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Effect of dietary zinc deficiency on reproductive function in male rats: Biochemical and morphometric parameters

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The effect of zinc on reproductive function was studied in rats fed with a zinc-deficient diet and control rats fed with a standard diet.

Serum testosterone (T) levels were measured before and after the injection of HCG (human chorionic gonadotropin). Serum gonadotrophin levels were measured before and after the injection of GnRH (gonadotropin releasing hormone). In addition, structural parameters were studied and morphometric techniques were used to study the seminiferous tubules. The findings were used to investigate the possible relationship between biochemical and morphometric changes. Body weight (BW) gain, zinc content of testes and weight of the testes, seminal vesicle, and prostate were significantly lower in zinc-deficient rats compared with controls. Serum zinc concentrations and alkaline phosphatase activity were decreased in zinc-deficient rats compared with controls. Serum basal and stimulated FSH concentrations were similar in the two groups. Serum LH response to GnRH was higher in zinc-deficient rats. Serum basal T was lower in zinc deficient animals, but the response of T to HCG was similar in the two groups. The basal and luminal diameters, perimeter, and surface area of the seminiferous tubules as well as tubule volume, wall thickness, and cross-section area decreased in zinc-deficient animals in comparison with controls. Concentrations of zinc were significantly correlated with structural parameters and serum T levels. The changes in T levels correlated positively with the structural parameters and morphological findings. (J. Nutr. Biochem. 7:403–407, 1996.)

Keywords: dietary zinc deficiency; testosterone; FSH; LH; seminiferous tubule; male rats

Introduction

Zinc has been known to be essential for animals and humans for many years. The causes of zinc deficiency include, in addition to genetic disorders, malnutrition, alcoholism, malabsorption, chronic renal disease, and certain diuretic and chelating agents.[1]

Hypogonadism is a prominent manifestation of zinc deficiency in animals and humans.[2] Although many studies have investigated this relation, the underlying mechanisms are unclear. Zinc fingers are present in nuclear receptors for testosterone (T)[3] and in the protein gene of spermatocytes.[4,5] Decreased zinc concentrations were observed in the testes of rats with testicular feminization.[6] Zinc deficiency decreases serum and testicular T levels,[7,8] and also decreases hyaluronidase in both the testis and epididymis.[9] Other effects of zinc deficiency include modifications in essential fatty acid status and desaturation of microsomal membranes in testes,[10] decreased or increased serum gonadotropin levels,[11,12] and impaired development of accessory sex glands,[13] depleted seminal volume, and oligospermia.[14] Histological abnormalities include alterations in Leydig cells[15] and degeneration of the cellular layer constituting the seminiferous tubules.[16]

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We investigated the effects of zinc deficiency on the reproductive function in zinc-deficient and control rats by measuring plasma T levels before and after the injection of human chorionic gonadotropin (HCG), and plasma gonadotropin levels before and after the injection of gonadotropin releasing hormone (GnRh). In addition, structural and morphometric data were obtained in the seminiferous tubules to search for relationships between endocrine and structural changes.

METHODS AND MATERIALS

Animals

Male Wistar rats weighing 75–85 g at the beginning of the experiment were housed under controlled light and temperature conditions. Animals in the zinc-deficient group were fed with a zinc-deficient diet containing 12% humidity, 17% protein, 5.5% lipids, 5.4% fibers (cellulose), 1% vitamins, 6% minerals (1.25 mg zinc/kg diet), and 54.1% starch during 6 weeks until the day of the experiment. Rats in the control group, were fed with a standard pellet chow containing 10.54% humidity, 17.62% protein, 2.5% lipids, 8.5% fibers (cellulose), 1% vitamins, 6% minerals (95 mg zinc/Kg diet), and 53.30% starch during 6 weeks until the day of the experiment. Food and doubly distilled water were available ad libitum in both groups.

The rats were weighed weekly, and were carefully monitored and maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Blood collection

Basal blood was collected at 9.00 a.m. and was centrifuged at 4°C, in trace metal free plastic tubes. The serum was frozen and stored at −20°C until the assays were performed.

