The effects of boron administration on plasma leptin and lactate levels in ovariectomized rats which had acute swimming exercise

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Abstract

OBJECTIVES: The aim of this study was to investigate the effects of intraperitoneal (IP) boron administration on leptin and lactate levels in swimming exercise in ovariectomized rats.

METHODS: Eighty adult female rats were equally allocated into 8 groups. Group 1; Control, Group 2; exercise control, Group 3; ovariectomized control, Group 4; Boron control (2 mg/kg/day), Group 5; ovariectomized plus exercise, Group 6; exercise plus IP boron, Group 7; ovariectomized and exercise group plus IP boron, Group 8; ovariectomized plus boron.

RESULTS: Leptin levels in Group 1 were higher than those in Groups 2, 3 and 4 and lower than those in groups 5, 6, 7 and 8 ($p<0.01$). Leptin levels were higher in Group 3 than in Groups 2 and 4 and significantly lower than in all other groups ($p<0.01$). Lactate levels in Groups 2 and 4 were higher than those in all other groups ($p<0.01$). Lactate levels were significantly lower in Groups 1, 3, 5 and 8 than in all other groups ($p<0.01$). Lactate levels in Groups 6 and 7 were significantly lower than those in Groups 2 and 4 ($p<0.01$) and higher than those in groups 1, 3 and 5 ($p<0.01$).

CONCLUSION: The present findings demonstrate that ovariectomy and acute swimming exercise in rats led to a significant decrease in leptin levels and a significant increase in lactate levels and that boron administration prevented the increased in circulating lactate concentration induced by swimming exercise.

INTRODUCTION

Recently there has been an increase in the claims that boron element, which is widely used in metallurgy, is also important for human health (Naghi & Samman, 1993). In fact, boron is present in various areas of human life from food to some personal care products. Although it has been known since 1857 that boron element is present in plants, research into its effects in animal and human metabolism is more recent (Naghi & Samman, 1993).

ORIGINAL ARTICLE

Studies carried out to discover the functions of boron in the body show that it contributes to a number of vital functions like mineral and energy metabolism, brain functions, hormonal balance and bone growth and although its biochemical functions have not been exactly identified yet. It is recommended to take food rich in boron such as vegetables, fruits, broad bean family and dried fruits because of their benefits for the body (Nielsen, 1997).

Amateur and professional athletes alike use several ergogenic supplements that are claimed to increase sporting performance. In several branches of sports, many athletes add ergogenic supplements to their diets, while others make sure that basic elements are included in their diets (Ahrent, 2001). One of the elements that is investigated in terms of its relation to sporting performance is boron. It is reported that dietary boron supplement increases endogenic steroid production and side effects are seen only in high doses (Ahrent, 2001).

It is propounded that boron supplementation leads to an increase in testosterone levels and that dietary boron supplement may be beneficial for performance in individuals doing endurance exercise (Naghii, 1999). As it is reported, boron supplementation can be an ergogenic substance for athletes, since it increases steroid hormones and it is noted that the relation between boron and exercise should be investigated further (Naghii, 1999). However, no study was found in our literature search about boron-exercise-menopause relation.

The present study investigates not only the effects of boron administration on performance in ovariectomized and exercising female rats, but also the relation between boron administration and plasma leptin and lactate levels.

MATERIALS AND METHODS

Rats

Animal Materials and Groups: This study was carried out at Selcuk University (S.U), Experimental Medicine Research and Application Center (SUDAM) on 80 adult female rats weighing 200–250 g. of Spraque –Dawley species obtained from the same Center. SUDAM ethics committee approved the study protocol.

Experimental Design

Experimental animals were allocated to 8 groups as follows:

Group 1 (n=10) General Control Group: The group which was fed on a normal diet and which was not exposed to any procedure.

Group 2 (n=10) Exercise Control Group: The group that was fed on a normal diet for 6 weeks and made to do acute swimming exercise for 30 minutes at the end of the study period.

Group 3 (n=10) Ovariectomy Control Group: The group that was fed on a normal diet for 6 weeks three days after ovariectomy.

Group 4 (n=10) Boron Control Group: The group that was administered 2 mg/kg/day intraperitoneal boric acid per rat for 6 weeks.

Group 5 (n=10) Ovariectomy + Exercise Group: The group that was fed on a normal diet for 6 weeks after ovariectomy and that was made to do acute swimming exercise for 30 minutes at the end of the study period.

Group 6 (n=10) Boron + Exercise Group: The group that was administered 2 mg/kg/day intraperitoneal boric acid per rat for 6 weeks and that was made to do acute swimming exercise for 30 minutes at the end of the study.

