Patterns of food intake and self-selection of macronutrients in rats during short-term deprivation of dietary zinc

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Abstract

Although it has been known for more than 50 years that zinc (Zn) deficiency regularly and consistently causes anorexia in many animal species, the basic mechanism(s) that cause this phenomenon still remain(s) an enigma. The following studies describe feeding behavior in the early stages of zinc deficiency in the rat model. In one experiment, we used computerized feeding monitors that measured the intake of individual rats at 10-min intervals over 24-hr periods. Male rats were acclimated to the cages and fed a Zn-adequate egg-white-based diet, or a similar diet with <1.0 mg Zn/kg. Food intake was monitored for seven, consecutive 24-hr periods. The 24-hr food intake pattern of the Zn-deprived rats did not differ from the controls; they simply ate less food, mainly during the night hours, with no differences between groups during the day. Although Zn-deprived rats ate less food than controls, the percentage of total diet consumed during night and day did not differ between groups. In another experiment, we simultaneously offered male rats three isocaloric diets with different macronutrient compositions and with or without adequate Zn, and measured the amount of each diet selected during seven, 24-hr periods. The three diets contained either 57% protein from egg white, 30% fat from soybean oil, or 80% carbohydrate from a combination of starch, hydrolyzed starch, and sucrose. For the first four days on experiment, rats selected similar amounts of each diet. Then the Zn-deprived rats began to select only 50% as much of the protein diet as the controls. Similar results were obtained when the data were expressed on the basis of each macronutrient as a percentage of the total diet selected. Zn-deprived rats selected a diet that contained 8% protein, 73% carbohydrate, and 6% fat while the Zn-adequate rats selected 12% protein, 69% carbohydrate, and 6% fat. Fat intake was not affected by Zn-deprivation. The results confirm our previous findings, and are discussed in terms of Zn-deprivation blunting the pathways of signal transduction that involve the peptide hormones known to affect food intake regulation. Published by Elsevier Inc. All rights reserved.

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1. Introduction

One of the first observable signs of zinc (Zn) deficiency in the rodent is a reduction in food intake [1]. This phenomenon occurs three to four days after the animal first begins to consume a semi-purified diet with <1 mg Zn/kg. Then the animal begins a cyclic pattern of food intake with three to four day intervals. Although the effect of Zn deficiency on food intake has been known for more than 50 years, the biochemical mechanism involved in its initiation is still unknown. Work by Chesters and Will [2,3] first suggested that the Zn-deprived animals develop an aversion to protein in the diet. Later, Reeves and O'Dell [4] used a two-diet self-selection feeding paradigm where rats were given a choice between two separate diets, one high in protein and one high in carbohydrate. They found that in the early stage of Zn deprivation, rats consistently selected less of the high protein diet and more of the high carbohydrate diet than Zn adequate rats.

Subsequent studies by other investigators, however, employed a more sophisticated three-diet self-selection regimen and found that their rats selected differently [5]. In these studies, the animals were offered a choice among diets with either: 1) very high contents of carbohydrate with no protein, 2) high fat with no protein and little carbohydrate, or 3) high protein with little carbohydrate. Each diet contained a complement of vitamins and minerals to meet the nutrient requirement of the rat [6]. The results showed that Zn-deprived animals tended to select more protein and fat, and less carbohydrate than Zn-adequate animals. These findings were counter to those of Chesters [3] and Reeves and O'Dell [4].

Other studies have been done to determine the food intake patterns of Zn-deprived rats. By using automatic food intake monitors, and diet compositions similar to those used

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previously [5], Rains et al. [7] found that the initiation of feeding normally observed soon after the onset of darkness was delayed in the Zn-deprived rat compared with the Zn-adequate rat. In addition, the number of meals consumed per day, but not meal size, was reduced in Zn-deprived rats. They also observed, again, that Zn-deprived rats self-selected a higher fat diet than the Zn-adequate rats.

Because there seemed to be some discrepancies in the design and diet composition of these and other studies attempting to discern the change in feeding habits and patterns of Zn-deprived rats, we implemented our own studies. We used a three-diet feeding paradigm, but with different percentage compositions for the high fat, high carbohydrate, and high protein diets than those of Rains et al. [5,7]. Because we were interested in the intake pattern at the initiation of the deficiency, we only made observations during the early stages of depressed food intake, up to day-6 after first feeding the deficient diet. Running the experiment for longer periods only makes the deficiency more severe, which can complicate the interpretation of the results.

2. Materials and methods

All studies were approved by the Animal Use Committee at the Grand Forks Human Research Center and were carried out according to the guidelines set down by the National Research Council [8]. Rats were housed in a room that was controlled for temperature (22°C) and humidity (50%). Beginning at 18:00, lights were set to slowly dim from full light to total darkness in a thirty-minute period. Starting at 06:00, they gradually gained full light over a similar period.

2.1. Experiment #1

This experiment was designed to monitor the minute-by-minute change in food intake patterns of rats fed a control and a Zn-deficient diet over a specified number of days. Ten male Sprague-Dawley rats weighing 58 ± 5 g (mean±SD) were adapted to a Zn-adequate semi-purified diet similar to the AIN-93G diet [9]. However, egg white solids were substituted for casein, and the composition of the mineral mix was adjusted to account for the differences in mineral content of the two protein sources. Extra biotin was added to the diets to counteract the avidin present in the egg white. For a precise formulation of the diets, please refer to the publication by Reeves [10]. The control diet contained 30 mg of Zn/kg and the deficient diet contained <1.0 mg Zn/kg.

When the rats reached a body weight (BW) of about 70 g, they were placed individually into Plexiglas cages specifically designed to monitor food intake automatically and continuously (Columbus Instruments, Inc., Columbus, OH). Each cage unit was modified to accept a Plexiglas insert that rested about 2.5 cm above the bottom of the cage,

and above the rim of the feeding chamber. A cutout for the food container and several one-cm holes were drilled into the insert. These small holes allowed fecal pellets and urine to drop through to a layer of paper (Deluxe Techboard, Ancare Corporation, Bellmore, NY) placed in the bottom of the cage. Each cage measured 42 cm square and 20 cm high. In the floor was a circular cup (10 cm in diameter) that contained four, three-cm holes through which the rat could access the contents of a food container. The food container (the bottom half of an acid-washed, 10-cm glass Petri dish) sat on a precision balance (Mettler, Model PB801) that was connected to a computer. With the use of a food monitoring computer program written specifically for the system, food intake was monitored continuously in specified intervals throughout the day and night. Data were saved to a file and then transferred to a spreadsheet for calculations. Food intake was monitored simultaneously with 10 units in 10-min intervals over 24 hr periods. Each period began at 0900 one day and ended at 0845 the next. The remaining 15-min was used to replenish the food containers with fresh diet.

