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Effect of Zinc Deficiency and Restricted Food Intake on Plasma and Pituitary LH and Hypothalamic LRF in Female Rats

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Adult female rats were given a low zinc diet (<1 ppm) for 8–9 weeks after which some of the females were repleted with zinc on either a restricted food intake or an ad lib. intake. Repletion times were 10 days in experiment 1, 25 and 32 days in experiment 2, and 25 days in experiment 3. At the end of each experiment blood was collected by heart puncture, and pituitary and hypothalamic tissue was removed. Luteinizing hormone-releasing factor (LRF) was measured by injecting pooled hypothalamic extracts into the carotid arteries of male rats and measuring luteinizing hormone (LH) in blood collected 5 min later. LH was measured by radioimmunoassay of blood plasma from males and females and on pooled pituitaries from females. Plasma LH in the zinc-deficient and restricted-intake females was generally lower than in the controls. Plasma progesterone (measured in experiment 3 only) was significantly lower in the deficient and restricted-intake groups than in the controls. The low plasma LH in the zinc-deficient and restricted-intake females did not appear to be due to a lack of LRF, however, since LRF was generally as high in these females as in the controls. Values for LH and LRF in the zinc-deficient females were never higher than those in the controls, whereas in the restricted-intake females there were instances in which values for LH (experiment 2, 25-day repletion) and LRF (experiment 2, 32-day repletion) were significantly increased compared to the controls. Since the females that were subjected only to food restriction were at times able to increase their production of LH or LRF, whereas the zinc-deficient females were not, zinc deficiency appears to have an effect on reproduction in addition to its effect on food intake.

Zinc deficiency has been associated with impaired reproduction in several species. (For a review, see Underwood, 1971). Prior to its effect on reproduction, however, a low zinc diet also causes a decrease in food consumption. Since decreased food intake also interferes with reproduction (Leathem, 1966), it is difficult to determine how much of the effect of zinc deficiency is due to an effect of zinc on food consumption. In these experiments, LRF, plasma and adenohypophyseal LH, and plasma progesterone were measured to determine the relative effects of zinc deficiency and restricted intake on reproduction in the female rat.

MATERIALS AND METHODS

Adult female Sprague-Dawley rats (180–200 g) were obtained commercially and housed in stainless steel cages under a 14 h light–10 h dark schedule. Animals were fed a low zinc diet (<1 ppm) previously described (Apgar, 1970), and given deionized water, supplemented where indicated with 100 ppm zinc as zinc acetate. All animals received the low-zinc diet with no additional zinc for the first 8 or 9 wk of an experiment. At the end of this time, the females were divided into three groups, two of which were repleted

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with zinc. Three experiments were done with repletion times of 10 days in the first experiment, 25 and 32 days in the second experiment, and 25 days in the third experiment. In each experiment one group, designated zinc-deficient, continued to receive the low zinc diet. A second group, designated control, received the low zinc diet plus zinc-supplemented water. The third group, the restricted-intake group, received the zinc-supplemented water and food equal to (in the first experiment) or less than that eaten by the females in the zinc-deficient group. Females in the restricted-intake group were individually paired with females in the zinc-deficient group. Because of the longer repletion periods in experiments 2 and 3, it was necessary to restrict the intake to approximately 80% of that of the deficient females to prevent weight gain in the restricted-intake females. The restricted-intake females in experiments 2 and 3 were therefore eating approximately 60% as much as the ad libitum controls.

Females that were given zinc and food ad lib. came into estrus on the fourth or fifth day after repletion was begun and continued to cycle thereafter. A few of the restricted-intake females had erratic cycles but most were acyclic as were the zinc-deficient females. To minimize variations due to cycling in the ad lib. controls, these females were killed before 10 h. Vaginal smear records indicated that only two out of ten females were likely to be in estrus, however.

At the end of an experiment the females were bled by heart puncture and decapitated, and the pituitary and hypothalamus were removed and weighed. The pituitaries were rapidly frozen and the hypothalami placed in acetone. Pituitaries from each treatment were pooled and adenohypophyseal LH prepared according to the procedure of Reichert and Midgley (1968) with modifications due to the small amount of tissue. Attempts were made in preliminary experiments to measure LH in homogenates of individual pituitaries. Increasing concentrations of the homogenate did not, however, give a linear response in our system. Since extracted LH did assay linearly, the pituitaries were pooled to provide sufficient material for extraction.

