

# Effect of fatiguing bicycle exercise on thyroid hormone and testosterone levels in sedentary males supplemented with oral zinc

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## Abstract

**OBJECTIVE:** The aim of this study was to determine how exercise affects thyroid hormones and testosterone levels in sedentary men receiving oral zinc for 4 weeks.

**METHODS:** The study included 10 volunteers (mean age,  $19.47 \pm 1.7$  years) who did not exercise. All subjects received supplements of oral zinc sulfate (3 mg/kg/day) for 4 weeks and their normal diets. The thyroid hormone and testosterone levels of all subjects were determined at rest and after bicycle exercise before and after zinc supplementation.

**RESULTS:**  $TT_3$ ,  $TT_4$ ,  $FT_3$ , and total and free testosterone levels decreased after exercise compared to resting levels before supplementation ( $p < 0.01$ ). Both the resting and fatigue hormone values were higher after 4 weeks of supplementation than the resting and fatigue values before supplementation ( $p < 0.05$ ).

**CONCLUSION:** The results indicate that exercise decreases thyroid hormones and testosterone in sedentary men; however, zinc supplementation prevents this decrease. Administration of a physiologic dose of zinc can be beneficial to performance.

## INTRODUCTION

Zinc, an important trace element, is the only metal found in almost all enzyme classes (Vallee & Falchuk, 1993). Therefore, zinc is essential for many reactions related to nucleic acid synthesis, protein, carbohydrate, and lipid metabolism (Vallee & Falchuk, 1993). Zinc has strong interactions with some hormones (Prasad, 1985), which is explained by the zinc-binding characteristic of many hormone receptors (Leblondel & Allain, 1989). Thyroid hormones and testosterone are among the most significant hormones in terms of their interactions with zinc (Leblondel & Allain, 1989; Prasad, 1985). Low values of zinc in hypothyroidism and high values

in hyperthyroidism point to a relation between zinc and thyroid hormones (Leblondel & Allain, 1989; Leblondel *et al.* 1992). High concentrations of zinc in the testes and accessory sex glands demonstrate that zinc plays an important role in the reproductive system (Kaya *et al.* 2006a; Kaya *et al.* 2006b; Levis-Jones *et al.* 1996; Prasad, 1985). Zinc maintains sperm membrane integrity, increases sperm motility, and regulates the spiral movement of the sperm tail (Levis-Jones *et al.* 1996; Prasad, 1985). Those reports emphasize a possible relation between zinc and testosterone.

Exercise has important effects on zinc metabolism or vice versa (Baltaci *et al.* 2003b; Kilic *et al.* 2006). Long-term resistance exercise significantly

reduced serum zinc levels in both male and female athletes (Haralambie, 1981). Reduced levels of zinc in individuals involved in resistance sports may be related to several mechanisms, the most important reason being a zinc-deficient diet (Khaled *et al.* 1999). It is also known that loss of zinc through perspiration and the skin is much higher in athletes than in those who do not participate in sports (Cordova & Alvarez-Mon 1995). The decreased serum zinc level also may result from increased urinary zinc loss resulting from breakdown of skeletal muscle protein observed in athletes who exercise regularly (Cordova & Alvarez-Mon 1995). As a result of the reduced serum zinc concentration in athletes, the muscle zinc concentration also decreases (Baltaci *et al.* 2003b; Cordova & Alvarez-Mon 1995). Because zinc is necessary for the activity of some enzymes in energy metabolism and because exercise decreases muscle zinc levels, resistance capacity and muscle tiredness may decrease. The relation between muscle tiredness and zinc is a topic that merits attention.

A relation between exercise and testosterone and thyroid hormones, which have important effects on energy metabolism, seems inevitable. However, the results of studies on this topic are contradictory. De Souza *et al.* (1994) reported that total and free testosterone levels of long-distance runners significantly decreased after exercise compared with short-distance runners and sedentary individuals. Similarly, Broocks *et al.* (1990) reported that testosterone levels decreased in exercising rats. However, other studies also reported significant increases in total and free testosterone levels after acute exercise (Bosco *et al.* 1996) and that total and free testosterone levels remained unchanged in rats made to do swimming exercise for 5 months (Woody *et al.* 1998). Similar contradictions are observed in studies about the relation between thyroid hormones and exercise. In a study that included eight men, the effects of different rates of oxygen consumption (47%, 77%, and 100%) and extended exhausting treadmill exercise on hormonal response were investigated. The investigators found an increase in thyroid stimulating hormone (TSH) depending on the intensity and duration of the exercise, but there was no change in the triiodothyronine ( $T_3$ ) and thyroxine ( $T_4$ ) levels (Galbo *et al.* 1977). It was reported that limited ischemia during bicycling for 45 minutes increased  $T_3$  levels but did not alter the thyroid-stimulating hormone (TSH) and free  $T_4$  levels (Viru *et al.* 1998). However, acute exercise significantly increased thyroid hormone levels, which was the result of exhaustion (Berchtold *et al.* 1978).