Rats were adapted to the cages over a 5-day period, during which food intake patterns were established and stabilized before the experiment began. To begin the experiment, the rats were divided into two groups of five rats each with similar body weights (108 ± 8 g; mean ±SD) with one rat per cage. One group continued to receive the AIN-93G-EGG diet with adequate Zn and the other group received a similar diet, but with a reduced amount of Zn (<1 mg/kg). Food intake was monitored for seven, 24-hr periods, beginning one day before first offering the Zn-deficient diet and ending seven days later. Animals were weighed each day, and the amount of food consumed by each rat during the following 24 hr was expressed as a percentage of the rat's body weight at the beginning of that 24-hr period. Deionized water was provided at all times in acid-washed glass bottles with silicone stoppers and stainless steel sipper tubes.

2.2. Experiment #2

This experiment was designed to determine how long it would take the Zn-deprived rats to reinitiate normal feeding patterns after their diets were supplemented with adequate Zn. The experimental design was similar to that in Experiment #1. After the deficient rats had gone through two depressed feeding cycles, and at the nadir of the second cycle, the rats were fed the Zn-adequate diet, and food intake was monitored for another four days.

2.3. Experiment #3

This experiment was designed to monitor the change in macronutrient selection patterns of rats fed control and Zn-deficient diets over a specified number of days. The experiment was repeated twice with similar results; hence, data from only one of the experiments are reported here.

Table 1
Diet Composition

Ingredients	High Protein		High Fat		High Carbohydrate	
	g/kg	kj/g of Diet	g/kg	kj/g of Diet	g/kg	kj/g of Diet
Cornstarch[a]	38.781	0.62	130.478	2.08	680.478	10.85
Hydrolyzed cornstarch[b]	100.0	1.59	100.0	1.59	100.0	1.59
Egg white solids[c]	700.0	11.18	61.7	0.99	61.7	0.99
Soy bean oil[d]	50.0	1.85	300.0	11.10	50.0	1.85
Granulated sucrose[e]	10.0	0.16	10.0	0.16	10.0	0.16
Fiber[f]	50.0	0	350.0	0	50.0	0
AIN-93G mineral mix[g]	35.0	0.11	35.0	0.11	35.0	0.11
AIN-93G vitamin mix[g]	10.0	0.16	10.0	0.16	10.0	0.16
Biotin premix[h]	3.705	0.06	0.309	0	0.309	0
Choline bitartrate[i]	2.5	0.04	2.5	0.04	2.5	0.04
TBHQ[j]	0.014	0	0.014	0	0.014	0
Total	**1000.0**	**15.77**	**1000.0**	**16.07**	**1000.0**	**15.75**

[a] Roquette America, Keokuk, IA.
[b] Dyetrose, Dyets, Inc., Bethlehem, PA.
[c] Teklad Madison, WI. Contained 81% protein.
[d] Wesson; Conagra Grocery Products Co, Irvine, CA.
[e] United Sugars Corp., Minneapolis, MN.
[f] Alphacel; Teklad, Madison, WI.
[g] See reference [10].
[h] Premix; 1.8 g D-biotin plus 998.2 g powdered sucrose.
[i] Teklad, Madison, WI. Contained 40% choline.
[j] Tert-butylhydroquinone; Aldrich Chemical Co., Inc., Milwaukee, WI.

Twenty male Sprague-Dawley rats, weighing 87 ± 5 g (mean $\pm$ SD), were placed in double wide stainless steel cages, one rat per cage. They were fed the AIN-93G-EGG diet for 4 days to adapt them to their new surroundings. Then they were offered free choice of three different diets, the compositions of which are shown in Table 1. Similar amounts of vitamins and minerals were provided in all three diets to meet the NRC micronutrient requirements for the rat [6]. Extra biotin was added to the diets to counteract the avidin present in the egg white.

The rats were allowed to select from all three Zn-adequate diets for a period of four days to allow them to establish a consistent feeding pattern of choice. Then half the rats were switched to similar diets containing <1.0 mg Zn/kg, while the other half continued to receive the Zn-adequate diets. Intakes of each diet were measured manually for 24-hr periods between the hours of 0800 and 0900 for seven more days. Natural spillage of the diets was minimal, but it was measured and accounted for in the final calculations. To avoid positional bias, each food cup was moved to a different place in the cage each day. Body weights were recorded daily at the same time as food intake was measured.

2.4. Statistical analysis of the data

Either a 3-way analysis of variance (ANOVA) or the Student's t statistic was used to determine differences between means. When each test was used is given in the Results section where appropriate.

3. Results

3.1. Experiment #1

Measurement of 24-hr food intake showed that Zn deficiency began to be expressed after three days of consuming the diet (Fig. 1). Both Zn-adequate and Zn-deprived rats consumed the same amount of diet/100 g BW up to day-3, then on the fourth day, food consumption dropped precipitously in the Zn-deprived rats. However, food intake in this group was back to the control level by day-6. A reduction ($p < 0.01$; Student's t test) in weight gain accompanied the reduction in food intake (Fig. 2).

On the third day after initiating the experiment, the 10-min cumulative food intake patterns of the Zn-deprived rats did not seem to be different from those of the Zn-adequate rats (Fig. 3; Day 3). Even on the fourth day, when Zn-deficiency signs presented (food intake depression), it seemed that intake patterns did not change significantly, only that the Zn-deprived group ate less diet than the control group. Food intake in both groups was minimal from about 0600 (lights on) until about1400 hr, when the rats began to feed (Fig. 3, Day-4). Then at 1800 hr (lights out) they began to increase their rate of intake again. The intake pattern of both groups was similar until about 1900 hr, when the rats receiving the Zn-deficient diet began to eat less food than those receiving the Zn-adequate diet. This pattern continued through days 5 and 6 (data not shown).