Group 7 (n=10) Ovariectomy + Boron + Exercise Group: The group that was administered 2 mg/kg/day intraperitoneal boric acid per rat for 6 weeks after ovariectomy and that was made to do acute swimming exercise for 30 minutes at the end of the study.

Group 8 (n=10): Ovariectomy + Boron Group: The group which was ovariectomy and boron supplemented 2 mg/kg/day for 6 weeks.

Ovariectomy Procedure

Ovariectomy was performed under general anesthesia (60 mg/kg ketamin and 5 mg/kg rompun). Ovariectomy procedure was as follows: After the hair on the back of the rats was shaved, appropriate asepsis and antisepsis was ensured by betadin. Following that, rats were placed in ventral position and the skin was incised from 1/3 upper point of the stretch between medial part of the back and tail. Spinal muscle was reached after releasing subcutaneous tissues. Peritoneal cavity was accessed via the muscles on the back wall of the abdomen. Ovaries were taken out together with lipid tissue. Ovaries were cleared of the lipid tissue and clamped, ligated and cut. After hemorrhage was checked, other organs were put into the peritoneal cavity. The muscle was sutured with 2/0 chrome catgut and the skin with 2/0 silk (Waynfort & Flecnel, 1994).

Boric Acid Administration

Boron administration was prepared so as to have 2 mg of boron in 0.5 ml serum physiologic. 2mg/0.5ml serum physiologic: 22.87 H_3BO_3 was put into a one-liter volumetric flask and complemented to 1 liter with serum physiologic. Intraperitoneal boron administration to relevant rats was made daily at 12.00 a.m. for 6 weeks.

Swimming Exercise

Swimming exercise was done in a 50 x 50 cm heat-resistant glass pool with a thermostat to fixate the temperature at 37°C. Exercises were done once for 30 minutes at the end of the injection period. Experimental animals were made to swim in pairs. After swimming exercise was completed, they were decapitated and blood samples were collected for analysis (Kaya et al. 2006).

Biochemical Analysis Lactate Determinations

2 ml. of blood samples put into fluoride-oxalate anticoagulant containing tubes were placed in ice blocks and within the short period of 15 minutes they were centrifuged at +4°C for five minutes at 3000 rotations to separate plasma. The separated plasma samples were studied in S.U. School of Medicine Central Biochemistry Laboratory with TECHNICON RA-XT brand autoanalyzer equipment using colorimetric method (Sigma Diagnostic lactic acid kits were used). Plasma lactate levels (read at 550 nm wavelength) were determined as mg/dl.

Leptin Determinations

Plasma leptin analysis was carried out using Rat Leptin RIA test kit (Linco trademark catalogue no: RL-83K). The results were presented as ng/ml. Limit sensitivity and limit linearity of the leptin analysis are 0.5ng/ml and 50ng/ml respectively.

Statistical Analysis

SPSS package software was used for statistical analysis. Kruskal-Wallis variance analysis and Mann-Whitney U test were carried out. Level of significance was set at $p<0.05$. Values of leptin and lactate in study groups were presented as Mean ± SD.

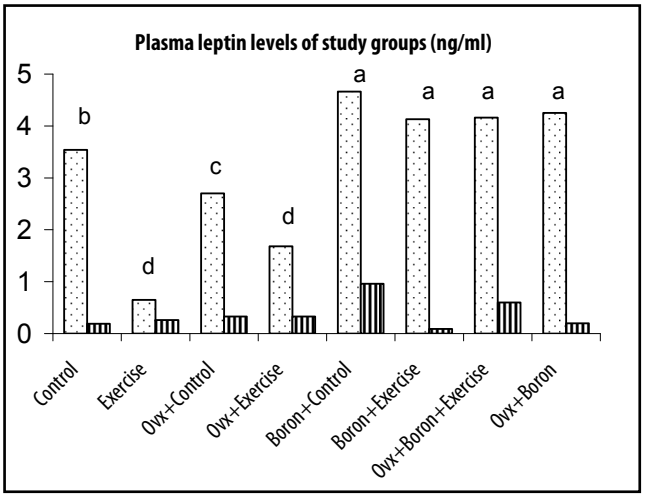

Figure 1: * Statistical significance (p<0.01) is a>b>c>d

Table 1. Plasma lactate levels of study groups

Groups	Lactate (mg/dl)*
G_1 =Generall Control (n=10)	26.04±4.59^c
G_2 =Control Exercise (n=10)	47.85±4.37^a
G_3 =OVX-Control (n=10)	23.87±2.12^c
G_4 =OVX-Exercise (n=10)	45.27±9.53^a
G_5 =Boron – Control (n=10)	25.71±7.66^c
G_6 =Boron-Exercise (n=10)	31.40±5.89^b
G_7 =OVX-Boron-Exercise (n=10)	32.78±8.74^b
G_8 =OVX-Boron (n=10)	25.50±5.90^c

