PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 170, 48–52 (1982)

Circadian Variation in Rat Plasma Zinc and Rapid Effect of Dietary Zinc Deficiency[1] (41395)

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Abstract. Adult rats on a 12-hr light/dark schedule fed a complete diet exhibited a circadian variation in plasma zinc, with a sharp decline of 25% between 8 and 12 hr after the beginning of the light cycle. In rats fed a zinc-deficient diet for 24 hr, plasma zinc concentration was similar to that of controls for the first 12 hr, but continued to fall further, and was significantly lower than controls after 14 hr. When rats were fed the zinc-deficient diet beginning at 8 hr of the light cycle, their plasma zinc concentration was significantly reduced 6 hr later. The results demonstrate the rapid effect of dietary zinc deficiency.

Earlier work from this laboratory showed that after 24 hr of a regimen severely deficient in zinc, the concentration of this element in the blood plasma of rats was greatly decreased (1). In pregnant rats, the drop in plasma zinc was 38% in the first 24 hr; in weanling males, it was 55%. The rapid effect of dietary zinc deficiency on plasma zinc concentration raised the question of the course of the decline during these first 24 hr, as well as the relationship of such a large change to any circadian variation in plasma zinc concentration.

Relatively little is known at present about circadian variations of zinc concentration in plasma. Marotta *et al.* (2) found diurnal changes of plasma zinc in rats under both normal and reverse lighting schedules. However, in their studies, the rats did not have food available to them for 2 to 10 hr before they were killed for blood samples, so that any diurnal variations under normal feeding conditions could not be discerned. Several investigators have studied changes in plasma zinc concentration in man during different periods of the day (3–7). Lifschitz and Henkin (8) found a circadian variation in serum zinc, with concentrations greater than the mean from 1000 to 2200 hr, and a sharp fall from 2200 to 0600 hr. The subjects in this study had a fairly normal pattern of feeding.

In the present investigation, we followed the concentration of plasma zinc during the first 24 hr after the institution of a zinc deficiency regime in rats. We evaluated the effect of dietary zinc deficiency on plasma zinc in relation to circadian variation and the pattern of food intake. The effect of fasting on plasma zinc concentration was also examined.

Materials and Methods. Virgin female rats of the Sprague–Dawley strain weighing 200 ± 10 g were obtained from a commercial source (Simonsen Laboratories, Gilroy, Calif.). They were housed in individual stainless steel cages on stainless steel racks and were provided with deionized distilled water and a complete purified diet containing soybean protein for a minimum of 7 days. Food and water were provided *ad libitum*. A 12-hr light/dark schedule beginning at 0800 was used throughout the experiment.

After the period of adaptation to the environment and the diet, the rats were randomly separated into groups. One group continued to be fed the complete purified diet containing 100 ppm zinc (control), while another group was fed from 0800 (t = 0) the purified diet without added zinc, which contained by analysis 0.4 ppm zinc (zinc deficient). Details of diet composition,

[1] This research was supported in part by NIH Research Grant HD-01743 from the National Institute of Child Health and Human Development.

0037-9727/82/050048-05$01.00/0

diet preparation, and the precautions taken to avoid contamination with zinc have been described previously (9). Groups of rats were anesthetized with ether and exsanguinated by cardiac puncture for plasma zinc analysis at 0800 (t = 0) and every 2 hr thereafter until 0800 (t = 24 hr). Between 1800 and 0200 a few single rats were killed and blood samples taken at hourly intervals. In two groups of rats, food intake was measured between 0800 and 0500.

In a second experiment, rats were given the zinc-deficient diet at either 0800 or 1600, while the control group continued to receive the control diet. The rats were killed at 2200 and blood was taken for plasma zinc analysis as described above. The effect of starvation on plasma zinc concentration was also measured. For this group of rats, food (but not water) was withdrawn at 0800; rats were killed and exsanguinated 2, 8, 24, and 48 hr later and plasma zinc concentration was analyzed. Zinc was analyzed by flame atomic absorption spectrophotometry using a Perkin–Elmer 370 spectrophotometer (Perkin–Elmer, Norwalk, Conn.).

Results. The rats fed the control diet exhibited a circadian variation in plasma zinc concentration (Table I and Fig. 1). From 0800 to 1600, the plasma zinc level remained constant, then it declined sharply between 1600 and 2000, from 138.8 to 104.1 μg zinc/dl plasma, a drop of 25%. From 2000 to 0400, plasma zinc concentration rose from 104 μg/dl to approximately 140 μg/dl, then fell slightly to its initial concentration by 0800 (t = 24 hr).