LRF was prepared from the pooled hypothalami by grinding with sand in cold acetone, centrifuging, and extracting the precipitate overnight with 2 M acetic acid. The acid extract was centrifuged, neutralized with concentrated NaOH, and the volume adjusted to 10 ml. Aliquots of the extract were injected into the carotid artery of adult male Long-Evans rats under ether anesthesia in experiments 1 and 2, and pentobarbital in experiment 3. One milliliter of the extract, equivalent to two hypothalami, was injected in experiment 1. The large amount of crude material caused respiratory difficulties, however; therefore the dose was reduced to 0.5 ml, equivalent to one hypothalamus, in experiments 2 and 3. Since preliminary results had indicated no difference in LH release from physiological saline or cortical extract, physiological saline was injected in the control males. *In vivo* assays similar in principle to that described here have been used by other investigators (Amoss and Guillemin, 1969; Debeljuk, Arimura and Schally, 1972).

LH was assayed by solid phase radioimmunoassay as described by Hobson and Hansel (1972). Rat LH standard, NIAMD-Rat LH-I-1 used for iodination, and NIAMD-Rat LH-Rp-1 used for the competing LH, was provided by the National Institute of Arthritis and Metabolic Diseases. The antibody used was anti-bovine LH prepared in a mare by Snook, Saatman, and Hansel (1971). The affinity of rat LH for the antiserum, although not as great as bovine LH, was still very high as is shown by Fig. 1. In each assay three plasma pools of known LH concentration were assayed. Variations in any of the three pools were never more than 0.2 ng/ml.

In experiment 3, plasma progesterone was determined by the competitive protein-binding assay of Murphy (1967) with a human male source of corticosterone-binding globulin.

RESULTS AND DISCUSSION

As shown in Table 1, plasma LH in zinc-deficient and restricted-intake females was generally lower than in the controls. In experiment 2, 25-day repletion, however, plasma LH in the deficient females was the same as in the controls and plasma LH in the restricted-intake females was higher than in the controls. Only in experiment 2, 32-day repletion, was the value for the plasma of the zinc-deficient females significantly less than that for the restricted-intake females. Female rats on 50% food restriction have been previously reported to have lower serum LH values than controls (Howland, 1972, 1971). Plasma progesterone, measured in experiment 3, was significantly lower in the zinc-deficient and restricted-intake females (27 ± 6 and 34 ± 7 ng/ml, respectively) than in the controls (46 ± 8 ng/ml), which is consistent with the lower plasma LH in the two groups.

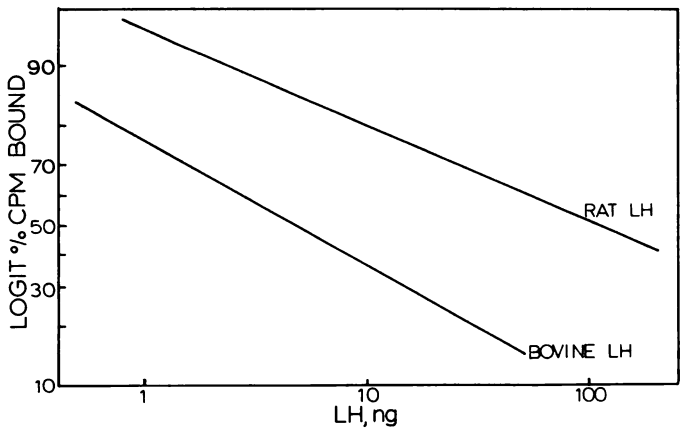

FIG. 1. Comparison of radioimmunoassay of rat and bovine LH using antibody to bovine LH prepared in a mare.

The lower plasma LH in the zinc-deficient and restricted-intake groups does not appear to have been due to decreased LRF, however. Only in experiment 1 was the apparent LRF of the zinc-deficient females significantly lower than the controls. In no case was the apparent LRF of the restricted-intake females significantly lower

TABLE 1

EFFECT OF LOW ZINC AND RESTRICTED-INTAKE DIETS ON PLASMA LH AND PROGESTERONE, APPARENT HYPOTHALAMIC LRF, ADENOHYPOPHYSEAL WEIGHT AND LH CONCENTRATION, AND BODY WEIGHT