Serum and testes zinc levels were determined by atomic absorption spectrophotometry (Perkin Elmer model 2100) according to a previously published method.[17] Serum alkaline phosphatase activity was determined in an automatic analyzer apparatus (Hitachi 704).

Hormone assay

Serum LH and FSH were measured using a double antibody Radioimmunoassay (RIA) procedure with materials provided by the NIADDK. The reference standard preparations were rats LH-RP2 for LH and rats FSH-RP2 for FSH. A commercial RIA NIADDK (Bethesda, MD, USA) was used for serum T determinations.

Hormone levels in serum from all animals were analyzed in duplicate and measured in the same assay. Intraassay coefficients of variation were 6% for LH, 8% for FSH, and 8% for T.

Both zinc-deficient and control animals were divided into two subgroups for tests of HCG and GnRH.

HCG response

In zinc-deficient and control animals, single blood samples were obtained from the tail under light ether anesthesia to determine basal T levels. One hour thereafter, an ip injection of HCG (Phisex Leo) 30 μg/100 g BW in 0.5 mL 0.9% NaCl was given, and 60 min after injection blood was collected by decapitation.

LHRH response

In zinc-deficient and control animals, single blood samples were obtained from the tail under light ether anesthesia to determine basal LH and FSH levels. One hour thereafter, an ip injection of LHRH 80 μg/100 g BW in 0.5 mL 0.9% NaCl was given, and 30 min after injection, blood was collected by decapitation.

Morphometric procedure

After decapitation, the testes, seminal vesicles, and prostate were removed from the animals and weighed on a precision balance. The testes were fixed in 5% formaldehyde. Sections were prepared by standard methods and stained with hematoxylin and eosin. Accepted morphometric procedures using an image processor (IBAS 2000 Sorin Biomedical, Vercelli, Italy) were used to obtain quantitative information on the seminiferous tubules. In each seminiferous tubule both basal and luminal diameters were measured across the minor and major axes, using an ocular micrometer calibrated with a stage micrometer under a 9 × 10 objective. Basal and luminal perimeter and basal and luminal area were also measured. Tubular cross-section area was calculated by the formula: basal area − luminal area. Tubular wall thickness in transverse section was calculated using the formula: mean basal diameter − mean luminal diameter. Seminiferous tubule volume was calculated as relative tubular area in testicular cross-section × testicular volume. Testicular volume in cubic centimeters was calculated according to Hess et al.[18]

In other groups of zinc-deficient and control rats, both testes were removed and frozen at −80°C in liquid nitrogen, then stored at −40°C for zinc analysis.[19]

Statistical evaluation

Significant differences between zinc-deficient and control rats were searched for with Student's *t* test. Pearson's correlation coefficient between different parameters was also determined.

Results

Serum zinc concentrations were significantly decreased in zinc-deficient rats in comparison with controls (74.65 ± 10.9 versus 169.40 ± 17.3 μg/dL, $P < 0.001$). Serum alkaline phosphatase activity decreased in zinc-deficient rats compared with controls (220 ± 58.5 versus 425 ± 85 I.U./L, $P < 0.001$). Zinc content of testes was significantly lower in the zinc-deficient in comparison with control rats (148.2 ± 14 versus 187.8 ± 12 μg/g dry weight, $P < 0.001$).

Zinc-deficient rats ate less food than control animals (3.396 versus 4.416 g/day). Body weight gain; wet weight of testes, seminal vesicles, and prostates were significantly lower in zinc-deficient animals compared with controls (*Table 1*).

Serum basal T was significantly decreased in zinc-deficient animals in comparison with controls, whereas the response of T to HCG was similar in the two groups (*Table*

Table 1 Body, testes, prostate, and seminal vesicles (SV) weights (g) of zinc deficient and control rats

	Zn deficients	Controls
Body	170,9 ± 24,0 **	269,2 ± 29,00
Testes	2,3 ± 0.4 **	2,8 ± 0,29
Prostate	0,13 ± 0,06 **	0,67 ± 0,20
SV	0,49 ± 0,2 **	1,20 ± 0,20

Values are mean ± SD, $n = 20$ for each group.
** $P < 0,01$ vs control.

