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RESEARCH ARTICLE

IMPACT OF ZINC DEFICIENCY ON HORMONES SECRETED BY ANTERIOR PITUITARY OF MALE WISTAR RATS

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ABSTRACT

This study was aimed to evaluate the effects of zinc deficiency on secretion of anterior pituitary hormones of male Wistar rats. Experiments were set for 2 and 4 weeks. For this study thirty albino male Wistar rats (pre-pubertal period) weighing 40-50 gm were divided into three groups: Zinc Control (ZC), Zinc Deficient (ZD) and Pair Fed (PF). ZC and PF groups were fed with 100 ppm zinc and ZD group were fed with 1.00 ppm zinc in the diet. For hormonal assay blood samples were collected from experimental group through cardiac puncture by using heparinized disposable syringe. Serum and plasma were stored at -20ºC until assayed. Serum FSH, LH, Prl, TSH, GH and plasma ACTH were determined by Chemi Luminescent Immuno Assay (CLIA). The results showed that serum FSH, LH, GH, PRL, TSH as well as plasma ACTH level exhibited a significant ($P<0.05$) decrease when ZD (2- and 4- weeks) group were compared with their respective controls. However serum GH level showed a non-significant decrease on comparison between PF and ZD (2- and 4- weeks) groups. Meanwhile, prolactin level exhibited a non-significant decrease on comparison between 2 PF and 2 ZD and significant decrease when comparison was carried between 4 PF and 4 ZD. In conclusion, it has been observed that a low level of zinc affects secretion of pituitary hormones thus affecting overall physiology and functioning of the body. The impact of zinc deficiency was more pronounced after four week of experiment.

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INTRODUCTION

Zinc, is a critical dietary essential trace element for humans and animals which is involved in numerous metabolic processes, i.e., as a catalyst, structural element, regulatory ion and in signaling pathways (Homma, 2016 and Prasad, 1995). It regulates normal growth and development, immune function, wound healing, proper sense of taste and smell, protein synthesis, DNA synthesis and cell division (Fabris, 1995 and Maret, 2006 and Solomons, 1998). Zinc is also required for maintaining the structural integrity of approxiamtely 300 metalloenzymes (Coleman, 1992). The human genome encodes approximate 2500 transcription factors or 8% of the human genome (Andreini 2006 and Maret 2013). Zinc is mostly concentrated in the testes, muscles, liver, bones, kidney and liver, with highest concentrations in the prostate and eye (Pfeiffer, 1982 and Roohani, 2013). The average daily requirement of zinc is 15-20 mg/day (Halsted, 1974 and Chasapis, 2012).

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Dietary sources of zinc are red meat, poultry, fish, other seafood, legumes, nuts, whole grains, and dairy products (Rimbach, 1996). Zinc is absorbed by small intestine, in which fibers, phosphates, calcium and copper interfere its absorption (Krebs, 2000). Acrodermatitis enteropathica is the inherited or acquired form of zinc deficiency (Kawamura, 2016). Zincplays vital role in the hormone secretion and present in secretory granules of cells of the endocrine and exocrine organs i.e. testes, prostate, epididymis, pancreas, submandibular glands, Paneth cells and bones (Danscher, 1996; Sorensen, 1997; Stoltenberg, 1996; Frederickson, 1987 and Danscher, 1985). The anterior pituitary is involved in the synthesis, storage and release of peptide hormones (Bonnemaison, 2016). In rat pituitary gland, zinc is present in very high concentration ~74ng/mg of dry weight showed by Particle induced X-ray emission (PIXE) measurement (Thorlacius-Ussing, 1987). Zinc is also involved in the synthesis of prolactin (Login, 1983). In human and rat pituitary gland, soluble dimer complexes are formed in secretory vesicles of hormones and zinc ions are required for their proper aggregation (Cunningham Cunningham, 1991). Zinc deficiency is a common problem in developing countries but many are unaware of the effects of zinc deficiency in their body.

Thus, to explore the impact of zinc deficiency on pituitary hormone secretion we set out this experiment because it affects every physiological aspects of the body.

MATERIALS AND METHODS

Thirty albino male Wistar rats (30-40 days of age, pre-pubertal period) with a mean weight of 45 gm were used in this study. The animals were housed at 22°C temperature, with a equal light-darkness period (12: 12 h L/D cycle) and were allowed free access to standard rat pellet diet (Aashirwad Industries Ltd., Chandigarh) and tap water. Animals were subdivided into three groups. Zinc Control (ZC) group - 100 ppm zinc was provided in basal diet and tap water was given ad libitum, Zinc Deficient (ZD) group- 1.00 ppm zinc was provided in basal diet and dimeneralized water was given ad libitum and Pair Fed (PF) group - received the same diet given to the control group but the amount of basal diet was equal to the average amount of diet consumed by zinc deficient group in the previous day and tap water was given ad libitum. PF group was run for study of starvation effects of the diet. The basal diet was prepared according to ICN Research Diet protocol (1999) and by addition of zinc sulphate (concentration of zinc in the diet was adjusted to 1.0 ppm and 100 ppm). The experiments were set for 2 and 4 weeks. Animals from experimental groups were anaesthetized under light ether anaesthesia. There after, 5 ml of blood sample were collected from each experimental animal by cardiac anesthetic puncture using heparinized disposable syringe, collected in plastic vials and blood was left in sterile plastic vial for twenty minutes for allowed to clot at room temperature. Then every blood sample was centrifuged at 2500 rpm for ten minutes in Remi-R8C laboratory centrifuge. Serum/plasma were collected by pipette and stored at -20^{0}C till hormonal assay by using Chemi Luminescent Immuno Assay (CLIA) Hormone Test kits, the average was recorded. The results were analyzed statistically and data are expressed as mean ± SEM. After that One way Analysis of Variance (ANOVA) was carried out and if significant difference was found then post-hoc test (Duncan's Multiple Comparison test) was carried out. P<0.05 were considered significant.

