Zinc Status and Serum Testosterone Levels of Healthy Adults

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ABSTRACT

Zinc deficiency is prevalent throughout the world, including the USA. Severe and moderate deficiency of zinc is associated with hypogonadism in men. However, the effect of marginal zinc deficiency on serum testosterone concentration is not known. We studied the relationship between cellular zinc concentrations and serum testosterone cross-sectionally in 40 normal men, 20 to 80 y of age. In four normal young men (27.5 ± 0.5 y), we measured serum testosterone before and during marginal zinc deficiency induced by restricting dietary zinc intake. We also measured serum testosterone in nine elderly men (64 ± 9 y) who were marginally zinc deficient before and after 3 to 6 mo of supplementation with 459 μmol/ d oral zinc administered as zinc gluconate. Serum testosterone concentrations were significantly correlated with cellular zinc concentrations in the cross-sectional study (lymphocyte zinc versus serum testosterone, $r = 0.43, p = 0.006$; granulocyte zinc versus serum testosterone, $r = 0.30$, $p = 0.03$). Dietary zinc restriction in normal young men was associated with a significant decrease in serum testosterone concentrations after 20 weeks of zinc restriction (baseline versus post-zinc restriction mean $\pm$ SD, 39.9 ± 7.1 versus 10.6 ± 3.6 nmol/L, respectively; $p = 0.005$). Zinc supplementation of marginally zinc-deficient normal elderly men for six months resulted in an increase in serum testosterone from 8.3 ± 6.3 to 16.0 ± 4.4 nmol/L ($p = 0.02$). We conclude that zinc may play an important role in modulating serum testosterone levels in normal men. *Nutrition* 1996;12:344–348

Key words: zinc, serum testosterone, elderly

INTRODUCTION

Zinc plays a key role in reproductive physiology.[1–4] Children with geophagia and those who subsist on cereal proteins alone become zinc deficient and exhibit both growth retardation and hypogonadism, which are reversed by zinc administration.[2–6] Furthermore, zinc deficiency has been associated with low serum testosterone concentrations in men with uremia, sickle cell anemia, and infertility.[7–13]

Mild or marginal zinc deficiency is probably common throughout the world, including the USA.[14,15] The effects of a mild deficiency of zinc in humans as observed in the experimental model studies have been described in previous publications.[15–18]

In a recent study of healthy elderly subjects in Detroit, approximately 30% had marginal or mild deficiency of zinc based on analysis of zinc concentrations in lymphocytes and granulo-

cytes.[14] The mean dietary zinc intake of the entire group was calculated to be 137.7 μmol/d (the recommended dietary allowance [RDA] in the USA is 229.5 μmol/d). The dietary intake of other essential nutrients was within RDA. In normal men, serum testosterone concentrations decrease with age primarily as a result of decreased testicular secretion.[19–22] It seems possible that the age-related decrease in serum testosterone could, in part, be secondary to a marginal deficiency of zinc.

The relationship between serum testosterone and plasma zinc concentrations has been reported,[23] but the results were inconclusive, as plasma zinc concentrations are not a sensitive indicator of body zinc status, particularly in cases of marginal zinc deficiency.[16,17] Using recently developed methods for assessment of zinc status by measuring the zinc concentrations in peripheral blood cells (lymphocytes and granulocytes), we

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examined the relationship between cellular zinc and serum testosterone concentrations in a cross-sectional study in normal men. In addition, we also determined the effect of marginal zinc deficiency (induced by dietary zinc restriction) on the serum testosterone concentration in normal young men. The effect of zinc supplementation on cellular zinc and serum testosterone concentrations in elderly men with marginal zinc deficiency was also determined.

