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Full Length Research Paper

Improving effect of zinc supplementation on pituitary gonadotropins secretion in smokers

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This study aimed to investigate the effect of zinc supplementation on testicular tissue as a feed-back mechanism of the pituitary secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) on cigarette smoker subjects. Total of twenty subjects participated in this study as per the national bioethics committee guidelines. Venous blood samples were collected for determination of serum level of LH and FSH before and after zinc supplementation. LH and FSH determination were done using enzyme-linked immunosorbent assay (ELISA). A t-test was used to compare the means of two groups, P value was considered significant if ≤ 0.05. Results showed that LH serum levels were elevated in smokers as compared to the control group (p 0.05). Similar observation was found in FSH levels (p<0.05). After one month of zinc supplementation, LH levels decreased in smokers group (after: 4.14 ± 2.38 mIU/ml, before: 7.43 ± 4.32 mIU/ml; p<0.05). FSH levels also decreased after zinc supplementation in smokers group (after: 1.50 ± 1.12 mIU/ml, before: 3.88 ± 1.57 mIU/ml; p<0.05). The improving effect of zinc supplementation on LH and FSH pituitary and testicular steroid secretions for smoker subjects is obviously observed. These findings can be implemented to contribute to the outcome of zinc treatment associated with smoking.

Key words: Zinc, biometals, smoking, gonadotropins, luteinizing hormone (LH), follicle-stimulating hormone (FSH), trace elements.

INTRODUCTION

Cigarette smoke contains a large number of oxidative compounds. Both zinc and antioxidants delay the progression of oxidative degeneration, possibly by preventing cellular damage. Zinc is involved in numerous aspects of cellular metabolism, for example, it is required for the catalytic activity of approximately 100 enzymes. Pituitary gonadotropins include luteinizing hormone (LH) and follicle-stimulating hormone (FSH), are essential for reproduction. While LH stimulates synthesis and secretion of testosterone hormone, FSH is critical for sperm production. Excessive secretion of FSH and/or LH is the most common result of gonad failure. Zinc, an essential trace element necessary for sustaining all animal life, is thought to protect cells from drought and disease. There are numerous functions in which zinc plays an important role such as prostate gland function and reproductive organ growth, immune function, protein synthesis , wound healing, DNA synthesis and cell division (Heiserman, 1992; Solomons, 1998; Prasad, 1995; Heyneman, 1996). It also supports normal growth and development of fetus during pregnancy and after birth in childhood and adolescence (Fabris and Mocchegiani, 1995; Maret and Sandstead, 2006). Zinc plays key role in different sensory functions like proper sense of taste

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and functions like proper sense of taste and smell (Prasad et al., 1997). Tissues with high rate of new cell development such as bone marrow, immune system cells and the lining of the gut, especially required zinc (David, 2006). Zinc involves in numerous aspects of cellular metabolism, approximately 100 enzymes require zinc for their catalytic activity (Sandstead, 1994). There are over 200 enzymes which contain zinc as a cofactor, and about the same number of transcription factors, zinc-containing enzymes are used by the body to regulate growth and development, promote fertility, aid digestion, and synthesize nucleic acid. Severe zinc deficiency is known to depress immune function (Navarro et al., 1994). The body requires zinc to develop and activate T-lymphocytes (Wintergerst et al., 2007). Individuals with low zinc levels have shown reduced lymphocyte proliferation response to mitogens and other adverse alterations in immunity that can be corrected by zinc supplementation (Beck et al., 1997). Both zinc and antioxidants delay the progression of age-related macular degeneration (AMD) and vision loss, possibly by preventing cellular damage in the retina (Marshall, 2000; Evans, 2006). Cigarette smoke contains a large number of oxidative compounds. These compounds appear to be the most potent compounds which can exert oxidative stress. Oxidative stress includes the generation of reactive oxygen species (ROS) by different cell type such as neutrophils and macrophages which may be present in increased number or activated form.