The food intake of the control rats followed a pattern different from that of their plasma zinc concentration (Fig. 1). From 0800 to 1600, the rats ate almost nothing. Thereafter, the cumulative food intake increased rapidly and continued to increase for the rest of the 24-hr period. The food intake of the rats fed the zinc-deficient diet did not differ significantly from that of the controls.

In the rats fed the zinc-deficient diet, on the other hand, the pattern of plasma zinc concentration was quite different from that of the controls (Table I and Fig. 1). As in the controls, the level of plasma zinc remained relatively stable from 0800 to 1600, and then declined. However, in the rats fed the zinc-deficient diet, unlike the controls,

TABLE 1. CIRCADIAN VARIATION IN PLASMA ZINC CONCENTRATION IN RATS FED EITHER CONTROL (100 ppm Zn), OR ZINC-DEFICIENT (0.4 ppm Zn) DIETS

	Diet group					
	Control		Zinc deficient			
Hour	N	Plasma zinc (μg/dl)	N	Plasma zinc (μg/dl)	df	t
0800	9	134.5 ± 3.09[a]	9	134.5 ± 3.09[b]		
1000	2	131.5 ± 11.00				
1200	6	128.9 ± 3.94	4	134.0 ± 7.48	8	0.64
1400	6	134.9 ± 6.12	4	138.3 ± 7.00	8	0.35
1600	6	138.8 ± 6.45	5	138.2 ± 7.35	9	0.06
1800	6	124.6 ± 7.25	5	121.6 ± 12.50	9	0.21
2000	6	104.1 ± 5.07	5	105.7 ± 11.58	9	0.14
2200	6	115.9 ± 5.01	5	82.1 ± 6.07	9	4.29*
2400	6	119.7 ± 5.68	5	62.5 ± 4.55	9	7.14*
0200	6	136.8 ± 4.41	5	79.4 ± 3.80	9	9.50*
0400	2	142.8 ± 4.75	1	79.5		
0600	2	129.3 ± 2.25	1	80.50		
0800	4	134.3 ± 7.73	4	61.3 ± 6.80	6	7.09*

[a] Data are shown as mean ± SEM.
[b] Same rats as 0800 control diet group (t = 0 control).
* Significantly different by Student's t test at $P < 0.01$.

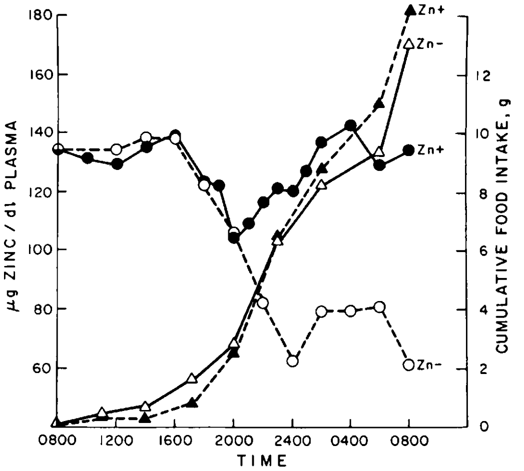

FIG. 1. Plasma zinc (μg/dl) and cumulative food intake (g) in rats given complete or zinc-deficient diets for 24 hr. ●, Plasma zinc, rats fed complete control diet; ○, plasma zinc, rats fed zinc deficient diet; ▲, cumulative food intake, rats fed complete control diet; △, cumulative food intake, rats fed zinc-deficient diet.

plasma zinc continued to drop after 2000, and was significantly lower than that of the controls at 2200. It continued to drop until 2400, when its concentration was only 62.5 μg/dl, significantly lower than that of the controls at this time. In fact, it was only 52% of the control value, and only 46% of the initial level at 0800. Thus, the plasma zinc concentration of rats was made significantly lower than that of controls by feeding them a zinc-deficient diet for 14 hr beginning at 0800. After 2400, the plasma zinc concentration of the rats fed the zinc-deficient diet rose slightly, and then fell again to 61.2 μg/dl at 0800 (t = 24 hr).

When rats were given the zinc-deficient diet at 0800 and killed at 2200, a time span of 14 hr, their plasma zinc concentration was significantly lower than that of rats fed the control diet for the same period (Table II). However, when the zinc-deficient diet was started at 1600, and the rats were killed at 2200, a time span of 6 hr, their plasma zinc concentration was also significantly lower than that of controls and was identical to that of the rats fed the deficient diet for 14 hr. Thus, 6 hr of the zinc deficiency regimen, if it was started at 1600, produced as great a decrease in plasma zinc as did 14 hr of this deficiency regime if started at 0800.