* Statistical significance (p<0.01) is a>b>c

RESULTS

Plasma leptin levels were summarized in Figure 1. Plasma leptin levels of Groups 5, 6, 7 and 8 were higher than those in all other groups ($p<0.01$, Figure 1). Plasma leptin levels in the general control group (Group 1) which was not exposed to any procedure were higher than those in Groups 2, 3 and 4 and lower than those in Groups 5, 6, 7 and 8 ($p<0.01$, Figure 1). Plasma leptin levels in ovariectomy control group (Group 3) were higher than those in Groups 2 and 4 and significantly lower than those in all other groups ($p<0.01$, Figure 1). Plasma leptin levels in control exercise group (Group 2) and ovariectomy exercise group (Group 4) were not different; but the levels in these two groups were found significantly lower than those in other groups ($p<0.01$, Figure 1).

Plasma lactate levels of the study groups were presented in Table 1. The comparison of plasma lactate levels among groups showed that the levels in Groups 2 (control exercise) and 4 (ovariectomy exercise) were higher than those in all other groups ($p<0.01$, Table 1). Plasma lactate levels in these two groups were not different from each other. Plasma lactate levels were significantly lower in Groups 1 (general control), 3 (ovariectomy control), 5 (boron control) and 8 (ovariectomy+boron supplemented) than in other groups ($p<0.01$), but the levels in the mentioned groups (Groups 1, 3, 5, 8) were not different from each other (Table 1). Plasma lactate levels in Groups 6 (boron exercise) and 7 (ovariectomy boron exercise) were significantly lower than those in Groups 2 (control exercise) and 4 (ovariectomy exercise) ($p<0.01$, Table 1) and higher than those in Groups 1, 3, 5 and 8 ($p<0.01$, Table 1). There was no significant difference between plasma lactate levels of Groups 6 and 7.

DISCUSSION

Results of the studies investigating possible relationship between female reproduction system and leptin in rats have been controversial. While it is claimed that female reproductive hormones have an important role in regulating leptin levels (Shimuzi et al. 1997; Ozcelik et al. 2004), there are also studies reporting that estrogen does not have a significant effect on leptin production (Watanobe et al. 1999). It can be said that there is no agreement between the results of studies on the re-

lation between ovariectomy and leptin in rats. Pinilla et. al. (1999) claimed that ovariectomy increased leptin levels in rats, while Shimizu et. al. (1997) showed that leptin secretion was inhibited in ovariectomized rats. The present results demonstrate that ovariectomy suppresses leptin secretion in rats. In this respect, our findings are consistent with those of Shimizu et. al. (1997). In the study that claimed that ovariectomy increased leptin secretion (Pinilla et al. 1999), estrogen was administered to rats after ovariectomy caused an increase in leptin levels. However, in the present study, we did not make hormone replacement in rats after ovariectomy. The contradiction between our findings and those of Pinilla et al. (1999) probably results from this difference in the experimental procedure.

Our findings demonstrate that an acute exercise significantly inhibits plasma leptin levels in rats. Although the results are contradictory, it is generally acknowledged that physical activity leads to changes in leptin secretion. Contrary to the findings claiming that exercise increased leptin concentration (Unal et al. 2005; Van-Aggel-Leijssen et al. 1999), Hickey and Calsbeek (2001) reported that exercise did not alter leptin levels. Similarly, Hilton and Loucks (2000) also concluded that exercise did not inhibit leptin production. Another study noted a decrease in leptin levels 9 hours after acute exercise (Nindl et al. 2002). The reports of the above-mentioned researchers appear not to support our findings. However, our findings are consistent with the results of the researchers (Karamouzis et al. 2002; Zaccaria et al. 2002), who reported that there were significant decreases in leptin levels after intensive exercise in long-distance swimmers and marathon runners. Pagano et. al. (1999) showed that leptin levels decreased by about 30% in rats following a 30-minute acute swimming exercise and this study can be said to be the most important study supporting our results. That is because we also investigated the effects of a 30-minute acute exercise on leptin levels. The study is fairly important since we can make a one-to-one comparison between this study and ours.