Table 2 Effect of Zn deficiency on basal and estimulated values of testosterone (T), FSH and LH

	Zn deficient	Controls
Basal T	0,23 ± 0,13*	0,51 ± 0,16
T after HCG	11,38 ± 4,71	7,77 ± 1,86
Basal LH	1,18 ± 0,41	1,34 ± 0,29
LH after GnRH	7,20 ± 2,00*	5,21 ± 1,11
Basal FSH	3,76 ± 1,04	3,54 ± 0,64
FSH after GnRH	6,43 ± 2,70	5,45 ± 1,45

Values are mean ± SD, $n = 10$ for each group, $^*P < 0,05$ vs control. Data are expressed in ng/ml.

2). Serum basal LH and FSH were similar in zinc-deficient and control animals. Serum LH and FSH increased after GnRH in all animals studied. In zinc-deficient rats, GnRH elicited a significantly higher increase in LH than in controls, whereas the response of FSH to GnRH was similar in the two groups.

Mean basal and luminal diameters, basal and luminal perimeter, and basal and luminal areas of the seminiferous tubule are shown in *Table 3*. There was a significant decrease in these values, as well as in the tubular cross-section area, tubular wall thickness, and tubule volume in zinc-deficient animals in comparison with controls.

There was a significant positive correlation between T and zinc levels and structural parameters, but no correlation between these parameters and changes in FSH or LH levels (*Table 4*). In addition, there was a significant positive correlation between testosterone levels and morphometric parameters in the seminiferous tubules (*Table 5*).

Discussion

Feeding rats a low zinc diet for 6 weeks markedly reduces zinc content of testes, serum zinc concentrations, and alkaline phosphatase activity. Plasma zinc concentrations coupled with measurement of alkaline phosphatase activity has been suggested as an useful index of zinc deficiency.[20] The results indicate that the measure of both parameters may serve as a diagnostic test of zinc deficiency in humans. The weight of the testes, prostate, and seminal vesicles were

Table 3 Effect of Zn deficiency on morphometric parameters of seminiferous tubules. Areas (A), Perimeters (P), Diameters (D), Volume

	Zn deficient	Control
Basal D (mm)	0,2419 ± 0,0527*	0,2641 ± 0,0498
Luminal D (mm)	0,0764 ± 0,0323*	0,1006 ± 0,0330
Tubular thickness (mm)	0,0474 ± 0,0090*	0,0678 ± 0,0075
Basal P (mm)	0,7563 ± 0,0578*	0,7980 ± 0,0451
Luminal P (mm)	0,2873 ± 0,0355*	0,3152 ± 0,0497
Basal A (mm^2)	0,0408 ± 0,0125*	0,0506 ± 0,0130
Luminal A (mm^2)	0,0061 ± 0,0041*	0,0081 ± 0,0043
Cross-section A (mm^2)	0.0392 ± 0.0061*	0.0430 ± 0.0048
Volume (mm^3)	5.8 ± 1.2***	10.3 ± 1.6

Values are mean ± SD, $n = 20$ for each group, $^*P < 0,05$, $^{***}P < 0.001$ vs control.

Table 4 Correlation coefficient between hormonal or Zn levels and body, testes, and prostate seminal vesicles (SV) weights

	T	FSH	LH	Zn
Body	0.64**	0.2	0.1	0.23
Testes	0.62**	0.2	0.2	0.61**
Prostate	0.65**	0.1	0.2	0.85***
SV	0.65**	0.2	0.3	0.69**

$^{**}P < 0.01$, $^{***}P < 0.001$.

significantly reduced in zinc-deficient rats (*Table 1*), which corresponds to previous reports.[12,13,21] We found a positive correlation between these structural parameters and serum T and zinc levels (*Table 4*). Because body growth and the food consumption in zinc-deficient rats were low, inanition alone may be directly responsible for the effects of zinc-deficiency on male reproductive organs. Earlier studies[8,12] have found that caloric restriction did not inhibit normal growth and maturation of the testes in adult male rats. Dietary zinc deprivation also impaired gonadal growth in sexually immature rats when compared with pair-fed control rats.[22] Therefore, in zinc-deficient rats, other, more specific changes are probably responsible for the reductions, at least in testicular weight.