The food intake patterns of individual rats are shown in Figure 4. The individual dots in this figure represent the amount of food consumed in each 10-min interval. The data
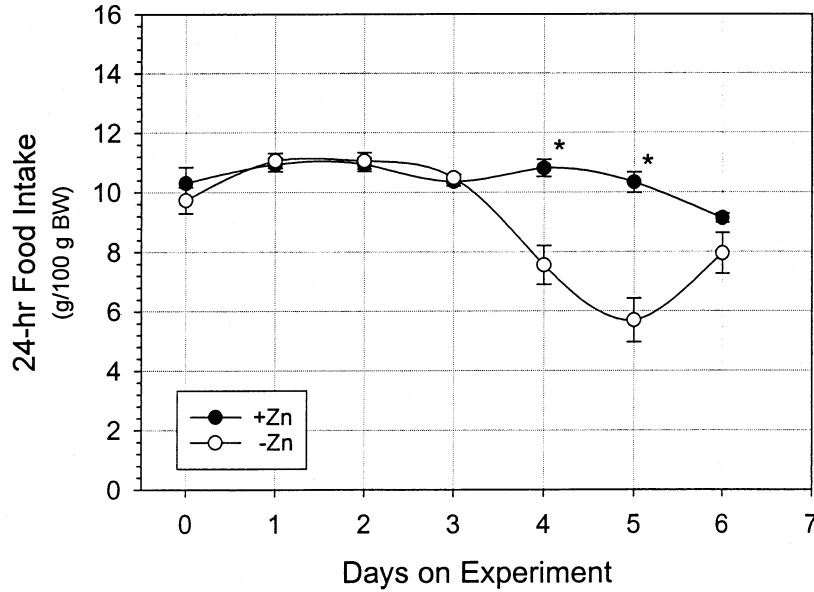
Fig. 1. Twenty-four-hr food intake in Zn-adequate and Zn-deprived rats with time, expressed as a percentage of body weight. Symbols are means $\pm$ SEM for five rats per group. The * indicates that groups are significantly different; P <0.001 (Student's t test), at those time points. *Experiment 1.*

show that rats are quite individualistic in their food intake patterns. The only apparent consistency was that all rats had more eating bouts and consumed more food during the dark hours than the light hours. There was no discernible delay in feeding in the Zn-deprived group at the onset of darkness. In addition, the individual patterns of intake during the onset of Zn deficiency (Day-4), did not change appreciably from the previous day (Day-3); Zn-deprivation simply caused the rats to eat smaller meals and less food (Fig. 4; Table 2).

When food intake was expressed as a percentage of total

intake for the light and dark periods of days 3, 4, and 5, there were no significant differences between light and dark between the Zn-deprived and control rats (Table 2). Each group consumed about 30% of its diet during the day and 70% at night. However, during nighttime on days 4 and 5, the Zn-deprived rats consumed less food than the Zn-adequate rats. There was no difference between groups during daytime.

When we assessed the number of 10-min intervals (bouts) in which at least 0.1 g of food was consumed, we found that Zn deprivation had no effect during the light or
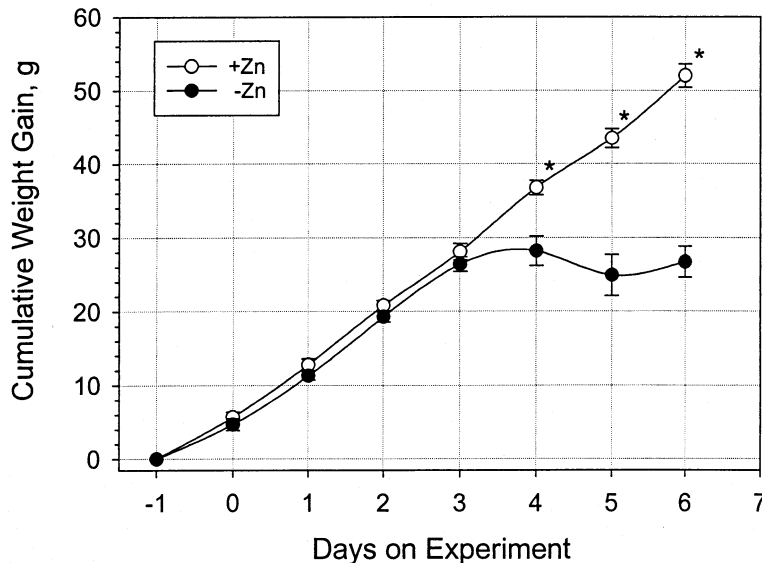

Fig. 2. Cumulative weight gain of Zn-adequate and Zn-deprived rats with time. Symbols are means $\pm$ SEM for five rats per group. The * indicates that groups are significantly different (P <0.001; Student's t test) at those time points. *Experiment 1.*
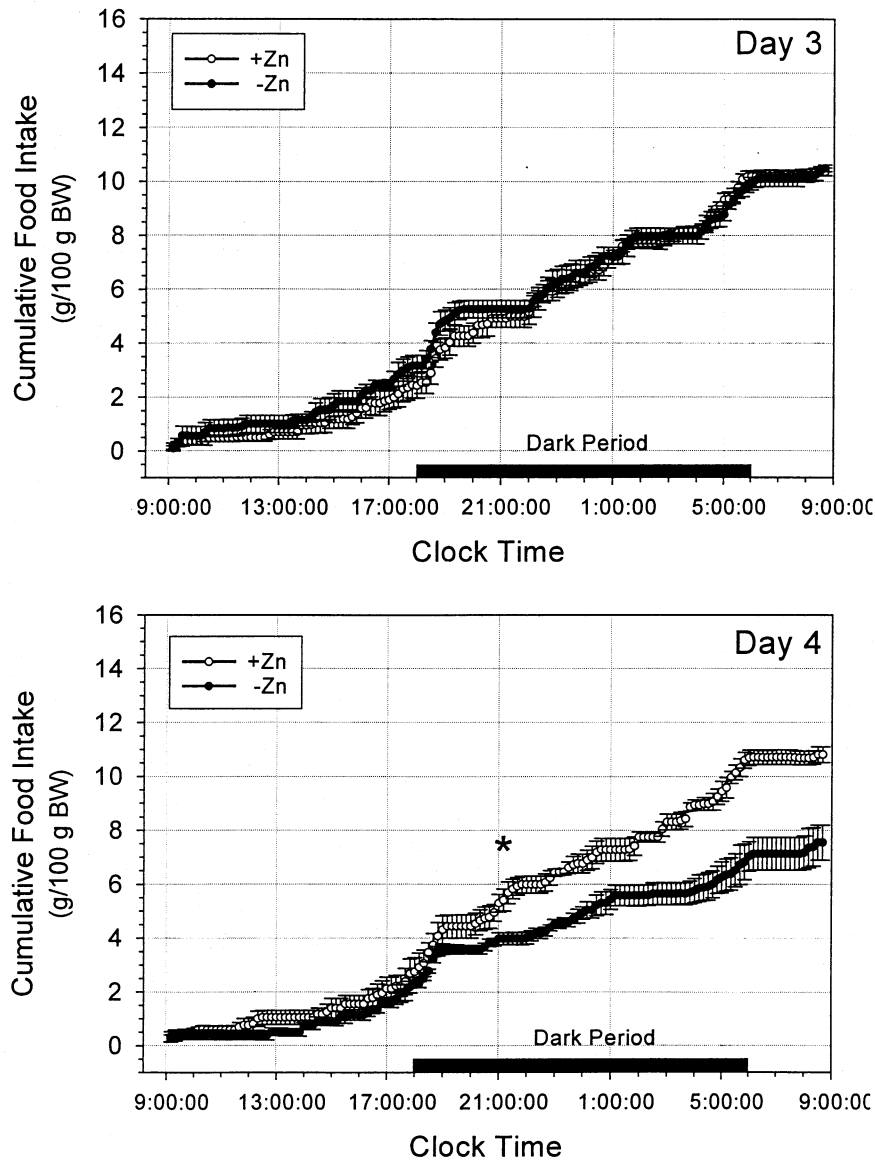
Fig. 3. Two 24-hr periods of cumulative food intake at 10 min intervals in Zn-adequate and Zn-deprived rats, expressed as a percentage of body weight. Symbols are means ± SEM for five rats per group. The * on the graph at day-4 indicates that the groups differ significantly (P <0.02; Student's t test) beginning at that time point and continuing thereafter. *Experiment 1.*