MATERIALS AND METHODS

Cross-Sectional Study

Forty normal men between the ages of 20 and 80 y participated in this study. The subjects in the cross-sectional study were recruited from two sources: a) Twenty-two adult men (ages 20-50 y) were medical students, laboratory personnel, house staff, and staff physicians working in the Detroit Medical Center, Wayne State University. The study protocols were approved by the Wayne State University Human Investigation Committee. The nature of the study was explained, and participants signed informed consent forms prior to enrollment in the study. These subjects were not taking medications, were in good health, and actively engaged in their professional duties; and b) eighteen elderly subjects (ages 50–80 years) who were participating in the Health Promotion Project supported by the W.K. Kellogg Foundation were recruited. The details of the elderly population have been presented below in another section. There was no overlap between the elderly subjects recruited for the cross-sectional study and those recruited for participation in the zinc supplementation study. Serum testosterone and plasma, lymphocyte and granulocyte zinc concentrations were determined in the same blood sample drawn between 0900 h and 1100 h after an overnight fast. We measured serum testosterone in one sample as recommended by Hain et al.[24]

Experimental Human Zinc Deficiency

All human subjects were volunteers recruited under the guidelines of the Wayne State University and University of Michigan Human Investigation Committees as appropriate for the locations of the studies.

Four normal adult men aged (mean ± SD) 27 ± 0.5 y were recruited for induction of mild zinc deficiency by dietary means according to methods previously published.[16,17] All subjects were nonsmokers and were deemed healthy on the basis of medical history and routine admission evaluation at the University Hospital, The University of Michigan, Ann Arbor, Michigan. All volunteers maintained their routine levels of physical activity throughout the study. The volunteers ate all their meals at the metabolic unit of Clinical Research Center, University Hospital, the University of Michigan, Ann Arbor, Michigan. The diets were prepared by the Research Dietitian and administered under her supervision daily.

Subjects received the usual hospital diet during the first month of the study. The average daily zinc content of the hospital diet was (mean ± SD) 192.3 ± 3.5 μmol/d. In order to induce zinc deficiency, subjects were placed on a semipurified diet based on texturized soy products (General Mills Company, Minneapolis, MN). The details of the experimental diet and induction of human zinc deficiency in volunteers have been previously published.[16] The soy products were washed with 0.5% disodium EDTA, and the EDTA-washed food products were rinsed at least six times with distilled deionized water. Proteins (soy chicken and soy hamburgers) used for the low-zinc diet were cooked and prepared with distilled deionized water in bulk and stored at −20°C. Stored food for each subject was defrosted, weighed, and heated in a microwave oven. Remaining dietary components were purchased in bulk weekly

with the exception of vitamin and mineral mixtures. Vitamins and mineral supplements were provided throughout the experiments to provide recommended levels as established by the National Research Council for all nutrients except zinc. The daily dietary intake of zinc during the zinc-restricted period (24 wk) ranged from 64.3 to 85.7 μmol. The diet supplied all essential nutrients according to RDA except zinc. The average copper intake throughout the study was 45 μmol/d. Serum testosterone and plasma and cellular zinc concentrations were measured twice before and twice after 8 and 20 weeks of dietary zinc restriction. The data were averaged for each period for statistical analysis.

Zinc Supplementation in Elderly Men

Subjects enrolled in "A Model Health Promotion and Intervention Program for Urban Middle Aged and Elderly Americans," funded by the W.K. Kellogg Foundation, were assessed for nutrition and zinc status.[14] This study was approved by the Human and Animal Investigation Committee of Wayne State University, and proper informed consent was obtained from each participant before enrollment in the study. The Health Promotion program was advertised in the local newspapers. Volunteers enrolled in the program were between the ages of 50 and 80 years. They were ambulatory and in good health. They were not taking any medications known to affect zinc metabolism. Individuals with chronic or debilitating disease or conditions (e.g., uncontrolled high blood pressure, chronic renal disease or cardiac disease, liver failure, diabetes, or psychologically incompetent) or other conditions that would preclude their active participation in the project were excluded from the study.

Sixty-five percent of the population were women; 59% were Caucasians, 39% Afro-Americans, and 2% Asian and Hispanic. The majority (64%) of the participants were married. The population was well educated (48% held baccalaureate or graduate/professional degrees), and most subjects were economically secure (65% had an annual household income >$25,000). Of 118 elderly men (mean ± SD, 62 ± 7 years) in whom zinc concentrations in both lymphocytes and granulocytes were available, 36 had lower values (lymphocytes <769.1 nmol/10^{10} cells and granulocytes <644.5 nmol/10^{10} cells). We have defined marginal zinc deficiency based on the concentrations of zinc in lymphocytes and granulocytes one SD below the mean for normal younger age group adults.[25] The participation of subjects for supplementation study depended largely on whether they were willing to participate. Nine men (mean ± SD, 64 ± 9 y) were randomly selected for zinc supplementation studies. They were carefully screened for dietary intake of nutrients by a trained research dietitian. Except for zinc and copper, the intake of all other nutrients, including iron, met RDAs. The mean zinc intake was 69% of RDA, and the copper intake was 58% of RDA. Although the mean intake of copper was low, their plasma copper was increased. These subjects were specifically instructed to discontinue all other nutritional supplements before beginning the formal zinc supplementation program.