Elevated serum FSH is a reliable indicator of germinal epithelial damage, and is usually associated with severe oligo-spermia or azoo-spermia of bad prognosis. In men with gonadotropins, insufficiency testosterone cannot maintain spermatogenesis contrary, therefore normal levels of both FSH and LH are required to achieve quantitatively normal sperm-production (Martin-du, 2012). In the present study, the effect of cigarette smoke on pituitary secretion of FSH and LH will be assayed and the effect of zinc supplementation on pituitary secretion of these hormones will be monitored in cigarette smoker subjects.

MATERIALS AND METHODS

Ten healthy male and ten smoker male subjects were invited to participate in the study. Each subject gave written informed consent for the study. The study has been designed ethically according to National Committee of BioEthics at King Abdulaziz City for Science and Teschnology (NCBE/KACST) and Al Jouf University (JU) bioethics committee guidelines. The study took place at Aljouf University facilities, Al Jouf province, KSA. The age of the subjects ranged between 19 and 26 years. The duration of smoking ranged between 4 and 7 years ago (20 cigarettes/day). Ten healthy male nonsmoker subjects were also included as control group who were matched to the smokers in age and socioeconomic status. For each subject of smoker and control groups, the following procedures were carried out: medical history collection, general clinical examination, venous blood sample collection for determination of serum levels of LH and FSH. Zinc supplementation was then started to smoker subjects for one month as one tablet daily of stress tablets with zinc, manufactured by Wyeth Canada. The oral tablet contains zinc sulfate (23.9 mg) and copper (3 mg), prescribed to be taken after meal with continuing the same smoking rate (20 cigarettes/day). High zinc intakes can inhibit copper absorption, sometimes producing copper deficiency and associated anemia (Whittaker, 1998; Broun et al., 1990). Considering the fact, dietary supplement formulations should contain high levels of zinc and copper (Broun et al., 1990). Venous blood samples were collected for determination of serum level of LH and FSH after zinc supplementation. Quantitative determination of LH and FSH serum levels were done using Accu-Bind enzyme-linked immunosorbent assay (ELISA) microwells (product code 625-300 for LH and product code 425-300 for FSH).

Estimation of serum zinc levels

They were measured by an atomic absorption spectrophotometry (mode 12380; Perkin Elmer). The monochromatic slit was adjusted to 0.7 and the wave length was set to the zinc resonance line at 213.9 nm (Pekarek et al., 1972).

Statistics

All the statistical analyses were processed using Statistical Program of Social Sciences (SPSS) for windows, version 21.0. Values of the measured parameters were expressed as mean value ± standard deviation (SD) and the difference between each of the two groups was determined using unpaired student's t-test, and the significance was considered at p values as less than 0.05. Analysis of variance (ANOVA) was used to compare the means of more than two groups.

RESULTS

The LH serum level was found to have a very high significant increase in smokers group before zinc supplementation compared to nonsmokers group as illustrated in Table 1 ($p<0.05$). LH serum level was found with very low significant decrease in smokers group after zinc supplementation as compared with smokers before zinc supplementation ($p<0.05$), while the LH serum levels after zinc supplementation shows nonsignificance difference as compared with nonsmokers as shown in Table 1 and Figure 1. Similarly, FSH serum level was found also with very high significant increase in smokers before zinc supplementation compared to nonsmokers group ($p<0.05$) as shown in Table 2. FSH serum level was also found with very high significant decrease in smokers group after zinc supplementation as compared with nonsmokers ($p<0.05$). And the FSH serum levels after zinc supplementation shows nonsignificance difference as compared with nonsmokers ($p>0.05$) as shown in Table 2 and Figure 2. Also, zinc serum level was also found with very high significant decrease in smokers before zinc supplementation compared to nonsmokers group ($p<0.05$) as shown in Table 3. Zinc serum level was also found with very high significant increase in smokers group after zinc supplementation as compared with smokers before zinc supplementation ($p<0.05$). The

Table 1. Comparison between serum level of LH in smokers (before and after Zn supplementation) and nonsmokers

Parameter	Smokers		Nonsmokers	ANOVA	
	Before Zn	After Zn		F ratio	P value
Serum level of LH (mIU/ml)	7.05 ± 0.45[a]	3.29±0.41[bc]	3.02 ± 0.25	345.0	<0.05***

(a) Very high significant differences between smokers before Zn supplementation and non smokers($p<0.05$); (b) Very high significant differences between smokers after Zn supplementation and smokers before Zn supplementation($p<0.05$); (c) Nonsignificant differences between smokers after Zn supplementation and nonsmokers ($p>0.05$).