the dark periods (Table 3). However, there were significantly (P <0.05) fewer and fewer feeding bouts as the days progressed. In addition, and as expected, there were significantly (P <0.0001) more feeding bouts during the night than during the day for both treatments.

3.2. Experiment #2

This experiment was similar to experiment #1, except that at the lowest point of the second cycle (day-9) of food intake, the Zn-deprived rats were refed the Zn-adequate diet. The results show that food intake did not rebound in the refed rats until two days after refeeding

began, and then it overshot the intake of the control group (Fig. 5). Part of the overshoot was because of the manner in which food intake was expressed. The body weights of the Zn-deprived rats were smaller than the controls on day-10. However, because they ate as much as, or more than, the control rats who weighed more than the deprived rats, the amounts of diet consumed per 100 g BW were exaggerated for the Zn-deprived rats on days 11 and 12. By day-13, the amount of diet consumed was back to near the control values.

We looked at the mean intake at 10-min intervals one day after Zn feeding was reestablished, and again the patterns looked similar between groups (Fig. 6; Day-10).
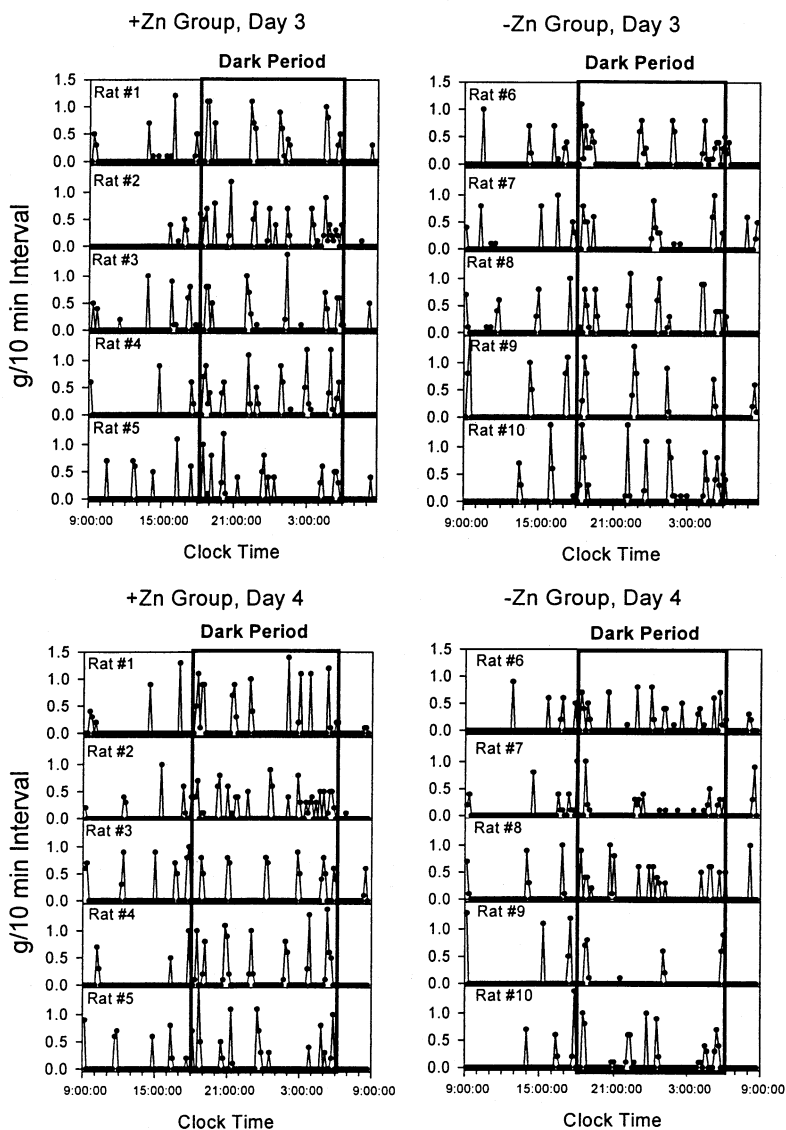
Fig. 4. Two 24-hr periods of food intake recorded for individual Zn-adequate and Zn-deprived rats. Each small dot represents the amount of food consumed in the previous 10 min interval. Day-3 represents the 24-hr period just before the rats began to show signs of Zn-deprivation, i.e., food intake depression. Day-4 represents the first 24-hr period after the rats began to show depressed food intake. *Experiment 1.*

However, the variability among individual rats was quite large in the Zn-deprived group refed Zn, compared with the Zn-adequate group. Two days after refeeding the Zn-adequate diet to the Zn-deprived rats, they were eating more than rats that had been fed Zn continuously, but the intake patterns remained similar (Fig. 6; Day-11)

Table 2
Food intake of Zn-deprived and Zn-adequate rats during the light and dark cycles.[a] *Experiment 1.*

Diet	Period	Day 3		Day 4		Day 5	
		(g/100g BW)	(% of Total)	(g/100g BW)	(% of Total)	(g/100g BW)	(% of Total)
+Zn	Light	3.7 ± 0.7	28 ± 5	3.9 ± 0.8	27 ± 6	3.9 ± 0.8	26 ± 5
	Dark	9.7 ± 0.6	72 ± 5	10.3 ± 0.6*	73 ± 4	11.0 ± 0.8*	74 ± 5
−Zn	Light	4.5 ± 0.5	35 ± 4	3.8 ± 0.3	39 ± 3	3.3 ± 0.6	34 ± 6
	Dark	8.5 ± 0.9	65 ± 7	6.0 ± 1.0	61 ± 10	6.4 ± 1.3	66 ± 13

[a] Food intake expressed as the mean ± SEM for five rats per group. During the dark period at days four and five, Zn-deprived rats ate less (* =P<0.05) food during the dark period than the Zn-adequate rats. There were no differences between groups during the light period. Percentages of total daily intake did not differ between treatment groups for the light or dark periods, or among days.