We obtained the highest plasma leptin levels in boron control, boron + exercise and Ovx-boron-exercise. Plasma leptin levels in these three groups were higher than those in all others. However, the levels in these three groups were not different from each other. We could not find a study in the literature investigating the relation between boron and leptin. However, our findings demonstrate that boron administration prevented the inhibition in leptin levels brought about by exercise or ovariectomy. It is stated that dietary boron supplement in athletes increases endogenic steroid production, is beneficial for performance and that side effects can be observed only when taken in high doses (Ahrendt, 2001). It is reported that boron mineral is an additional element that can provide muscle enhancement to athletes during endurance exercise. This claim is based on the study that showed that 3 mg of boron supplement increased estradiol and testosterone levels (Sheng et al. 2001). However, subsequent studies reported that 2.5 mg/day boron supplement for 7 weeks did not have any ergogenic value in testosterone levels, body composition and resistance during endurance exercise (Kreider, 1999). Studies conducted on athletes verified that boron supplement increased serum testosterone, but showed that it did not affect muscle development (Ferrando and Green, 1993).

It is known that leptin is plays an important role in the regulation of energy balance (Hickey & Calsbek, 2001). It is inevitable that leptin, which is an important hormone for the energy balance, is related with exercise and effective on performance (Hickey & Calsbek, 2001). In the study hereby, boron administration resulted in an increase in leptin levels. Boron administration at least prevents the reduction in leptin levels caused by both exercise and ovariectomy. However, further studies are needed to explain the mechanisms playing a role in boron-exercise, boron-leptin and boron-female reproductive system relations.

The highest plasma lactate levels in the present study were found in control exercise and Ovx-exercise rats. Plasma lactate levels in boron-exercise and Ovx-boron-exercise were significantly lower than those in Groups 2 and 4. These findings of ours suggest that boron administration in exercise leads to inhibition of lactate levels. However, lack of any relevant study investigating the relationship between boron administration and lactate makes it difficult to discuss the findings we obtained.

In the study carried out by Baltaci et. al.(2003), a negative relation was reported between plasma lactate levels and leptin in rats which were made to do acute swimming exercise. In other words, as leptin levels increase, lactate levels decrease and vice versa. In the present study we also found that leptin levels in the groups that received boron were higher than those in other groups. Parallel to this, we found reduced lactate levels in groups with high leptin levels. However, the point that needs to be addressed here is whether boron administration affects lactate by increasing leptin levels. The findings put forth in this study demonstrate that boron administration increased leptin levels and inhibited lactate levels. Studies about the relation between boron and exercise concentrate generally on the relation of this element to testosterone hormone, muscle development and bone metabolism (Beattie & Peace, 1993; Naghi, 1999; Sheng et al. 2001). Even this aspect of the studies' results suggests that boron administration could have positive effects on physical performance. Likewise we aimed to investigate primarily the effects of boron administration on exercise in ovariectomized rats. Recent studies make it evident that administration of estrogen is not sufficient in menopause treatment, and that several minerals including boron should be administered as treatment (Nielsen et al. 1987).

Various studies investigating the interaction between boron and reproductive system show that boron

administration contributes to such significant processes as endocrine system, calcium and bone metabolism and bone development. The present findings are likely to make an additional contribution to what is known on this topic.

CONCLUSION

Depending on the findings obtained in this study it can be said that

1. Ovariectomy in rats leads to an inhibition of leptin secretion;
2. Acute swimming exercise significantly inhibits plasma leptin;
3. Boron administration prevents the inhibition of leptin levels in both ovariectomized rats and acute swimming exercise; and
4. Boron administration prevented the increase in circulating lactate concentrations induced by swimming exercise.

Parallel to the information above, it can be concluded that physiologic doses of boron administration can be beneficial for performance.

