basically, the amount of soybean-oil in the LFD was reduced to 1/2 that in the AIN-93G, and lard (14%) was added to the HFD, substituting for cornstarch. The LFD and the HFD contained soybean-oil (3.5%), and soybean-oil (7%) and lard (14%), respectively. The protein energy ratio of both the LFD and the HFD was prepared to be the same as the AIN-93G diet. Therefore, the LFD and the HFD contained 20% and 25% casein, respectively. ZnCO$_3$ was omitted from the AIN-93G mineral mixture. Thirty mg/kg of Zn was added to the ZnD diet to make the ZnA diet. The ZnD diet and the ZnA diet contained 0.75 and 30.75 mg Zn/kg, respectively.

All procedures were performed in accordance with the Animal Experimentation Guidelines of the Laboratory Animal Care Committee of Seitoku University.

**Experimental design.** Thirty-two 4-wk-old male rats were fed the AIN-93G (15) diet containing 7% soybean-oil for 3 d before the experiment to allow acclimatization to their new environment. Then, the rats were divided into two equal groups (ZnD and ZnA groups). All rats were placed on a two-choice diet program in which they self-selected from two food cups. The ZnP group received the ZnD-LFD and the ZnD-HFD and the ZnA group received the ZnA-LFD and the ZnA-HFD during the experimental period. After 21 d of the experimental period, 10 rats in each of the two groups were sacrificed. The remaining ZnP rats recovered sufficient amounts of Zn when they were switched to the ZnA-LFD and the ZnA-HFD, whereas the ZnA group continued on the two ZnA diets for 7 d (Zn recovery period). After the Zn recovery period, all rats were anesthetized without fasting after the feeding period. Blood was col-

**Table 1. Composition of experimental diets.**

| Ingredient          | LFD  | HFD  |
|---------------------|------|------|
| Casein              | 20.0 | 25.0 |
| l-Cystine           | 0.3  | 0.4  |
| Cornstarch          | 56.45| 37.25|
| Sucrose             | 10.0 | 6.6  |
| Soybean oil         | 3.5  | 7.0  |
| Lard                | —    | 14.0 |
| Cellulose           | 5.0  | 5.0  |
| Mineral mixture     | 3.5  | 3.5  |
| Vitamin mixture     | 1.0  | 1.0  |
| Choline bitartrate  | 0.25 | 0.25 |
| tert-Butylhydroquinone | 0.0014 | 0.0014 |

| Energy (kcal/g)     | 3.7  | 4.6  |
| Protein energy ratio (%) | 20.8 | 20.8 |
| Fat energy ratio (%) | 9.1  | 42.4 |
| Carbohydrate energy ratio (%) | 70.2 | 36.8 |

1The composition of all ingredients is given in grams per 100 g of diet.
2The mineral mixture and the vitamin mixture were based on AIN-93G formulation (15). ZnCO$_3$ was omitted from the AIN-93G mineral mixture.

**Table 2. Food intake, body and perirenal fat tissue weights and plasma Zn, lipid and hormone concentrations of ZnP and ZnA rats self-selecting HFD and LFD for 21 d.**

| Group                  | ZnA               | ZnP               |
|------------------------|-------------------|-------------------|
| Energy intake (kcal/21 d) | 933±45            | 617±24**          |
| HFD intake (g/21 d)     | 124±8             | 101±9*            |
| LFD intake (g/21 d)     | 98±7              | 41±16**           |
| Ratio of HFD intake (%) | 55.9±7            | 71.1±14*          |
| Body weight (g)         | 145±5             | 89±4**            |
| Perirenal fat tissue weight (g/100 g) | 0.68±0.16 | 0.12±0.02** |
| Plasma                 |                   |                   |
| Zn (µg/dL)             | 298±41            | 103±33**          |
| Triacylglycerol (mg/dL) | 127±25            | 29±10**           |
| Total-cholesterol (mg/dL) | 92±12       | 77±18             |
| Glucose (mg/dL)        | 179±31            | 148±17            |
| Insulin (ng/mL)        | 2.33±0.74         | 1.38±0.25*        |
| Leptin (ng/mL)         | 2.47±0.29         | 1.44±0.18**       |

Values represent mean±SD (n=10). Significantly different from ZnA, *p<0.05, **p<0.01. Zn: zinc, ZnD: zinc-deficient, ZnA: zinc-adequate, HFD: high-fat diet, LFD: low-fat diet.
lected by heart puncture with a heparinized syringe. After centrifugation (1,000 × g, 30 min), plasma was removed and stored at −80°C until analysis. Perirenal fat tissue was removed and weighed.