The zinc supplementation consisted of 459 μmol elemental zinc/d (zinc gluconate, Labcatal, Paris). Although our intent was to continue the supplement for six months, four of the subjects were not compliant after 3 mo. Zinc concentrations in plasma, lymphocytes, and granulocytes and serum testosterone concentrations were measured in blood samples twice before and twice after the supplementation periods; the data were averaged for each period for statistical analysis.

Body Weight

Body weights were available in 16 subjects who participated in the cross-sectional study, and body weight was closely monitored in all four younger subjects in whom a dietary deficiency

of zinc was induced. In the elderly subjects who were supplemented with zinc, we were not only able to record body weight and height but we determined the lean body mass and percent body fat by electrical impedance.

Plasma and Cell Zinc Assays

Zinc was measured in plasma, lymphocytes, and granulocytes by previously established techniques[14,16,17] with precautions to avoid contamination during collection, preparation, and analysis. For determination of zinc in cells, we used an atomic absorption spectrophotometer (IL-551-AA1AE model) equipped with a graphite furnace (model 655 with the Fastac-1 Autosampling System).[16,17] The plasma zinc concentration (mean ± SD) for normal subjects of both sexes between the ages of 20 and 50 y in our laboratory is 1626.4 ± 136.8 nmol/dL ($n = 20$). The lymphocyte and granulocyte zinc concentrations for normal subjects of both sexes between the ages of 20 and 50 y in our laboratory are 860.5 ± 97.3 nmol/10^{10} cells and 722 ± 79.0 nmol/10^{10} cells ($n = 65$).[25]

Serum Testosterone Assays

The serum samples were obtained between 9-11 a.m. and all samples were assayed in one batch after the completion of our study.

Serum testosterone was measured in duplicate using commercially available kits (Coat-a-Count total testosterone kit, DPC, Los Angeles, CA). The sensitivity of this assay was 0.14 nmol/L and the inter-assay and intra-assay coefficients of variation were 9.2% and 5%, respectively.

The serum testosterone concentration (mean ± SD) for normal men in our laboratory is 10-35 nmol/L.

Sex Hormone Binding Globulin (SHBG)

Serum SHBG was assayed in five elderly subjects who were supplemented with zinc. Baseline and post-supplemented serum samples were analyzed by immunoradiometric assay. The normal ranges by this technique are 12-75 nmol/L.

Statistical Analysis

Linear regression analysis was used for the evaluation of the relationship between serum testosterone and cellular zinc concentrations in the cross-sectional study. Analysis of variance was used to compare the baseline results with the results after zinc depletion in young men in whom zinc deficiency was induced by dietary means, and to compare baseline and post-supplementation data obtained from the elderly. The p values were set at ≤0.05 for statistical significance.

RESULTS

Body weight was recorded in 16 subjects in the cross-sectional study. There was no statistically significant correlation between body weight and any of the variables measured (lymphocyte zinc, granulocyte zinc, and serum testosterone concentration). Subjects in whom zinc deficiency was induced by dietary means were also monitored for body weight. Throughout the study, their weight change was no more than 2 kg (not significant statistically). We also monitored serum albumin and cholesterol levels throughout the study, which showed no significant changes. Total body fat was assessed in the elderly subjects who were supplemented with zinc, and body fat percent did not correlate with either zinc status or serum testosterone concentration. Serum albumin and total cholesterol showed no significant difference between initial and post-supplemented samples.

The lymphocyte zinc and serum testosterone concentrations in the normal 20- to 80-year-old men were positively correlated

($r = 0.43, p = 0.006$) (Fig. 1). The granulocyte zinc and serum testosterone concentrations were also positively correlated in these men ($r = 0.30, p = 0.03$) (not shown). There was, however, no correlation between plasma zinc and serum testosterone concentrations in these men.