Table 2. Comparison between serum level of FSH in smokers (before and after Zn supplementation) and nonsmokers.

Parameter	Smokers		Nonsmokers	ANOVA	
	Before Zn	After Zn		F ratio	P value
Serum level of FSH (mIU/ml)	3.45 ± 0.67[a]	2.01 ± 0.47[bc]	2.00 ± 0.18	31.89	<0.05***

(a) Very high significant differences between smokers before Zn supplementation and non smokers ($p<0.05$); (b) Very high significant differences between smokers after Zn supplementation and smokers before Zn supplementation ($p<0.05$); (c) Nonsignificant differences between smokers after Zn supplementation and nonsmokers ($p > 0.05$).

Table 3. Comparison between serum level of Zn in smokers (before and after Zn supplementation) and nonsmokers.

Parameter	Smokers		Nonsmokers	ANOVA	
	Before Zn	After Zn		F ratio	P value
Serum level of zinc (µg/dl)	84.21 ± 13.70[a]	107.35 ± 5.68[bd]	115.03 ± 8.7	26.03	<0.05***

(a) Very high significant differences between smokers before Zn supplementation and non smokers ($p<0.05$); (b) Very high significant differences between smokers after Zn supplementation and smokers before Zn supplementation ($p<0.05$); (d) Significant differences between smokers after Zn supplementation and nonsmokers ($p<0.05$).

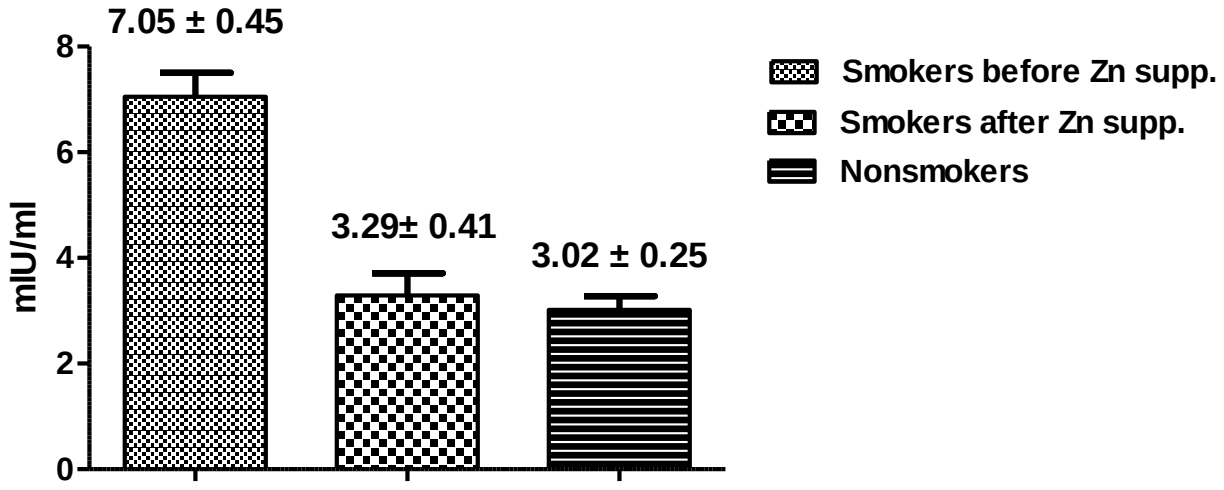

Figure 1. Comparison between serum level of LH (mIU/ml) in smoker (before and after Zn supplementation) and nonsmokers.