**Analytical methods.** Triacylglycerol (TG), total-cholesterol (T-cho), Zn, insulin and leptin concentrations in plasma samples were measured using test kits (TG: Triglyceride E-test Wako, T-cho: Cholesterol E-test Wako, Zn: Zn-test Wako, Insulin: Rat Insulin ELISA Kit, Leptin: Rat Leptin ELISA Kit) (Wako Pure Chemical Industries, Ltd., Osaka, Japan).

**Statistical analysis.** Values were expressed as the mean±SD. For comparison between the groups fed the ZnD diet and the ZnA diets, the non-paired Student’s t-test was used. The paired Student’s t-test was used to evaluate significance in each group. Differences were considered significant at p<0.05.

**RESULTS**

During the experimental period, rats that consumed the ZnD-LFD and the ZnD-HFD showed typical symptoms of Zn-deficiency, such as sparse and coarse hair, poor appetite and limited movements. However, rats that consumed the ZnA-LFD and the ZnA-HFD appeared to be healthy. Plasma-Zn concentration of the ZnD rats was significantly lower than that of the ZnA rats (p<0.01) (Table 2).

Energy intake during the 21-d experimental period of the ZnD group was about 34% lower than that of the ZnA group (p<0.01) (Table 2). At the end of the experimental period, body and perirenal fat tissue weights of the ZnD group were lower than those of the ZnA group (p<0.01). The HFD and the LFD intakes during the experimental period of the ZnD group were lower than those of the ZnA group (p<0.05 and p<0.01, respectively). The ratio of the HFD intake [HFD intake (g)/total intake (g)] in the ZnD group was significantly lower than that in the ZnA group (p<0.05).

Daily energy and food intakes of the ZnA and the ZnD groups selected from a two-choice diet of the LFD and the HFD are shown in Fig. 1. For the ZnD groups, the energy intake began to decrease 4 d after being fed the ZnD diet and a temporary decrease occurred on day 5 (Fig. 1A). Thereafter, energy intake of the ZnD group recovered nearly to that of the ZnA group on day 6, and showed a cyclical 3- to 4-d pattern of decreased energy consumption after feeding on the ZnD diet. However, energy and food intakes of the ZnA group did not show the cyclical pattern (Fig. 1A and B). Although the HFD intake of the ZnD group did not show the cyclical pattern, the LFD intake showed a cyclical 3- to 4-d pattern of decreased LFD intake (Fig. 1C). The cyclical pattern of decreased LFD intake in the ZnD group was essentially the same as that of decrease in energy intake (Fig. 1A and C).

At the end of the experimental period, although no significant difference in plasma T-cho concentration was observed between the two groups, plasma TG concentration of the ZnD group was lower than that of the ZnA group (p<0.01) (Table 2). Although no significant difference in plasma glucose concentration was observed between the two groups, plasma insulin and leptin concentrations of the ZnD group were lower than those of the ZnA group (p<0.05 and p<0.01, respectively).

When ZnD rats had recovered sufficient Zn through the provision of the ZnA-LFD and the ZnA-HFD, energy...
intake was restored to normal levels within 2 d of consuming the diets containing Zn and the difference in both the LFD and the HFD intakes disappeared between the ZnD and the ZnA groups (Table 3). When ZnD rats had recovered sufficient Zn, the body weight of the ZnD rats recovered nearly to that of the ZnA rats during the 7-d recovery period (Fig. 2). The difference in plasma Zn, TG, insulin and leptin concentrations between the two groups disappeared after the 7-d recovery period (Table 3).

**DISCUSSION**

Utilizing the two-choice diet selection of the LFD and the HFD, we found different preferences and selection patterns for the LFD between the ZnD and the ZnA rats. Our data clearly demonstrates that, although the ZnA rats self-selected uniformly from the two diets, the reduced intake due to Zn-deficiency is caused entirely by reduced intake of the LFD. Reeves and O’Dell reported that rats, deprived of dietary Zn and given a choice between two diets containing 10% and 50% protein, quickly begin to select a lower protein diet than that selected by controls fed adequate Zn (16). As rats fed the ZnD diet were shown to have developed an aversion to protein, in the present study, protein calorie of the LFD and the HFD was fixed at 20.8%.