Figure 2 shows the changes in the plasma, lymphocyte, and granulocyte zinc and serum testosterone concentrations in the four men in whom a mild dietary deficiency of zinc was induced. Plasma zinc or copper concentrations did not change throughout the study. The mean (± SD) lymphocyte zinc concentrations at baseline and after 8 and 20 weeks of zinc restriction were 872.5 ± 60.8, 714.4 ± 45.6, and 577.6 ± 30.4 nmol/10^{10} cells, respectively ($p = 0.0007$). The mean (± SD) granulocyte zinc concentration at baseline and after 8 and 20 weeks of zinc restriction were 729.6 ± 60.8, 653.6 ± 30.4, and 501.6 ± 121.6 nmol/10^{10} cells, respectively ($p = 0.01$). The respective serum testosterone concentrations (mean ± SD) at baseline and post-zinc restriction at 8 and 20 weeks were 39.9 ± 7.1, 23.5 ± 12.0, and 10.6 ± 3.6 nmol/L ($p = 0.01$).

Following zinc supplementation for three months in the elderly men, the serum testosterone concentration increased from 8.3 ± 6.3 to 14.2 ± 3.6 nmol/L ($n = 7$). In those men, the zinc concentrations in lymphocytes and granulocytes also increased, but the plasma zinc concentration was not altered (Table I). Following six months on zinc supplementation, the serum testosterone was 16.0 ± 4.4 nmol/L ($n = 5$). Whereas the baseline plasma zinc concentrations in those men were normal, their baseline lymphocyte and granulocyte zinc concentrations were decreased in comparison to those found in young men (which formed the basis for diagnosing marginal zinc deficiency in the elderly subjects).

The serum SHBG at baseline and at the end of six months following zinc supplementation in five elderly subjects were (mean ± SD) 37 ± 3.08 and 41.8 ± 5.17 nmol/L, respectively ($p = 0.448$).

DISCUSSION

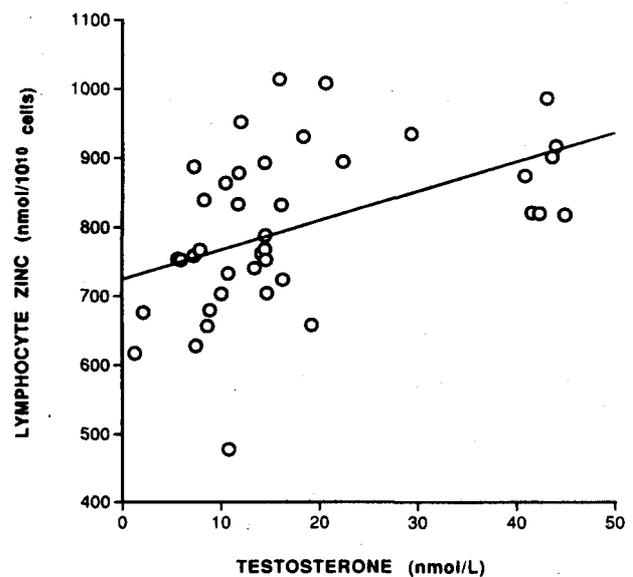We found a positive correlation between cellular (lymphocyte and granulocyte) zinc concentrations and serum testoster-

FIG. 1. Relationship between lymphocyte zinc and serum testosterone concentrations in 40 normal men ages 20 to 80 y ($r = 0.043, p = 0.006$).
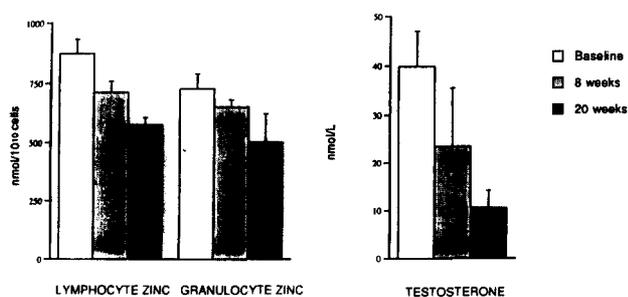
FIG. 2. Mean (± SD) plasma, lymphocyte, and granulocyte zinc and serum testosterone concentrations in 4 normal men before and after 8 and 20 weeks of dietary zinc restriction.

one concentrations in normal men (as defined by clinical and laboratory criteria, see METHODS), suggesting that zinc may play a role in regulating testosterone secretion. We also found that by inducing a specific marginal dietary deficiency of zinc, a decrease in serum testosterone in normal young men resulted and that zinc supplementation to marginally zinc-deficient elderly men (as defined earlier) increased serum testosterone levels.