An overall evaluation of the available information on this topic reveals that there is a relation between zinc and exercise; exercise, thyroid hormones, and testosterone; and zinc, thyroid hormones, and testosterone. The aim of the present study was to examine the effect of fatiguing exercise on thyroid hormones and testosterone levels in sedentary men who received oral zinc supplements for 4 weeks.

## MATERIAL AND METHODS

### Physical characteristics of subjects and zinc administration

The study included 10 male students (mean age,  $19.47 \pm 1.7$  [mean  $\pm$  SD] years) who did not actively exercise. The mean height of the subjects was  $178.7 \pm 5.3$  cm and the mean weight was  $71.56 \pm 8.87$  kg. All subjects received oral zinc sulfate (3 mg/kg/day) supplements for 4 weeks in addition to their normal diets. All subjects were healthy and volunteered for the study. The study protocol was approved by the local ethics committee.

### Exercise testing

A Sensor Medics 2900 Metabolic Measurement Cart Device was used to perform the fatiguing exercise (aerobic) tests on a bicycle ergometer at the Department of Physiology of (Meram Medical School) Selcuk University. The subjects' maximal respiratory gas parameters were recorded for about 1 minute each and then the subjects did a 3-minute, 40-watt-load warm-up. The initial load heart rates of the subjects were recorded using a Polar Sport Tester, so that the heart rate 1 minute after warm-up was 120–130 beats per minute. After warm-up, an initial load was applied to subjects on 12 W OK AS IS and then the load was increased each minute. The pedaling speed was set at 60 rpm, and the test was continued until the pedal rate fell below 50 rpm or until the subject was unable to continue pedaling.

### Collection of blood samples

Blood samples were collected four times from each subject for hormone analyses. For the first measurement, blood was taken to determine the resting hormone parameters before zinc supplementation began. For the second measurement, blood samples were collected for hormone analysis just after fatiguing exercise and before zinc supplementation. For the third measurement, blood samples were taken for resting hormone analyses 4 weeks after the start of oral zinc sulfate supplementation (3 mg/kg/day). For the fourth measurement, blood samples were collected for hormone analyses immediately after exhausting exercise 4 weeks after the start of oral zinc sulfate supplementation (3 mg/kg/day). The blood samples were obtained at the same time intervals in both resting periods (at 9.30 a.m.) and after fatiguing exercise (just after the resting measurements).

### Biochemical analyses

Blood samples collected from the subjects were centrifuged for 10 minutes at 2500 rpm. Total  $T_3$ , total  $T_4$ , free  $T_3$ , free  $T_4$ , TSH, and free and total testosterone analyses were conducted on the serum samples. Analyses were carried out in Central Biochemistry Laboratory of Selcuk University Meram Medical School of Medicine.

All analyses, except for that of free testosterone, were carried out in an Immulite 2000 auto-analyzer. Total  $T_3$  analyses were carried out using an Immulite brand

(catalogue number L2KT32) test kit according to the competitive enzyme immunoassay method; results are presented as ng/dl. Total  $T_4$  analyses were done using an Immulite brand (catalogue number L2KT42) test kit according to the competitive chemiluminescent enzyme immunoassay method; the values are expressed as  $\mu\text{g/dl}$ . The free  $T_3$  analyses were carried out using an Immulite brand (catalogue number L2KF32) test kit according to a competitive analogue-based immunoassay method; the values are presented as pg/ml. The free  $T_4$  analyses were conducted using an Immulite brand (catalogue number L2KF42) test kit according to the competitive analogue immunoassay method; the results are expressed as ng/dl. The TSH analyses were carried out using an Immulite brand (catalogue number L2KT52) test kit according to an immunometric assay method; the results are presented as MIU/ml. The total testosterone analyses were carried out using an Immulite brand (catalogue number L2KTT2) test kit according to a competitive immunoassay method; the results were presented as ng/dl. The free testosterone analyses were done in a gamma counter using a Coat-A-Count brand (catalogue no TKTF1) test kit by radioimmunoassay method; the results are expressed as pg/ml.

#### Statistics

Statistical evaluation was conducted using Minitab software for MS Windows. Arithmetic mean values and standard deviations (SD) of all parameters were calculated. Variance analysis was used to establish the differences among groups. The least significant difference was used to compare the mean values of groups that were found to be significant by variance analysis. The level of statistical significance was  $p < 0.05$ .