Rains and Shay investigated the differences in macronutrient preference in ZnD and ZnA rats from complete macronutrient selection by simultaneously providing each animal with three different diets, each containing carbohydrate-, fat- or protein-rich diets (17). They reported that the total metabolizable energy intake in ZnD rats was between 20 and 35% lower than that in ZnA rats and carbohydrate intake accounted for essentially 100% of the lower energy intake. At the onset of the study, both ZnD and ZnA groups consumed approximately 70% of their total intake from carbohydrate, 10% from fat and 20% protein. Although the ZnA group continued to exhibit this intake profile throughout the study, the ZnD group decreased carbohydrate intake to 49% of total intake, increased fat intake to 25%, and increased protein intake to 26% (18). These results are consistent with those of our study, which found that the LFD intake decreased in ZnD rats paralleled with the reduction in total intake (Fig. 1), although protein calorie of the LFD and the HFD was fixed at 20.8%.

In any case, our data clearly demonstrates that the ZnD rats showed significantly increased preference for the HFD. However, as perirenal fat tissue weight and plasma TG concentration decreased with Zn-deficiency, we supposed that the low plasma TG concentration in
the ZnD group was likely a result of the reduced food intake and the low body fat mass of the group. Kwon et al. reported that, although food intake decreased in marginal ZnD (3 mg/kg diet) rats and pair-fed ZnA (30 mg/kg diet) rats compared to ZnA (30 mg/kg diet) rats, growth was unaffected in the pair-fed rats but had decreased in the ZnD rats (19). Moreover, the food efficiency ratio [weight gain (g)/food eaten (g)] of the ZnD rats was lower than that of the pair-fed rats (20, 21).

Although the provision of two separate diets to each rat for self-selection did not allow pair feeding, in this study, we calculated the energy utilization ratio, which was the ratio of weight gain (g) to the energy eaten (kcal), and assessed the utilization of energy consumed. The energy utilization ratio in the ZnA and the ZnD groups was 0.089 and 0.044, respectively. The ratio of the rats deprived dietary Zn was extremely inefficient, suggesting that Zn directly works on one or more factors affecting energy utilization efficiency. However, when rats offered the ZnD diet were returned to the ZnA diet, the energy and the LFD intakes were restored after day 2 of feeding and their body and perirenal fat tissue weights and plasma TG concentration increased rapidly. However, it is unclear what metabolic mechanisms may be driving the increase in the energy and the LFD intakes.

Peripheral hormones like leptin and insulin affect food intake and energy consumption through their receptors in the hypothalamus (9, 22, 23). Although leptin was reduced in the plasma of the ZnD rats, lower body fat and decreased plasma insulin concentrations are correlated with lower concentration of leptin (24, 25). Mangian et al. suggested that Zn-deficiency decreased leptin levels, whereas Zn supplementation increased them (26). Reduced leptin levels indicated that leptin was responding normally, signaling low body fat levels during Zn-deficiency. It might be that leptin was not a dominant factor in the development of Zn-deficiency induced anorexia.

Injection of insulin in the third ventricle of rodents leads to a decrease of food intake and body weight (27). A receptor mediated transport system passes insulin from the plasma into the brain (28). Within the brain, synthesis of neuropeptide Y (NPY) is inhibited by insulin (27). Hsu et al. and Hubner and Gershoff have shown that rats deprived of adequate dietary Zn secrete less insulin and that the rats have a lower tolerance to a glucose load than rats receiving Zn (29, 30). Because Zn is essential for insulin synthesis and Zn deficiency seems to impair release of insulin from the pancreas (29), this may be one of the reasons that the LFD intake decreased in ZnD rats paralleling the reduction in energy intake.

Although the effects of dietary Zn and dietary fat are usually investigated in separate studies, both nutrients are known to alter membrane and tissue fatty acid composition (31, 32). Altered fatty acid composition may influence several processes related to insulin resistance such as hormone binding and signal transduction, and availability of precursor molecules for lipid synthetic and catabolic pathways (33). By observing the LFD and the HFD preferences during development of and recovery from Zn-deficiency, we provide evidence that contributes to a better understanding of anorexia resulting from consumption of Zn-deficient diets. Further research is necessary to clarify the relationship between lower appetite and Zn-deficiency.

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