	Experiment 1	Experiment 2		Experiment 3
	10-day repletion	25-day repletion	32-day repletion	25-day repletion
Plasma LH (ng/ml)				
Zinc-deficient	78 ± 4 (19)[a]	65 ± 9 (10)	3 ± 3 (9)	13 ± 4*** (11)
Restricted-intake	76 ± 4 (19)	113 ± 22* (10)	32 ± 6* (9)	5 ± 1 (11)
Control	115 ± 10* (19)	68 ± 16 (9)	46 ± 7** (9)	47 ± 16* (11)
Apparent LRF,[b] (ng/ml)				
Zinc-deficient	205 ± 15 (11)	114 ± 14 (8)	160 ± 7 (8)	64 ± 7 (10)
Restricted-intake	242 + 18*** (11)	146 ± 13 (8)	280 ± 43* (7)	87 ± 7 (10)
Control	277 ± 25* (11)	110 ± 12 (7)	154 ± 18 (8)	76 ± 10 (7)
Saline-injected control	101 ± 9** (11)	99 ± 11 (8)	71 ± 5* (8)	34 ± 9* (10)
Adenohypophyseal LH concentration (ng/μg protein)[c]				
Zinc-deficient	Not measured	47	48	135
Restricted-intake	Not measured	47	48	153
Control	Not measured	42	44	111
Adenohypophyseal weight (mg)				
Zinc-deficient	Not measured	7.7 ± 1.0 (10)	5.5 ± 0.6 (9)	7.7 ± 0.4 (12)
Restricted-intake	Not measured	8.0 ± 1.0 (10)	6.3 ± 0.8 (8)	7.9 ± 0.6 (12)
Control	Not measured	11.9 ± 0.6* (10)	11.7 ± 1.0* (9)	12.9 ± 0.9* (12)
Body weight (g)				
Zinc-deficient	188 ± 4 (20)	174 ± 4 (10)	176 ± 4 (9)	155 ± 4 (12)
Restricted-intake	191 ± 3 (20)	172 ± 4 (10)	179 ± 6 (8)	157 ± 4 (12)
Control	233 ± 4* (20)	235 ± 3* (10)	253 ± 6* (9)	222 ± 6* (12)

[a] Mean ± standard error. Number of observations is given in parentheses.

[b] Measured by rise in plasma LH in males injected with female hypothalamic extract.

[c] Based on absorption at 280 nm. LH values are based on pooled samples.

* Significantly different ($P < 0.05$, by Duncan's multiple range test) from unmarked and double-asterisk marked values within each experimental group.

** Significantly different ($P < 0.05$, by Duncan's multiple range test) from all other values within each experimental group.

*** Not significantly different from unmarked and single-asterisk marked values.

than the controls, and in experiment 2, 32-day repletion, it was significantly higher than the controls. The extracts in experiment 2, 25-day repletion, were apparently inactive, since none of the means were significantly different from the saline-injected controls. Low plasma progesterone, as observed in experiment 3, could result in increased LH owing to a decreased negative feedback as appears to be the case in heifers fed rations that are deficient in energy (Gombe, 1972). However, plasma LH levels were low in both the zinc-deficient and restricted-intake females, despite the fact that they had amounts of LRF equal to the controls.

Although the concentration of LH in the pituitaries of the three groups was not different (Table 1), the total content of LH in the pituitaries of the zinc-deficient and restricted-intake females would have been less because of the significantly smaller size of their pituitaries. It may be that the smaller pituitaries bound less of the LRF, or the LRF in these females may have remained stored in the hypothalamus and never reached the pituitaries. A 50% restriction of food intake has been previously reported to cause no change in the concentration of LH in the pituitary (Howland, 1972, 1971) and to lower the concentration of LH in the pituitary to one-third of that in the controls (Piacsek and Meites, 1967). The more recent results of Ibrahim and Howland (1972) show that pituitary LH concentrations are higher in starved ovariectomized rats than in fed ovariectomized rats. Collectively, the results seem to indicate that the ability of the pituitary to synthesize gonadotropins is not impaired by restricted intake.

From our experiments, it would appear that low plasma LH is a frequent, though not invariable, characteristic of both zinc deficiency and restricted food intake in the rat. Millar *et al.* (1960) also concluded from hormone replacement experiments in young male rats on a zinc-deficient diet for 6–8 wk after weaning that pituitary gonadotropin output was inhibited in zinc deficiency, primarily due to the restricted food intake of the zinc-deficient males.

Although it seems clear that restricted intake and zinc deficiency both cause a decrease in LH secretion, we were unable to show that this decrease was due to a reduction in hypothalamic LRF, as suggested by the *in vitro* measurements of LH release carried out by Piacsek and Meites (1967). This question deserves further study, especially in view of the recent suggestion of Ibrahim and Howland (1972) that impaired hypothalamic function in underfed intact rats may be due to a change in hypothalamic sensitivity to steroid inhibition. The results reported here also indicate that the effects of food restriction and zinc deficiency are not necessarily the same and that a lack of zinc has an effect on reproduction in addition to its effect on food consumption.

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