## RESULTS

After exercise, the total  $T_3$ , total  $T_4$ , and free  $T_3$  levels decreased significantly ( $p < 0.01$ ) before zinc supplementation. After 4 weeks of zinc supplementation both during rest and after exercise, the total  $T_3$ , total  $T_4$ , and free  $T_3$  values were higher than before supplementation. Contrary to the pre-supplementation phase, the total  $T_3$ , total  $T_4$ , and free  $T_3$  levels did not decrease after fatiguing exercise (Table 1).

The highest free  $T_4$  and TSH values were obtained during rest after zinc supplementation ( $p < 0.05$ ). There was no significant difference in free  $T_4$  and TSH levels between resting and fatigue after exercise before zinc supplementation and the free  $T_4$  and TSH levels after zinc supplementation (Table 1).

Total testosterone and free testosterone levels measured before zinc supplementation decreased significantly during fatigue compared with at rest ( $p < 0.05$ ). After zinc supplementation, the total testosterone and free testosterone levels at rest and after exercise were higher than before supplementation ( $p < 0.05$ ). Total testosterone and free testosterone levels after zinc supplementation did not differ during rest and fatigue (Table 2).

## DISCUSSION

The results of studies examining how thyroid hormones are affected by exercise are inconsistent. In a study of weightlifters, McMurray *et al.* (1995) found that exercise did not change the  $T_3$  concentration, while the  $T_4$  concentration increased in the 20 minutes after exercise. However, TSH, free  $T_3$ , free  $T_4$ , total  $T_3$ , and

**Table 1.** Serum thyroid hormone levels of the study subjects.

Measurements	BEFORE SUPPLEMENTATION		AFTER SUPPLEMENTATION	
	Before exercise (resting)	After exercise (fatigue)	Before exercise (resting)	After exercise (fatigue)
Total $T_3$ (ng/dl)	93.42±14.69 <sup>b</sup>	84.97±12.58 <sup>c</sup>	105.64±15.45 <sup>a</sup>	100.28±10.48 <sup>a</sup>
Total $T_4$ ( $\mu\text{g/dl}$ )	7.86±0.97 <sup>b</sup>	6.28±0.66 <sup>c</sup>	8.98±1.34 <sup>a</sup>	8.92±1.39 <sup>a</sup>
Free $T_3$ (pg/ml)	3.45±0.62 <sup>b</sup>	2.98±0.69 <sup>c</sup>	4.02±0.52 <sup>a</sup>	3.98±0.70 <sup>a</sup>
Free $T_4$ (ng/dl)	1.40±0.28 <sup>b</sup>	1.38±0.12 <sup>b</sup>	1.61±0.32 <sup>a</sup>	1.50±0.35 <sup>b</sup>
TSH (MIU/ml)	1.79±0.86 <sup>b</sup>	1.43±0.63 <sup>b</sup>	2.58±1.44 <sup>a</sup>	2.26±1.23 <sup>b</sup>

a>b>c - different letters in same line are significant for all parameters ( $p < 0.05$ ).

**Table 2.** Serum total and free testosterone levels of the study subject.

Measurements	Total testosterone (ng/dl)	Free testosterone (pg/ml)
Before supplementation (resting period)	657.10±168.96 <sup>b</sup>	21.95±6.70 <sup>b</sup>
Before supplementation (fatigue)	612.90±160.80 <sup>c</sup>	16.57±4.51 <sup>c</sup>
After supplementation (resting period)	756.20±165.98 <sup>a</sup>	32.04±8.48 <sup>a</sup>
After supplementation (fatigue)	749.75±171.20 <sup>a</sup>	33.50±5.61 <sup>a</sup>

a>b>c - different letters in same column are significant as statistic ( $p < 0.05$ ).

total  $T_4$  concentrations increased significantly immediately after acute exercise, which was attributed to fatigue (Bosco *et al.* 1996). Nonetheless, another study reported that long-term exercise of moderate intensity did not have an important impact on thyroid hormones (Berchtold *et al.* 1978). Ischemia (a 15–20% decrease in blood flow) during bicycling for 45 minutes increased the  $FT_3$  concentrations but did not change the TSH and free  $T_4$  concentrations (Viru *et al.* 1998).

In the present study, total  $T_3$ , total  $T_4$ , and free  $T_3$  levels decreased after fatiguing exercise before zinc supplementation. However, no such difference was observed in the free  $T_4$  and TSH levels, which indicates that total  $T_3$ , total  $T_4$ , and free  $T_3$  are significantly inhibited just after exhaustive exercise in sedentary people. Our findings do not agree with the results of the researchers cited previously. However, in a study on female gymnasts, Jahreis *et al.* (1991) found that intense exercise for 3 days significantly reduced the  $T_3$  concentration. It was reported that exercise together with restricted food intake inhibited the  $T_3$  concentration in rats (Broocks *et al.* 1990). Another study reported that physically active men had lower thyroid hormone levels than sedentary men (Ravaglia *et al.* 2001). The findings of the researchers reporting that thyroid hormones decreased after exercise are consistent with the reduced total  $T_3$ , total  $T_4$ , and free  $T_3$  concentrations that we observed after fatiguing exercise.