Hartoma[23] reported a positive correlation between serum testosterone and serum zinc concentration only in the elderly subjects and not in the younger age group. In our study, by using cellular zinc, we were able to show a correlation between cellular zinc and serum testosterone in our subjects ages 20 to 80 years. In cases of marginal deficiency of zinc, plasma zinc concentrations may remain normal.[15-17] In previous studies of marginal zinc deficiency in humans, we found that measurement of zinc in lymphocytes and granulocytes reflected zinc status much more accurately.[14-17,25]

In rats with zinc deficiency, hypogonadism results primarily from Leydig cell failure.[26,27] Zinc enhances human chorionic gonadotropin-induced production of cAMP and consequently testosterone in rat testes.[28] Additionally, zinc may increase the conversion of androstenedione to testosterone in the periphery.[29] Zinc also interferes with the metabolism of testosterone by decreasing its hepatic clearance and reducing hepatic 5 alpha reductase activity.[30] Finally, zinc may increase not only the serum concentration of testosterone but also may affect the action of androgens, since the DNA-binding domain of the androgen receptors is a cysteine-rich zinc-finger protein.[31]

Aging in normal men is accompanied by a decrease in serum testosterone concentration.[19] Although the primary event in the

age-related changes of the hypothalamic-pituitary-testicular axis may be a decrease in luteinizing hormone (LH) secretory burst amplitude, primary Leydig cell failure with a secondary increase in LH pulse frequency and decreased LH pulse amplitude represents a more widely accepted mechanism.[19,20] Since zinc deficiency causes primary Leydig cell failure in animals, and marginal zinc deficiency is so prevalent among elderly men[14,15] and we have shown that zinc supplementation raises the serum testosterone concentrations in elderly subjects, it is possible that zinc deficiency may be involved in the age-related decrease in serum testosterone.

Although we did not measure LH and follicular stimulating hormone (FSH) in this study, our previous studies in the rats showed that the baseline serum LH and FSH as well as their response to gonado-tropin releasing hormone (GnRH) administration were higher in the zinc-deficient animals, but their testosterone response was lower in comparison to controls.[32] GnRH stimulation in zinc-deficient sickle cell anemia patients resulted in a significantly greater increase of serum LH and FSH than in normal controls, even when their serum testosterone concentration remained low.[9] In zinc-deficient sickle cell anemia patients and chronic uremics undergoing hemodialysis, baseline LH and FSH were higher than the LH and FSH of controls and the serum testosterone concentration was low.[9] A significant increase in serum testosterone and sperm count, and a significant decrease in serum LH and FSH were observed after zinc supplementation in sickle cell anemia and hemodialysis patients.[7,9,33,34] These observations support the concept that the effect of zinc is primarily on testicular testosterone secretion. Martin et al.[35] have suggested that in young male sheep, the zinc-specific effect is localized within the testis where it reduces the development of the capacity to produce testosterone, leading to low intratesticular concentrations of testosterone, a critical factor for the growth, development, and function of the seminiferous tubules.

We assayed serum testosterone from single samples obtained at any one time with one exception. In one study, averages of two samples were used for analysis. Many investigators measure serum testosterone in three samples collected at 20- or 30-minute intervals. It has been shown, however, that the hormone level obtained from one sample has such a high predictive value that drawing more than one sample may be redundant.[24]

Although a seasonal variation of testosterone concentrations has been demonstrated, the magnitude of this variation does not exceed 7-15%.[31] A greater than 95% change in testosterone concentration in our zinc depletion study cannot be accounted for by the circannual variation of plasma testosterone.[31] How-

TABLE I.