Table 1. Hormone assay of male Wistar rats after 2 week experiment (Mean ± SEM)

Groups	2 ZC	2 PF	2 ZD
FSH (mIU/ml)	1.12 ± 0.002	1.11 ± 0.002	0.57 ± 0.004 b*c*
LH (mIU/ml)	0.11 ± 0.003	0.11 ± 0.004	< 0.10 b*c*
GH (uIU/ml)	1.26 ± 0.007	1.23 ± 0.009	0.85 ± 0.02 b*
PRL (ng/ml)	0.65 ± 0.002	0.62 ± 0.001	0.40 ± 0.001 b*
ACTH (pg/ml)	10.53 ± 0.02	10.48 ± 0.01	7.70 ± 0.35 b*c*
TSH (uIU/ml)	0.98 ± 0.003	0.97 ± 0.002	0.77 ± 0.004 b*c*

* Significant (P < 0.05)
* Significant (P < 0.05)
a - ZC Vs PF
b - ZC Vs ZD
c - PF Vs ZD

RESULTS

The index study showed that after two and four weeks of oral zinc supplementation in albino wistar rats, statistically significant (p<0.05) decrease in serum levels of follicle stimulating hormone (FSH), leutinizing hormone (LH), growth hormone (GH), prolactin (PRL), thyroid stimulating hormone (TSH) as well as adrenocorticotrophic hormone (ACTH) was observed in ZD group when compared with their control groups.

Table 2. Hormone assay of male Wistar rats after 4 week experiment (Mean ± SEM)

Groups	4 ZC	4 PF	4 ZD
FSH (mIU/ml)	1.36 ± 0.004	1.35 ± 0.003	0.34 ± 0.004 b*c*
LH (mIU/ml)	0.12 ± 0.002	0.11 ± 0.003	< 0.10 b*c*
GH (uIU/ml)	3.14 ± 0.02	3.09 ± 0.01	1.95 ± 0.06 b*
PRL (ng/ml)	0.99 ± 0.003	0.89 ± 0.004	0.19 ± 0.003 b*c*
ACTH (pg/ml)	12.43 ± 0.08	11.42 ± 0.05	9.25 ± 0.04 b*c*
TSH (uIU/ml)	1.04 ± 0.03	0.99 ± 0.02	0.55 ± 0.04 b*c*

a - ZC Vs PF
b - ZC Vs ZD
c -PF Vs ZD

No significant differences were observed in serum levels of GH when comparison was carried out between PF and ZD (2- and 4- weeks) groups. The results also revealed non-significant decrease of serum prolactin level when 2 PF was compared with 2 ZD. However, a significant (p< 0.05) decrease of serum prolactin level were observed when 4 PF was compared to 4 ZD group (Table 1 and 2).

DISCUSSION

In the index study, serum levels FSH and LH hormones were significantly decreased when compared to their respective control groups. Several authors have reported the role of zinc in the synthesis and secretion of gonadotrophin-releasing hormone, LH and FSH and sex hormones, including testosterone (Egwurugwu, 2013 and Joshi, 2014). Decreased output of pituitary gonadotropins and androgen production after zinc deficiency were observed in male rats by Howland (1971), who reported smaller size of the pituitaries and decreased total LH content in the pituitaries (Howland, 1971). It may be possible that smaller pituitaries bind lesser to LRF or LRF, which may remain stored in the hypothalamus and never reach the pituitaries (Howland, 1971). Contrary to this Root *et al.* (1979), observed increased levels of LH in zinc deficient mature rats (Root, 1979), while contrary results were reported by the author Hafiez *et al.* (1989), who revealed no effect of zinc deficiency on FSH and LH levels in the serum of zinc deficient rats (Hafiez, 1989). Contrary to these observations, Lei *et al.* (1976) reported high levels of FSH and low levels of LH in zinc deficient rats (Lei, 1976). Author observed inverse correlation between serum zinc and FSH levels in men (Ebisch, 2006). Zinc administration produced controversial results on FSH and LH secretion among various studies. Serum FSH and LH levels decreased in ZD group in the index study may be due to degenerative changes associated with zinc deficiency in the pituitary gland and decreased output of releasing hormones from the hypothalamus after zinc deficiency.