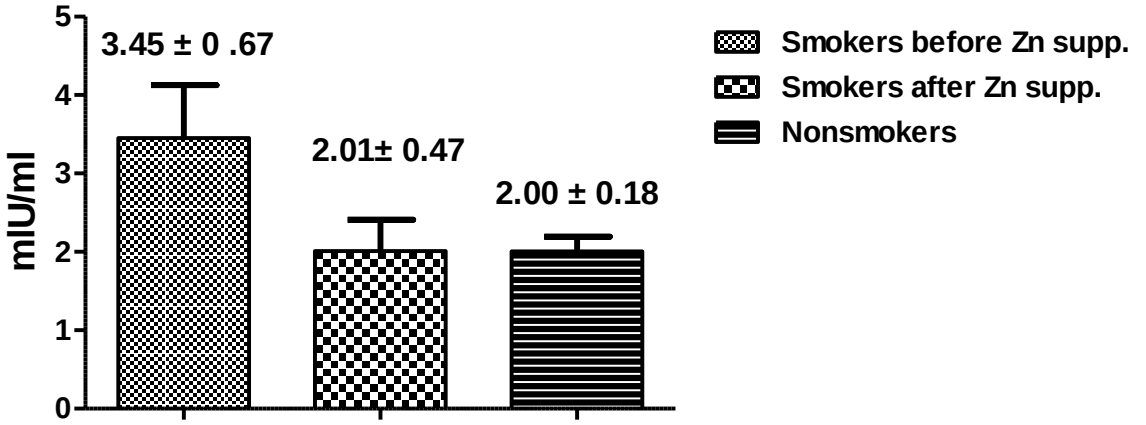

Figure 2. Comparison between serum level of FSH (mIU/ml) in smoker (before and after Zn supplementation) and nonsmokers.

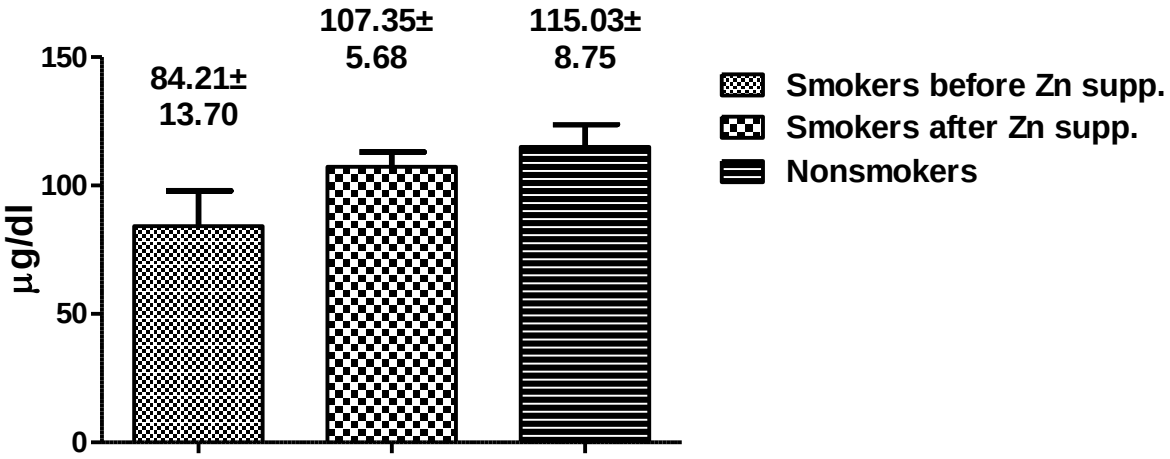

Figure 3. Comparison between serum level of Zinc (µg/dl) in smoker (before and after Zn supplementation) and nonsmokers.

zinc serum levels after zinc supplementation shows significance difference as compared with nonsmokers ($p<0.05$) as depicted in Table 3 and Figure 3.

DISCUSSION

The improving effect of zinc supplementation on LH and FSH pituitary and testicular steroid secretions for smoker subjects is obviously observed in this study. These findings can contribute to the outcome of zinc treatment associated with smoking and zinc involvement in relevant biological processes. Zinc has potential to influence many metabolic functions and to impact a range of diseases (Hughes and Samman, 2006). Testicular toxicants such as polycyclic aromatic hydrocarbons exert their effects at least in part by free radical-dependent mechanisms. Although, the role of trace elements and the mechanism of their kinetics in relation to these diseases remain controversial, the serum copper/zinc ratio (CU/Zn) has been claimed to be highly sensitive in the diagnosis of the diseases in question (Hiura et al., 2003). The decrease in the zinc levels may be due to the decreased intake of zinc, disturbed intestinal absorptionof zinc, decreased serum albumin as a carrier of zinc, decreased storage of zinc in liver and increased urinary excretion of zinc (Lin et al., 2006). Pro-oxidant states often result in impairment of this defense. The testis tissue and sperm function are particularly vulnerable to per-oxidative injury produced by reactive oxygen species. Defective sperm function of infertile men is associated with increased lipid per-oxidation and impaired function of antioxidant enzymes in