Results of previous studies point to a positive relation between zinc and thyroid hormones (Leblondel & Allain, 1989; Leblondel *et al.* 1992). The assertion that free and total  $T_3$  levels, which are lower in people with a zinc deficiency, returned to normal after zinc supplementation and high serum  $rT_3$  levels decreased thereafter (Napolitano *et al.* 1990) shows that zinc has a role in thyroid hormone metabolism. Identification of a 67% decrease in type 5-deiodinase enzyme activity in the liver in zinc deficiency (Kralik *et al.* 1996) is evidence of the role that zinc plays in converting  $T_4$  to  $T_3$ . Reports to the effect that thyroid hormones were reduced in zinc deficiency (Baltaci *et al.* 2004) and thyroid hormone levels increased with zinc supplementation (Baltaci *et al.* 2003a) exemplify the dramatic effect of zinc on thyroid hormones. Our study demonstrates that 4 weeks of zinc supplementation increased thyroid hormones in sedentary males. This increase was observed in all total  $T_3$ , total  $T_4$ , free  $T_3$ , free  $T_4$ , and TSH levels. These findings are consistent with the results of the above-mentioned researchers. However, these parameters were significantly inhibited in fatiguing exercise before zinc supplementation in sedentary men, indicating that zinc supplementation prevents the inhibition of thyroid hormones by acute, fatiguing exercise observed in sedentary men.

Resting free and total testosterone levels before zinc supplementation were significantly higher than after fatiguing exercise, indicating that fatiguing exercise performed by sedentary men significantly suppresses the free and total testosterone levels. In fact, results of studies

on the relation between exercise and testosterone show that there is no agreement on this topic. Besides studies reporting that exercise did not change the free and total testosterone levels (Berchtold *et al.* 1978; Ravaglia *et al.* 2001; Viru *et al.* 1998; Woody *et al.* 1998), others reported that exercise significantly increased the free and total testosterone levels (Bosco *et al.* 1996; Galbo *et al.* 1977; Humpeler *et al.* 1980). The inhibition we observed in the free and total testosterone levels just after fatiguing exercise before zinc supplementation is inconsistent with the findings of these researchers. Dobrzanski *et al.* (1981) showed that there was a significant decrease in serum testosterone after submaximal exercise that did not change for 45 minutes after exercise. The study of Dobrzanski *et al.* (1981) is the most appropriate study with which we can compare ours in terms of study method and exercise type, and their results are consistent with ours. A decrease in testosterone levels also was reported in mountaineers (Friedl *et al.* 1988) and wrestlers (Roemmich & Sinning, 1997).

Zinc, which is an important trace element, is the only metal found in almost all enzyme classes (Vallee & Falchuk, 1993). The presence of high concentrations of zinc in the testes and accessory sex glands shows that it plays an important role in the reproductive system (Wong *et al.* 2001). It was reported that a diet deficient in zinc alone led to hypogonadism (Prasad, 1985) and that there was a positive relation between zinc and testosterone (Fuse *et al.* 1999); Prasad *et al.* (1996) also reported a similar finding. Borderline zinc deficiency for 6 weeks was reported to decrease testosterone levels but did not affect luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels in rats (Hamdi *et al.* 1997). LH and FSH production was significantly inhibited in female rats fed a zinc-deficient diet (Bedwal & Bahuguna, 1994), while Om and Chung (1996) found that zinc deficiency significantly inhibited both testosterone and LH in male rats. Martin *et al.* (1994) also reported a similar finding. Our findings indicate that resting free and total testosterone levels after 4 weeks of zinc supplementation were significantly higher than the levels measured before zinc supplementation. The increase observed in testosterone levels as a result of zinc supplementation is consistent with the findings of researchers who studied the relation between zinc and testosterone. It should be underscored that while fatiguing exercise before zinc supplementation inhibited free and total testosterone levels, this inhibition was not observed in fatiguing exercise after zinc supplementation.

Exercise affects zinc metabolism or vice versa (Baltaci *et al.* 2003b). Because zinc is necessary for the activity of some enzymes in energy metabolism and because exercise decreases muscle zinc levels, there may be a decrease in resistance capacity and muscle tiredness. The relation between muscle tiredness and zinc is a topic that merits attention.

In conclusion, our study demonstrates that 1) fatiguing exercise inhibits thyroid hormones and testosterone concentrations in sedentary men, 2) zinc supplementa-

tion (3 mg/kg/day) for 4 weeks leads to increased thyroid hormones and testosterone levels, 3) zinc supplementation prevents the inhibition of thyroid hormones and testosterone levels in sedentary men caused by fatiguing exercise, and 4) administration of a physiologic dose of zinc may benefit performance.

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