ZINC CONCENTRATIONS IN PLASMA, LYMPHOCYTES, AND GRANULOCYTES AND SERUM TESTOSTERONE CONCENTRATIONS BEFORE AND AFTER ZINC SUPPLEMENTATION FOR THREE AND SIX MONTHS IN ELDERLY MEN (MEAN ± SD)

	Zinc			
	Plasma nmol/dL	Lymphocytes nmol/10^{10} cells	Granulocytes nmol/10^{10} cells	Testosterone nmol/L
Before	1748 ± 197.6 (8)	684 ± 46.5 (8)	501.6 ± 76 (9)	8.3 ± 6.3 (9)
After three months	1647.8 ± 162.6 (8)	765.0 ± 112.4 (8)	676.9 ± 75.6 (9)	14.2 ± 3.6 (7)
After six months	1807.2 ± 521.7 (5)	809.9 ± 46.4 (5)	700.4 ± 115.9 (5)	16.0 ± 4.4 (5)
Analysis of Variance p	0.66	0.05	0.009	0.02

Numbers in parentheses indicate the number of subjects tested.

ever, our data need further confirmation given the lack of a control group.

In addition to the correlation between cellular zinc and serum testosterone concentrations in the cross-sectional part of our study, the association of induced marginal zinc deficiency with decreased serum testosterone concentrations, and a significant increase of serum testosterone concentration following zinc supplementation in the elderly, underscores the importance of zinc for maintenance of serum testosterone in apparently healthy adults. Our data are in agreement with an earlier study when a more severe dietary zinc deficiency was induced in human volunteers. In that experiment, zinc supplementation reversed the effects of zinc deficiency on gonadal functions.[33]

CONCLUSION

Zinc status, as assessed by measurements of cellular zinc concentrations, is correlated with serum testosterone concentrations in normal men. Marginal deficiency of zinc results in decreased serum testosterone, and zinc supplementation raises

serum testosterone in men with mild zinc deficiency. Zinc status appears to represent an important determinant of serum total testosterone levels. Further studies are needed to elucidate the effect of zinc status on the testosterone and gonadotropin concentrations as well as to clarify its relationship with the decreased androgen levels accompanying aging. Finally, inasmuch as a marginal deficiency of zinc is fairly common,[15] zinc status must be ascertained in normal men with decreased serum testosterone concentrations.

REFERENCES

1. Favier AE. The role of zinc in reproduction. Biol Trace Elem Res 1992;32:363
2. Prasad AS, Halsted JA, Nadimi M. Syndrome of iron deficiency, anemia, hepatosplenomegaly, hypogonadism, dwarfism and geophagia. Am J Med 1961;31:532
3. Prasad AS, Miale A Jr, Farid Z, et al. Zinc metabolism in patients with the syndrome of iron deficiency, anemia, hypogonadism and dwarfism. J Lab Clin Med 1963;61:537
4. Castro-Mangana M, Collip PJ, Chen SY, et al. Zinc nutritional status, androgens and growth retardation. Am J Clin Nutr 1981;135:322
5. Ghavami-Maibodi SZ, Collip PJ, Castro-Mangana M, et al. Effect of oral zinc supplementation on growth, hormonal levels and zinc in healthy short children. Ann Nutr Metab 1983;27:214
6. Nishi Y, Hatano S, Aihara K, et al. Transient partial growth hormone deficiency due to zinc deficiency. J Am Coll Nutr 1989;8(2):93
7. Mahajan SK, Abbasi AA, Prasad AS, et al. Effect of oral zinc treatment on gonadal function in hemodialysis patients. Ann Intern Med 1982;97:357
8. Antoniou LD, Sudhakar T, Shahloub RJ, et al. Reversal of uremic impotence by zinc. Lancet 1977;II:895
9. Abbasi AA, Prasad AS, Ortega J, et al. Gonadal function abnormalities in sickle cell anemia. Studies in adult male patients. Ann Intern Med 1976;85:601
10. Marmar JL, Katz S, Partaiss DE, et al. Semen zinc levels in infertile and post vasectomy patients and patients with prostatism. Fertil Steril 1975;26(II):1057
11. Hartoma RT, Nahoul K, Netter A. Zinc plasma androgens and male sterility. Lancet 1977;II:1125
12. Caldamone AA, Freytag MK, Cockett ATK. Seminal zinc and male infertility. Urology 1979;13:280
13. Netter A, Hartoma R, Nahoul K. Effect of zinc administration on plasma testosterone, dihydrotestosterone and sperm count. Arch Androl 1981;7:69
14. Prasad AS, Fitzgerald JT, Hess JW, et al. Zinc deficiency in elderly patients. Nutrition 1993;9:218
15. Prasad AS. Clinical spectrum and diagnostic aspects of human zinc deficiency. In: Prasad AS, ed. Essential and toxic trace elements in human health and disease. New York: Alan R. Liss; 1988:3
16. Rabbani P, Prasad AS, Tsai R, et al. Dietary model for production of experimental zinc deficiency in man. Am J Clin Nutr 1987;45:1514
17. Prasad AS, Meftah S, Abdallah J, et al. Serum thymulin in human zinc deficiency. J Clin Invest 1988;82:1202
18. Mahajan SK, Prasad AS, Brewer GJ, et al. Effect of changes in dietary zinc intake on taste acuity and dark adaptation in normal human subjects. J Trace Elem Exp Med 1992;5:33