spermatozoa (Fridovich and Freeman, 1986; Aitken et al 1989). Smoking is associated with a high significant reduction in sperm output and motility (Handelsman et al., 1984). Superoxide anions are potent oxidants (Halliwell and Gutteridge, 1999) moderated by the activities of various forms of superoxide dismutase (SOD) (Zou et al., 2002). Zinc is postulated to influence oxidants via its role as a cofactor for Cu, Zn-SOD (O'Dell, 2000; Powell, 2000). Both low and high zinc intakes impact Cu, Zn-SOD activity (Ruz et al., 1992). Increase in zinc intake raises plasma and erythrocyte SOD (Davis et al., 2000). Zinc supplementation decreased plasma lipid peroxides; therefore, adequate zinc intake could play an important role in the prevention and/or modulation of diseases. Hypozincemia and marked hypercupremia have been reported in patients with digestive, hepatic, breast, and lung cancers (Maeda et al., 2005).

The evaluated levels of the LH and FSH pituitary secretions in the male subjects showed a significant increase in serum level of LH in smokers group compared to control group ($p<0.05$) and a significant increase in serum level of FSH in smokers group as compared to control group ($p<0.05$). These results are in accordance with literature reviews. Such trend in LH and FSH secretions is an indicator of the testicular dysfunction in smoker subjects.

After one month zinc supplementation, the serum levels of LH and FSH were measured in smokers group. LH serum level significantly decreased in smokers group after zinc supplementation in comparison to the level before supplementation ($p<0.05$). FSH serum level also significantly decreased in smokers group after zinc supplementation compared to the level before supplementation ($p<0.05$). This is an indicator about the improvement of testicular function of smoker subjects as a feedback regulation. Decreased blood levels of gonadotropins usually reflect elevation of steroid feedback. The improvement of LH pituitary secretion in smokers group after zinc supplementation is statistically nonsignificant in comparison to control group ($p>0.05$). Similarly, the improvement of FSH pituitary secretion of smoker group in comparison to control group is not significant ($p>0.05$).

Zinc supplementation is associated with decreased oxidative stress and improved immune function among possible mechanism for its disease prevention (Prasad and Kucuk, 2002). Baltaci et al. (2006) reported that LH serum levels in rats having zinc supplementation (3 mg/kg/day) were lower than castrated rats. Free and total testosterone serum levels in zinc supplemented rats were higher than in control group. Nakayama et aI. (2002) reported a progressive decrease in serum zinc levels in patients with liver diseases from chronic hepatitis, alkaline phosphatase activity in zinc supplemented adult male group. Sunar et al. (2009) studied 40 adult female rats, which were allocated to 4 groups; control (group 1), ovariectomized control (OVX) (group 2), OVX-zinc-supplementation (group 3), OVX-zinc deficient (group 4). Estrogen and progesterone levels in group 3 were higher than those in groups 2 and 4. Group 3 had the highest serum zinc level. The findings by Sunar et al. (2009) also reflect the improving effect of zinc on LH and FSH pituitary secretion. Testicular dysfunction existed in smoker subjects before zinc supplementation which is supported by the elevation in serum levels of LH and FSH with zinc supplementation. The improving in testicular steroid secretion in smoker subjects as a feedback regulation appeared by a decrease in serum levels of LH and FSH. The decrease in secretion of LH and FSH is the most common result of gonad improvement. This reflects elevation of steroid feedback. Moreover, this may significantly contributes to the outcome of zinc therapeutic intervention in gonad dysfunction and oxidative stress conditions.

In conclusion and as a preventive measure, an implementation of short term plan to reduce the present health hazards of high levels of LH and FSH were recommended due to smoking. Further studies with higher sample size are warrantied to confirm these results for improving benefits of zinc supplementation to prevent certain health disorders may be necessary.

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