Table 3
Number of 10-mm intervals (bouts) in which Zn-deprived and control rats consumed food during the light and dark hours.[a] *Experiment 1.*

Diet	Period	Day 3	Day 4	Day 5
+Zn	Light	7.6 ± 1.5	7.4 ± 1.1	6.0 ± 1.3
	Dark	18.6 ± 1.8	18.2 ± 2.5	16.4 ± 1.7
	Total	**26.2 ± 1.8**	**25.6 ± 2.7**	**22.4 ± 1.9**
−Zn	Light	9.0 ± 0.8	7.2 ± 1.2	4.8 ± 0.9
	Dark	17.8 ± 2.2	15.2 ± 2.1	13.6 ± 2.2
	Total	**26.9 ± 2.6**	**22.4 ± 1.9**	**18.4 ± 2.7**

[a] Mean ± SEM for five rats per group. The food monitor was set to record food intake in 10-min intervals. Food intake was recorded if the rat consumed a minimum of 0.1 g. By a 3-way ANOVA, there was a highly significant ($P < 0.0001$) difference in the number of feeding bouts between the light and dark periods. Also, there was a significantly ($P < 0.05$) lower number of feeding bouts over time.

3.3. Experiment #3

In this experiment, rats were offered a choice among three different diets, one containing high protein, one containing high fat, one containing high carbohydrate, and all three either adequate or inadequate with respect to Zn concentration. All rats were fed the three diets containing Zn for the first few days of the experiment to allow them to establish a consistent intake pattern by choice before they were switched to the Zn-deficient diets.

With this design, rats began to show signs of Zn deficiency after about 3 days of consuming the diet. Zn-deprived and Zn-adequate rats consumed similar amounts of total diet up to day-2 of feeding (Fig. 7). However, by day-3, the deprived rats began to consume less of the total diet than the adequate rats. By day-4, they were consuming

only about 50% as much as the adequate rats. After that, we observed a minor cyclic food intake pattern that normally is characteristic of zinc deficiency in some animal species.

During the first day of feeding (Fig. 8; Day-(-3), the selection for fat and protein diets was quite variable among rats; however, by the fourth day (Day-0), the patterns and amounts of intake were more consistent, and averaged about 0.5 and 1.4 g/100 g BW/day, respectively. Intake of the carbohydrate diet seemed to be more consistent and averaged about 11 g/100 g BW/day. A similar pattern emerged when intakes of the macronutrients were expressed as percentages of the total diet (Fig. 9).

When food intake was separated into the amount of each diet consumed, we found that at the onset of the deficiency (day-(+4), as expressed by food intake depression, the Zn-deprived rats consumed only 50% as much of the high-
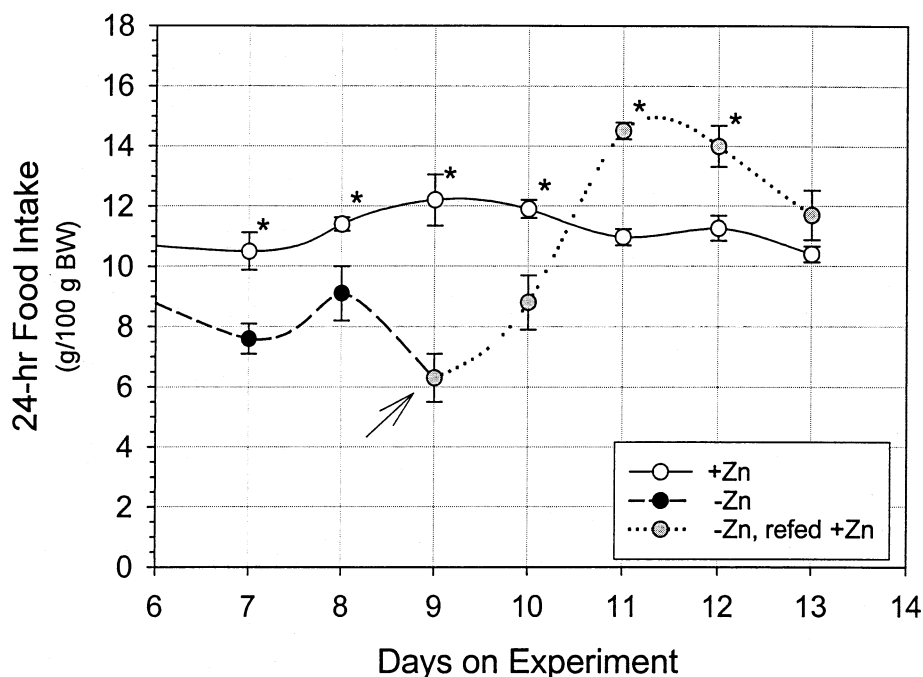

Fig. 5. Change in food intake after refeeding Zn-deprived rats a Zn-adequate diet. After rats had consumed a diet inadequate in Zn through two depressed feeding cycles, they were refed a Zn adequate diet (arrow). Symbols are means ± SEM for five rats per group. The * indicates that groups are significantly different ($P < 0.01$; Student's t test) at those time points. *Experiment 2.*
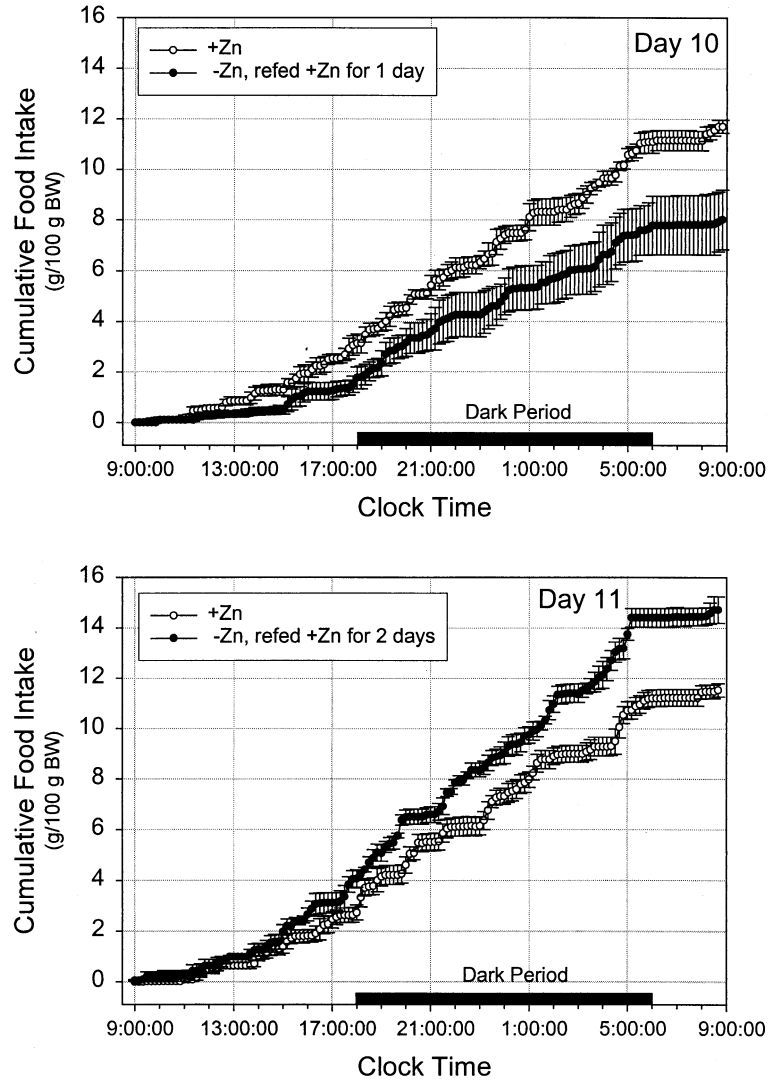
Fig. 6. Two consecutive 24-hr periods of cumulative food intake at 10 min intervals in Zn-adequate and Zn-deprived rats. At Day-10, rats previously deprived of Zn in the diet had received the Zn-adequate diet for one day. At Day-11, the previously deprived rats had received the Zn-adequate diet for 2 days. Data are expressed as the mean ± SEM for five rats per group. *Experiment 2.*