TABLE II. PLASMA ZINC AFTER 6 OR 14 hr OF ZINC-DEFICIENT DIET

Diet	*N*	Time started[a]	Time elapsed (hr)	Plasma zinc (μg/dl)
Control	12	0800	14	140 ± 5.1
Zn defic.	7	0800	14	118 ± 4.4*
Zn defic.	8	1600	6	119 ± 6.5*

[a] Time ended: 2200; all rats killed.
* Significantly lower than control, $P < 0.001$.

In the starved rats, plasma zinc showed no change at 1600, after 8 hr without food, and after 24 hr was still at its initial level (Table III). After 48 hr of starvation, however, the plasma zinc concentration was higher than it was initially, reaching a concentration of 150.8 μg/dl.

Discussion. These results demonstrate that normal rats experience a circadian variation in plasma zinc concentration, with a decrease of 25% at 12 hr after the beginning of a 24-hr light/dark period. This finding emphasizes the importance of careful attention to the timing of blood sampling for plasma zinc. Investigators may increase the variability of plasma zinc analyses if blood samples are not always taken at the same part of the circadian cycle. In addition to the necessity for consistency in the timing of blood sampling, these results suggest that the timing of blood sampling with reference to the light cycle should be identified in research reports, so that comparisons

TABLE III. PLASMA ZINC CONCENTRATION IN STARVED RATS[a]

Time killed	Hours starved	*N*	Plasma zinc (μg/dl)
	No.		
0800	0	9	134.5 ± 3.09[b]
1000	2	6	128.67 ± 5.49
1600	8	6	121.92 ± 7.32
0800	24	6	133.75 ± 5.16
0800	48	2	150.75 ± 3.25*

[a] Diet (100 ppm Zn) was withdrawn at 0800. Results are means ± SEM.
[b] See footnote *b* of Table I.
* Significantly different from 0 time group, $P < 0.05$.

among different studies or laboratories may be valid.

The pattern of plasma zinc concentration in normal rats appeared to be related to the food intake. From 0800 to 1600, when the plasma zinc level remained constant, the rats ate very little. Between 1600 and 2000, when food intake increased rapidly, plasma zinc declined sharply by 25%, and then rose to its initial concentration.

Results of the experiment in which the effect of starvation on plasma zinc concentration was investigated are consistent with these observations. Starvation did not cause a decline in plasma zinc concentration, but rather a constant value for 24 hr. After 48 hr, there was, in fact, a slight rise. Thus, the constant level of plasma zinc during the hours that the rats were not eating is similar to that shown by the starved rats, and can probably be explained by the release of zinc from body tissues as they are broken down during gluconeogenesis. Others (10, 11) have similarly found that during total starvation, humans showed no drop in plasma zinc concentration, but excreted increased amounts of urinary zinc. These results also demonstrate the important effect of food intake on the concentration of plasma zinc, and suggest that in clinical studies ignorance of the food consumption prior to blood sampling may result in an incorrect evaluation of zinc status.

The effect of starvation on plasma zinc concentration makes the results of Marotta *et al.* (2) difficult to evaluate, as the rats in their study did not have food available to them for 2 to 10 hr before they were killed.

It may be postulated that the drop in plasma zinc concentration at the time when the rats increased their food intake resulted from the suppression of tissue breakdown, accompanied as well by an outpouring of pancreatic enzymes (largely zinc containing) for digestion. The subsequent rise in plasma zinc may be conjectured to result finally from accumulation of dietary zinc.

The observation of the normal circadian cycle in plasma zinc concentration renders the effect of a zinc-deficient diet even more dramatic than was previously supposed. The plasma zinc concentration of the rats fed the deficient diet was the same as that of the controls until 2000; that is, it was relatively stable from 0800 to 1600, and then declined to 2000. However, unlike controls, in rats fed the zinc-deficient diet, plasma zinc continued to drop after 2000. Thus, the zinc concentration of the diet was crucial in determining whether or not the normal circadian cycle could occur. As both groups ate approximately the same amount of food, the rapid fall in plasma zinc level was apparently due to lack of zinc in the food consumed, rather than to the quantity of food.

The changes in plasma zinc concentration during the circadian cycle also determine the minimum duration of a zinc deficiency regimen required to produce a significant fall in plasma zinc. When the zinc-deficient diet was started at 0800, plasma zinc was significantly lower than that of controls 14 hr later. However, when the deficient diet was started at 1600, the time when the plasma zinc concentration starts to decline in the circadian cycle (and when the rats start eating), plasma zinc was significantly lower than in controls after only 6 hr. These findings provide dramatic evidence of the rapid effect of consumption of a zinc-deficient diet.

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Received December 16, 1981. P.S.E.B.M. 1982, Vol. 170.