The basal values of LH and FSH were similar in zinc-deficient and control rats. Contradictory data have been published on this point. Lei et al.[12] found that basal serum FSH values were much higher, and LH values much lower, in zinc-deficient rats than in controls, whereas Hafiez et al.[11] found similar values for LH and FSH in both groups.[22] Differences in the age of initiation and the duration of zinc-deficiency may explain the lack of consistent results.

After a single injection of GnRH, the response of FSH was similar in both groups, whereas the response of LH was greater in zinc-deficient than in control animals, possibly because of a decrease in the negative feedback of T on LH.[12] In zinc-deficient animals, LH reserves may be increased due to the lack of androgenic expression in gonadotrophic cells.[21,23]

Serum T levels were significantly lower in zinc-deficient than in control animals, in accordance with previous reports.[7,8,11] These data, together with the basal values of LH and FSH, suggest that the influence of zinc in the male reproductive system centers mainly on the testicular level. In this connection, zinc fingers have been found in the tes-

Table 5 Correlation coefficients between hormonal levels and morphometric parameters of seminiferous tubule

	T	FSH	LH
Basal (D)	0.52*	0.09	0.09
Luminal (D)	0.59*	0.06	0.28
Basal (P)	0.67**	0.21	0.37
Luminal (P)	0.56*	0.01	0.09
Basal (S)	0.54*	0.06	0.07
Luminal (S)	0.53*	0.05	0.23

D = Diameter, P = Perimeter, S = Surface, $^*P < 0.05$, $^{**}P < 0.01$.

tis-determining Y genes of the mouse, and in the protein gene of spermatocytes.[5,6] In zinc-deficient animals, testicular RNA, DNA, and protein biosynthesis are decreased,[24] and decreased activity of hydroxysteroid dehydrogenase, a key enzyme in T biosynthesis, has been reported.[25] In addition, Vera Gil et al.[26] located zinc in several cellular components in the Leydig cells, using histochemical techniques.

The response of T to HCG was similar in zinc-deficient and control animals. This indicates that the Leydig cells did not lose their capacity for T biosynthesis. The testes of zinc-deficient animals may be more refractory to LH than those of controls, making the basal values of T lower in zinc-deficient animals, but not after a supraphysiological dose of LH (HCG). In vitro studies have shown that zinc plays an important role in the HCG-stimulated production of cyclic AMP and T.[27]

In zinc-deficient rats we noted decreases in seminiferous tubular diameter, perimeter, and both basal and luminal areas (*Table 3*). Previous reports described severe atrophic changes in spermatogenic cells in zinc-deficient animals[28] or degeneration of the cellular layer constituting the seminiferous tubules.[16] However, morphometric data like those reported in this paper were not supplied. We interpret the decrease in luminal area as an indicator of reduced Sertoli cell fluid production,[29] as the Sertoli cell is the primary cell type that secretes fluid into the tubular lumen.[28] A unexpected finding in the present study was the lack of increase in basal FSH in zinc-deficient animals despite the decrease in Sertoli cell fluid, because FSH, although modulated by T and estradiol, is mainly affected by inhibin produced by Sertoli cells.[30] A possible explanation could be that the zinc-deficiency decreased only some proteins secreted by Sertoli cells. In addition to inhibin, other proteins such as ABP (androgen binding protein), transferrin, and testin are also secreted by Sertoli cells.[30,31]

The decrease in tubular cross-section area in our zinc-deficient rats may indicate a decrease in the number of germinal and Sertoli cells, in accordance with a previous report.[16]

We found a positive correlation between T levels and morphometric characteristics in the seminiferous tubules. In contrast, there was no significant correlation between structural changes and either serum LH or FSH levels, probably because of the complicated paracrine regulation of the testis and the importance of cell-to-cell interactions within the testis.[32]

In summary, we have found that in zinc-deficient rats there is a marked decrease in T levels and in the weight of the reproductive organs, in addition to morphological alterations in the seminiferous tubules.

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