protein diet as the adequate rats (Fig. 8). The amounts of fat and carbohydrate were not affected by Zn deficiency when expressed in this manner. However, when expressed as a percentage of the total diet selected, the Zn-deprived rats selected a diet with about 8% protein and the Zn-adequate rats selected one with about 12% protein (Fig. 9; days 4 and 5). The percentage carbohydrate selected was 73% and 69% in Zn-deprived and adequate rats, respectively. Fat selection was not affected. By the close of the experiment (days 6 and 7), the Zn-deprived rats were still eating slightly more carbohydrate than the controls, but the differences were not significant.

4. Discussion

The results of this study corroborated our original findings [4] that by giving a choice among diets with different macronutrient compositions, the Zn-deprived rat will choose a diet that is lower in protein content than one chosen by Zn-adequate rats. Studies by Chesters and Quarterman [1,2] were the first to show that Zn-deprived rats consume more of an egg white-based diet with a reduced protein content than one with an adequate amount of protein. We then expanded and corroborated those findings with a different design [4]. We showed that Zn-deprived rats self-selected a 22% protein diet compared with 30% protein for Zn-adequate rats, when given a choice between two nutritionally balanced diets where one contained 10% soybean protein and 77% glucose, and the other contained 50% soybean protein and 37% glucose.

Other investigators, however, have not been able to show responses similar to those we observed. For example, Griffith and Alexander [11] found that the cyclic feeding pattern seen in Zn-deprived rats consuming a normal protein diet
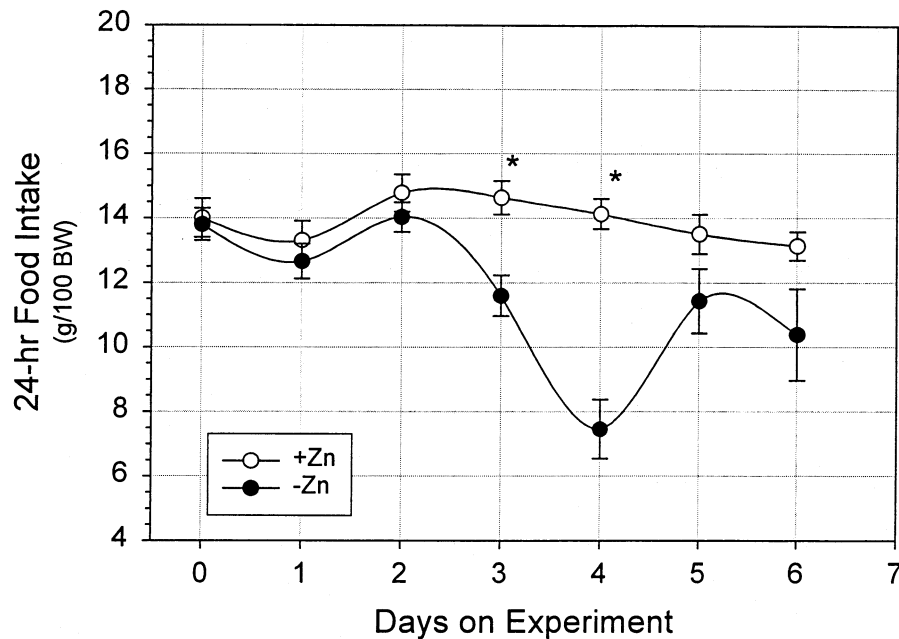
Fig. 7. Total food intake in Zn-adequate and Zn-deprived rats during six, 24-hr periods, expressed as a percentage of the body weight. Symbols are means ± SEM for 10 rats per group. The * indicates that groups were significantly different (P <0.01; Student's t test) by Student's t test at those time points. *Experiment 3.*

was dampened considerably when they were fed a low protein diet. However, the average daily intakes between diets were similar. Rains and Shay [5] and Rains et al. [7] used a three-diet feeding paradigm where Zn-deprived and control rats were offered diets containing high concentrations of either protein, fat, or carbohydrate. Their results showed that Zn-deprived rats, during the first seven days of the study, selected less of the high-carbohydrate diet, but the same amounts of both high-protein and high-fat diets than the control group.

It is not immediately apparent why their results were so different from those found by us [7] and Chesters [1,2]; however, differences in the composition of the diets might explain the lack of consistent responses. The diets of Rains and Shay [5] consisted mainly of over 90% of each macronutrient source, dried egg white, fat, or carbohydrate. The high-fat and high-carbohydrate diets contained no protein source, and it was not stated whether the protein diet contained an extra supply of biotin to counteract the avidin present in egg white. Vitamins and minerals were supplied as prescribed for the AIN-76 diet [12]. Although our diets contained a high proportion each of egg white solids (~70%), soybean oil (~30%), and carbohydrate (~80%), they also contained a portion of the other macronutrients as well. We also added extra biotin and used the AIN-93G vitamin and mineral mixes [9]. Although Rains and Shay [5] adapted their animals to the control diets for one week before feeding the Zn-deficient diet, the day-to-day variations in intakes of the high-protein and high-carbohydrate diets were quite large, even before signs of the deficiency were expressed. On the other hand, the variability in our

study seemed much less. Whether these differences in diet composition were enough to cause the different results between investigators remains unanswered.