REFERENCES

1 Ahrendt DM (2001). Ergogenic aids: counseling the athlete. *Am. Fam. Physician* **63:** 913–922.
2 Baltaci AK, Ozyurek K, Mogulkoc R, Kurtoglu E, Ozkan Y and Celik I (2003). Effects of zinc deficiency and supplementation on the glycogen contents and leptin levels of rats performing acute exercise. *Biol. Trace Elem. Res.* **96:** 227–236.
3 Beattie JH and Peace HS (1993). The influence of a low- boron diet and boron supplementation on bone, major mineral and sex steroid metabolism in women. *Br. J. Nutr.* **69:** 871–884.
4 Ferrando AA and Green NR (1993). The effect of boron supplementation on lean body mass, plasma testosterone levels, and strength in male bodybuilders. *Int. J. Sport Nutr.* **3:** 140–149.
5 Hickey MS and Calsbeek DJ. Plasma leptin and exercise : recent findings. *Sports Med.* **31:** 583–589.
6 Hilton LK and Loucks AB (2000). Low energy availability, not exercise stress, suppresses the diurnal rhythm of leptin in healthy young women. Am. J. Physiol. Endocrinol. Metab. **278:** E43–E49.
7 Karamouzis I, Karamouzis M, Vrabas IS, Christoulas K, Kyriazis N, Giannoulis E and Mandroukas K (2002). The effects of marathon swimming on serum leptin and plasma neuropeptide Y levels. *Clin. Chem. Lab. Med.* **40:** 132–136.
8 Kaya O, Gokdemir K, Kilic M and Baltaci AK (2006). Melatonin supplementation to rats subjected to acute swimming exercise: Its effect on plasma lactate levels and relation with zinc. *Neuro Endocrinol. Lett.* **27:** 263–266.
9 Kreider RB (1999). Dietary supplements and the promotion of muscle growth with resistance exercise. *Sports Med.* **27:** 97–110.
10 Naghii MR (1999). The significance of dietary boron, with particular reference to athletes. *Nutr. Health* **13:** 31–37.
11 Naghii MR and Samman S (1993). The role of boron in nutrition and metabolism. *Prog. Food Nutr. Sci.* **17:** 331–349.
12 Nielsen FH (1997). Boron in human and animal nutrition. *Plant. Soil.* **193:** 199–205.
13 Nielsen FH, Hunt CD, Mullen LM and Hunt JR (1987). Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. *FASEB J.* **1:** 394–397.
14 Nindl BC, Kraemer WJ, Arciero PL, Samatallee N, Leone CD, Mayo MF, and Hafeman DL (2002). Leptin concentrations experience a delayed reduction after resistance exercise in men. *Med. Sci. Sports Exerc.* **34:** 608–613.
15 Ozcelik O, Celik H, Ayar A, Serhatlioglu S and Kelestimur H (2004). Investigation of the influence of training status on the relationship between the acute exercise and serum leptin levels in obese females. *Neuro Endocrinol. Lett.* **25:** 381–385.
16 Pagano C, Marzolo M, Granzotto M, Ricquier D, Federspil G and Vettor R (1999). Acute effects of exercise on circulating leptin in lean and genetically obese fa/fa rats. *Biochem. Biophys. Res. Commun.* **255:** 698–702.
17 Pinilla L, Seoane LM, Gonzalez L, Carro E, Aguilar E and Casanueva FF (1999). Regulation of serum leptin levels by gonadal function in rats. *Eur. J. Endocrinol.* **140:** 468–473.
18 Sheng MH, Taper LJ, Veit H, Qian H, Ritchey SL and Lau KH (2001). Dietary boron supplementation enhanced the action of estrogen, but not that of parathyroid hormone, to improve trabecular bone quality in ovariectomized rats. *Biol. Trace Elem. Res.* **82:** 109–123.
19 Sheng MH, Taper LJ, Veit H, Thomas EA, Ritchey SJ and Lau KH (2001). Dietary boron supplementation enhances the effects of estrogen on bone mineral balance in ovariectomized rats. *Biol. Trace Elem. Res.* **81:** 29–45.
20 Shimizu H, Shimomura Y, Nakanishi Y, Futawatari T, Ohtani K, Sato N and Mori M (1997). Estrogen increase in vivo leptin production in rats and human subjects. *J. Endocrinol.* **154:** 285–292.
21 Unal M, Unal DO, Baltaci AK, Mogulkoc R and Kayserilioglu A (2005). Investigation of serum leptin levels in professional male football players and healthy sedentary males. *Neuro Endocrinol. Lett.* **26:** 148–151.
22 Van Aggel-Leijssen DP, Van Baak MA, Tenenbaum R, Campfield LA and Saris WH (1999). Regulation of average 24h human plasma leptin level; the influence of exercise and physiological changes in energy balance. *Int. J. Obes. Relat. Metab. Disord.* **23:** 151–158.
23 Watanobe H, Schioth HB, Wikberg JE and Suda T (1999). The melanocortin 4 receptor mediates leptin stimulation of luteinizing hormone and prolactin surges in steroid- primed ovariectomized rats. *Biochem. Biophys. Res. Commun.* **21:** 860–864.
24 Waynfort HB and Flecnell PA (1994). Ovariectomy in experimental and surgical technique in the rat. Second Edition, Academic Press, pp. 276–278.
25 Zaccaria M, Ermoloa A, Roi S, Englaro P, Tegon G and Varnier M (2002). Leptin reduction after endurance races differing in duration and energy expenditure. *Eur. J. Appl. Physiol.* **87:** 108–111.