Serum GH levels decreased significantly in the present study in zinc deficient groups after two and four week experiment. Certain authors have reported the role of zinc ions in synthesis, storage or release of growth hormone (MacDonald, 2000 and Miletta, 2013). Zinc facilitates proper storage and secretion of GH in secretory vesicles of rat pituitary with the help of zinc transporter ZnT5 (Petkovic, 2014). Our findings were supported by Rocha et al. (2015) who reported stimulated consumption of macronutrients, improved plasma alkaline phosphatase levels in serum and increased hormones of the GH-IGF1 (growth hormone-insulin-like growth factor 1) system in children after zinc administration (Rocha, 2015). Several authors have reported that zinc deficiency results in failure of GH secretion from the pituitary gland (Root, 1979; Martin, 1994; Cole, 2008). Hamza *et al* (2012) reported low levels of serum IGF-1 and insulin growth factor binding protein-3 (IGFBP-3) in children after zinc deficiency (Hamza, 2012). In the present study decreased serum GH levels in ZD group can be correlated with reduced activity of zinc transporters and decreased concentration of insulin like growth factor binding proteins due to zinc deficiency. In this study, serum levels of PRL were decreased significantly in zinc deficient rats when compared with their respective control groups. Certain authors have reported that PRL aggregation, intracellular processing and secretion were facilitated by zinc (Login, 1983 and Judd, 1984 and Greenan, 1990). Recently, it had been observed that zinc prevents the kallikrein-mediated cleavage of PRL (Lorenson, 1996). Our findings were supported by Hafiez *et al.* (1989) who reported low serum prolactin (PRL) levels in zinc deficient rats (Hafiez, 1989). Contradictory results were given by other author with no change in PRL levels in immature rats (Judd, 1984). Similar findings were observed by several authors who reported PRL is a zinc regulating hormone and changes in its concentration inhibit PRL secretion due to closure of its negative feedback loop (Koppelman, 1988). PRL secretion is also regulated by dopamine (Koppelman, 1989). Thus, zinc suppresses PRL from pituitary gland directly by reciprocal decrease in dopamine secretion (Koppelman, 1989). Decreased serum PRL levels in zinc deficient group show role of zinc in PRL secretion from pituitary but exact role is not known. It may be possible that in zinc deficient states, inhibitory or cleavage factors of PRL would be which increased attenuates decreased levels of serum PRL. In the index study, serum levels of ACTH decreased in ZD group as compared to their respective control group. Increased in weight of adrenal gland were observed in the zinc deficient rats is consistent with earlier reports which were associated with adrenal hypertrophy (Quarterman, 1979; Reeves, 1977 and Rothman, 1986). Du and Chen (2001) revealed that zinc at certain concentrations could facilitate the secretion of ACTH (Du, 2001). In an experiment, serum cortisol levels in ZD group were significantly increased contrary to decreased serum ACTH concentration in others. This suggested that zinc have influence on hypothalamic-hypophysial-adrenocortical axis (Yan-Qiang, 2001). In the present study, plasma ACTH level decreased in zinc deficient rats which are indicative of the fact that animals were under stress and cells responsible for ACTH secretion exhibited degenerative changes. Several authors reported role of zinc in T_3 binding to its nuclear receptor and in the formation and action of TRH (Pekary, 1991). In this study decreased levels of TSH was observed in ZD group.

Morley *et al.* (1980) also reported decreased content of hypothalamic thyrotrophin-releasing hormone (TRH), T_4 and T_3 in zinc deficient rats which may be possibly due to impairment production of T_3 in thyroid (Morley, 1980). Pekary *et al.* (1991), reported similar findings and suggested that decreased precursor peptides such as TRH-Gly-IR and TRH-Gly in the pituitary occur due to reduced TRH level in zinc deficient (1 ppm) rats (Pekary, 1991). In rats, author observed that the production of GH in pituitary is influenced by T_3 due to its regulation growth hormone gene (Samuels, 1988). Moreover, in zinc deficient rats thyroid hormone signaling is impaired which reduces the binding of thyroid receptor to DNA thereby influencing transcription of the target gene (Freake, 2001). On the other hand, Kralik *et al.* (1996) noted that concentrations of triiodothyronine (T_3) and thyroxine (T_4) decreased in serum after zinc deficiency as compared to controls. They noticed that the activity of 5-deiodinase enzyme in liver decreased which may result in reduction in conversion of T_4 to T_3 in zinc deficient states (Kralik, 1996). The present study revealed a decrease in serum TSH level after zinc deficiency indicative of the fact that the cell responsible for it has been affected due to reduced concentration of precursor peptides in the pituitary gland affecting synthesis of TSH and its target organ. Considering the role of zinc on the secretion of anterior pituitary hormones, it seems that zinc deficient states can affect the overall physiological aspects of the body.

Conclusion

The present study has revealed the role of zinc in producing pituitary hormones and zinc supplementation was associated with improved hormone profile. This study act as a preventive measure to reduce the present health hazards associated with low levels of hormone profile like FSH, LH, TSH, GH, PRL and ACTH due to zinc deficiency. Further research on applications of the zinc and related contributing mechanisms would elucidate the effects demonstrated in this preliminary study.

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Conflicts of interest

None of the author has any conflict of interest to report.

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