If indeed the Zn-deprived rat is self-selecting a diet with a lower amount of protein than the Zn-adequate rat, what is the biochemical mechanism that brings this about? It is unlikely that the initial change in plasma Zn concentration is the stimulus, because plasma Zn begins to fall almost immediately after the Zn deficient diet is consumed. Hurley et al. [13] showed that plasma Zn dropped to 45% of normal in only 8 hr after rats began to consume a Zn deficient diet. However, food intake was not affected until three or four days later. This suggests that some regulatory impulse must be generated and it must reach a critical level before the animal is induced to stop eating. Therefore, because of the time requirement, and because it takes much longer for low dietary Zn intake to affect tissue Zn than to affect plasma Zn, the regulatory impulse more than likely is stimulated by a change in organ or tissue Zn concentration.

What is the regulatory impulse? Zn plays a central role in the activation of numerous enzyme systems that synthesize and degrade bioactive peptides. Some of these peptides are involved in the regulation of food intake, and a few possible candidates have been suggested. Neuropeptide Y (NPY), for example, is one of the most potent appetite-regulating neuropeptides, and stimulates food intake behavior when injected into the paraventricular nucleus [14]. This peptide has received a lot of attention with regard to food intake regulation in Zn deficiency. In addition to increasing total food intake, NPY also seems to be associated with increased carbohydrate selection when rats are offered a choice
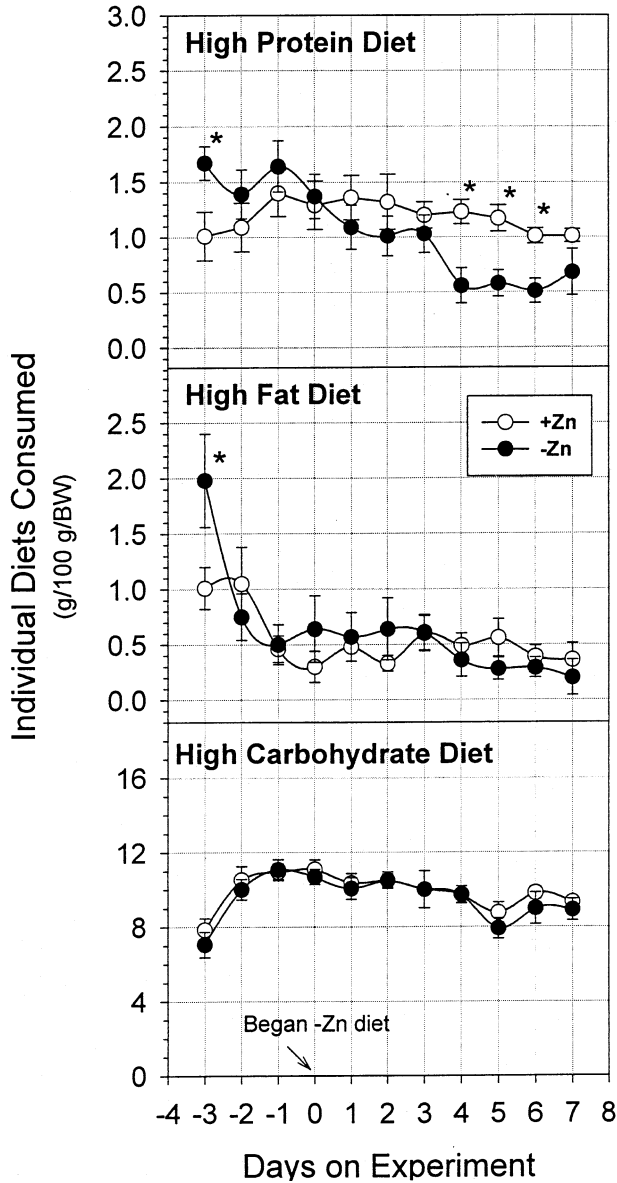
Fig. 8. Self-selection of diets by Zn-deprived and control rats when they were offered 3 separate diets with high concentrations of either protein, fat, or carbohydrate. Male rats were offered the three diets containing adequate Zn (30 mg/kg) simultaneously for four days to allow them to adapt to the selection paradigm. Then at day-0 on the graph, half the rats were offered similar diets, but with <0.1 mg Zn/kg. At day-4, the Zn deprived rats began to select less of the high protein diet than the control rats. Expressed in this manner, Zn deficiency had no effect on the selection of high fat or high carbohydrate diets. Symbols are means ± SEM for 10 rats per group. The * indicates that groups were significantly different by Student's t test (P <0.01) at those time points. *Experiment 3*.
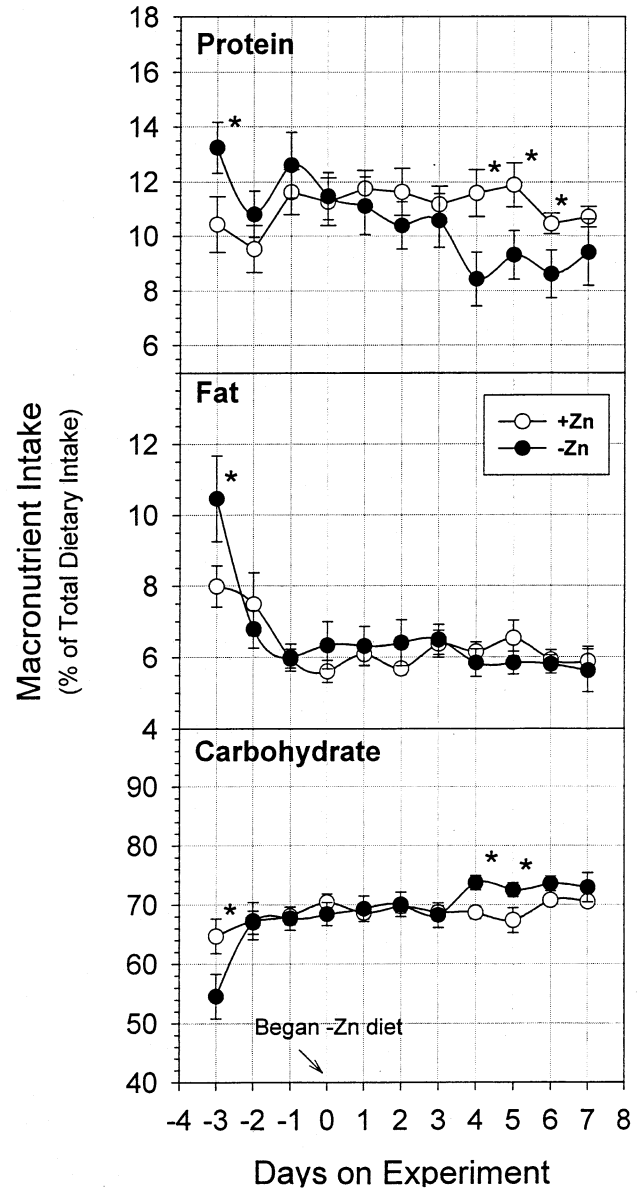
Fig. 9. Self-selection of macronutrients by Zn-deprived and control rats from three separate diets with high concentrations of either protein, fat, or carbohydrate. Male rats were offered the three diets containing adequate Zn (30 mg/kg) simultaneously for four days to allow them to adapt to the selection paradigm. Then at day-0 on the graph, half the rats were offered similar diets, but with <0.1 mg Zn/kg. When the macronutrient intake was expressed as a percentage of the total diet selected, at day-4, the Zn deprived rats began to select a diet with less percentage protein than the control rats. However, at the same time, they selected so that the diet was higher in carbohydrate than that selected by the control rats. Symbols are means ± SEM for five rats per group. The * indicates that groups were significantly different (P <0.01) by Student's t test at those time points. *Experiment 3*.