19. Vermeulen A, Kaufman JM. Role of the hypothalamic-pituitary function in the hypoandrogenism of healthy aging (Editorial). J Clin Endocrinol Metab 1992;74(6):1226A
20. Vermeulen A. Clinical Rev 24: Androgens in the aging male. J Clin Endocrinol Metab 1991;73:221
21. Davidson Jm, Chen JJ, Crapo K, et al. Hormonal changes and sexual function in aging men. J Clin Endocrinol Metab 1983;57:71
22. Pirke KM, Doerr P. Age related changes in free plasma testosterone, dihydrotestosterone and oestradiol. Acta Endocrinol 1975;80:171
23. Hartoma R. Serum testosterone compared with serum zinc in man. Acta Physiol Scand 1977;101:336
24. Hain J, Langevin R, D'Costa M, et al. Serum pituitary and steroid hormone levels in the adult male: one value is as good as the mean of three. Fertil and Steril 1988;49:123
25. Meftah S, Prasad AS, Lee D-Y, et al. Ecto 5' nucleotidase (5'NT) as a sensitive indicator of human zinc deficiency. J Lab Clin Med 1991;118:309
26. McClain CJ, Gayaler JS, VanThiel DH. Hypogonadism in the zinc deficient rat. Localization of the functional abnormalities. J Lab Clin Med 1984;104:1007
27. Root AW, Duckett G, Sweetland M, et al. Effects of zinc deficiency on Leydig cell function in sexually mature and immature male rats. J Nutr 1979;109:958
28. Nishi Y, Hatano S, Aihara K, et al. Effects of zinc ion on human chorionic gonadotropin stimulated in vitro production of cAMP and testosterone by rat testis. Pediatr Res 1984;18:232
29. Bermudez JA, Perex-Pasten E, Villalpando S, et al. Low plasma zinc and androgen in insulin dependent diabetes mellitus. Arch Androl 1986;16(2):151
30. Leake A, Chisholm GD, Habib FK. The effect of zinc on the 5 alpha reduction of testosterone by the hyperplastic human prostate gland. J Ster Biochem 1984;20(2):651
31. Grumbach MM, Conte FA. Disorders of sex in differentiation. In: Wilson JD, Foster WD, eds. Williams textbook of endocrinology, 8th Ed. Philadelphia: WB Saunders Co.; 1992:853
32. Lei KY, Abbasi A, Prasad AS. Function of pituitary-gonadal axis in zinc deficient rats. Am J Physiol 1976;230:1730
33. Abbasi AA, Prasad AS, Rabbani PI, et al. Experimental zinc deficiency in man. Effect on testicular functions. J Lab Clin Med 1980;96:544
34. Prasad AS, Abbasi A, Rabbani P, et al. Effect of zinc supplementation on serum testosterone level in adult male sickle cell anemia subjects. Am J Hematol 1981;10:119
35. Martin GB, White CL, Markey CM, et al. Effects of dietary zinc deficiency on the reproductive system of young male sheep: testicular growth and the secretion of inhibin and testosterone. J Reprod Fertil 1994;101:87