among three diets with different concentrations of the macronutrients [15,16]. Lee et al. [17] studied the effects of Zn deficiency on NPY and NPY mRNA concentrations in rat hypothalami, because they had shown previously that their Zn-deprived rats tended to select against carbohydrate, and had lower total food intake than controls in a three-diet feeding paradigm. Surprisingly, they found that NPY con-

centration and the relative abundance of NPY mRNA were significantly higher in the hypothalami of Zn-deprived rats than controls. However, they also found that control rats with a restricted dietary intake had elevated NPY in the hypothalamus, suggesting that reduced food intake in the

Zn-deprived rats was causing NPY elevation. In addition, they found no impairment in the feeding response in Zn-deprived rats with NPY infused into the paraventricular nucleus of the hypothalamus; thus, they concluded that NPY was not associated with the impaired feeding behavior in Zn-deprived rats.

Other investigators have reached similar conclusions. Williamson et al. [18] infused NPY into the hypothalamus of short-term Zn-deprived rats and found that the relative response was not different from controls, even though the total amount of food consumed was reduced, i.e., similar to that prior to infusion. They concluded that NPY was not related to feeding behavior in Zn-deprived rats, but that some other undefined factor was responsible for the loss of appetite.

Other peptides and hormones have been associated with feeding behavior. These include cholecystokinin (CCK) [19], the opioid receptor agonists [20], catecholamines [21], leptin [22], and others [23]. CCK is secreted into the blood from endocrine cells in the duodenum upon stimulation by partially digested food, especially certain proteins. CCK then acts through specific receptors to regulate stomach emptying and gut motility, and through both peripheral and central mechanisms, to regulate the intake of food [24]. CCK is metabolized by a Zn-dependent enzyme, aminopeptidase A [25], the activity of which might be reduced in Zn-deprived rats. In addition, Blanchard and Cousins [26] found by differential display that the relative concentration of intestinal CCK mRNA was enhanced in the Zn-deprived rat, giving a greater potential for CCK production. It is sheer speculation, but if the Zn-dependent CCK degrading enzymes were inhibited sufficiently, this could lead to a prolonged action of CCK. A sustained elevation of plasma CCK could lead to prolonged anorexia. However, eventually, anorexia would reduce CCK release, and once it was cleared from the plasma, feeding would begin again; thus, leading to the cyclic feeding pattern seen in the Zn-deficient rat.

The relationships of some of the food intake enhancing hormones to food intake regulation in the Zn-deficient rats have been studied [27,28]. Some of these include the endogenous opiates, norepinephrine, dopamine, and γ-aminobutyric acid (GABA). Infusing the agonists to the receptors of these enhancers into the ventricle of the brain stimulated feeding in the Zn-deprived rat to some extent, but not to the level found in the Zn-adequate or paired fed control rats. The authors theorized that Zn deficiency "produces a generalized decrease in receptor responsivity (sic) . . . .". Neither of these studies has been repeated or substantiated in other laboratories.

Megestrol acetate (MA), a synthetic progestin, has been shown to stimulate food intake and increase NPY in the hypothalamus [23]. Browning et al. [29] and Williamson et al. [30] showed that MA could stimulate a significant increase in food intake in both male and female rats deprived of dietary Zn for the short-term. However, MA severely reduced the growth rate of Zn-adequate male rats, but not

female rats. MA actually stimulated both food intake and weight gain in Zn-deprived female rats over a short period, but was unable to completely restore growth when given for longer periods. Hypothalamic NPY concentrations were elevated in male rats treated with MA, but there was no clear association between NPY and food intake.

Numerous investigators have found an association between Zn, food intake, and leptin. Leptin is a cytokine that is secreted by adipose tissue and plays a role in energy balance and body weight, and it has a positive correlation with the amount of body fat [31]. It has been shown that plasma leptin is lower in Zn-deprived rats than controls [32], and plasma Zn concentration is inversely related to plasma leptin in humans [33]. However, work by Gaetke et al. [34] suggested that the low plasma leptin concentrations in Zn-deprived rats was not caused by the deficiency per se, but by anorexia that normally accompanies the deficiency. Although low plasma leptin concentrations are usually inversely correlated with hypothalamic NPY in the Zn-deprived rat, there is no clear association with food intake regulation in this model.

Because the short-term Zn-deprived rat selects against dietary protein, part of the mechanism might involve an aberration in amino acid metabolism, which was suggested in the initial studies by Chesters and Will [2] nearly 30 years ago. However, this does not rule out the possibility that Zn-deprivation is disrupting the gut/brain neurophysiological axis [35]. The epithelial cells that line the gut are the first to receive the brunt of the deficiency. The cells turnover very fast (2 to 3 days), which, more than likely, would cause them to have low concentrations of Zn. Would this in itself affect both chemical and neurological signaling pathways to the rest of the body?

In summary, short-term Zn deficiency in the laboratory rat reduces food intake three to four days after the deficient diet is first consumed. In the current study, this reduction in food intake was found to be a simple reduction in the amount of food consumed, and not in a reduction in the number of feeding bouts or a change in any other observable feeding pattern. If the Zn-deprived rat is given a choice among three diets with different amounts of protein, fat, or carbohydrate, at the time food intake depression begins, it will reduce its selection of protein from 12% to 8% of the diet. At the same time, it will increase its intake of carbohydrate from 69% to 73% of the diet, while the intake of fat is not significantly affected. The biochemical mechanism for food intake depression in this model is still unknown, but might involve food intake regulating substances, such as CCK, whose concentrations are regulated by Zn-dependent enzymes.

Acknowledgments

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