ELSEVIER

The effect of boron supplementation on the distribution of boron in selected tissues and on testosterone synthesis in rats

M.R. Naghii and S. Samman

Human Nutrition Unit, Department of Biochemistry, University of Sydney, NSW, 2006, Australia

Boron has been shown to increase the concentration of oestrogen and testosterone in plasma. The aim of this study was to investigate further the effect of boron by determining the response to three levels of boron intake during an experimental period of 6 weeks. The concentration of plasma testosterone and its production in the testes were determined in addition to the distribution of boron in selected tissues. Boron was added to the drinking water as boric acid to provide 2, 12.5, and 25 mg boron/rat/d. Body weight gain was found to be higher at the lowest dose but no significant change was observed at the highest dose. The distribution of boron in all tissues reflected its level of intake with all tissues demonstrating an increase over time. Within 6 weeks, rats fed the lowest and intermediate doses appeared to have a favorable effect on the indices examined, whereas the toxic testicular effects indicated by significant increases in the plasma follicle stimulating hormone concentration and testicular atrophy was associated with the higher dose (25 mg). The synthesis of testosterone by the testicular homogenates in vitro *from its immediate precursor, androstenedione in the presence of boron was determined, but there did not appear to be any clear relationship between dietary boron and testosterone production in vitro. The effect of boron on steroidogenesis and testicular function and development appears to be proportional to the dose and subsequent boron concentration in the testes.* (J. Nutr. Biochem. 7:507–512, 1996.)

Keywords: boron; tissue concentrations; testosterone; gonadotrophin hormones; rats

Introduction

The extent and nature of the possible physiological effects or potential risk(s) of boron in animals and humans are still at a primary stage and require further investigation.[1,2] High doses of boron are encountered as a result of industrial exposure or in animal models that are used to mimic such events. Such exposure results in the impairment of reproductive function, reduced semen quality[3–7] and an increased incidence of male infertility (according to Trasenko 1972, as quoted by Ku et al[7]). Although the mechanism of boron toxicity is unknown, a decreased concentration of plasma testosterone has been reported in some studies,[6,8] whereas other reports show evidence of testicular atrophy and inhibition of spermiation.[4–7] Some of these effects have been shown to be dose-dependent, however, in all cases pharmacological doses of boron had been used and only limited information is available on lower doses.

Under normal circumstances, boron (B) is found in plants and animals in small quantities and it is, therefore, considered as a trace element.[9] Although B has been accepted as a vital element for plants,[10] mounting evidence suggests that B may be an essential nutrient for animals.[11] The potential role for boron in nutrition and metabolism has been reviewed[1] and of particular interest is the demonstration by Nielsen and colleagues[12] that low doses of boron resulted in an increase in the plasma concentration of both oestrogen and testosterone as well as an increase in calcium retention in postmenopausal women. Similarly, in a short-term supplementation trial in men,[13] we have shown that 10 mg of B induces an increase in the plasma concentration of

Address reprint requests to Dr. S. Samman at Human Nutrition Unit, Department of Biochemistry, University of Sydney, NSW, 2006, Australia
Received February 9, 1996; accepted June 19, 1996.

655 Avenue of the Americas, New York, NY 10010

0955-2863/96/$15.00
PII S0955-2863(96)00102-7

oestrogen and testosterone. Although not all published trials have shown these effects,[14,15] the potential for an element found in trace amounts to affect steroid hormone metabolism clearly warrants further investigation.

The purpose of this study was to investigate the effect of three levels of boron, as boric acid, on the boron content of selected tissues, testicular responsiveness, steroid hormone concentrations, and gonadotrophin hormones in adult male rats. In addition, the effect of boron on the production of testosterone from androstenedione in vitro utilizing testicular tissues was studied.

Methods and materials

Animals and diets

Adult male Sprague-Dawley rats (6 to 8 weeks old; 200 to 300 g) were obtained from Combined University Laboratory Animal Services (Little Bay, Sydney, Australia). Ten animals in each group were placed in polycarbonate cages with 12:12 h light/dark cycles and constant temperature (25°C). The rats were provided free access to a commercially available pelleted diet (Y.S. Feeds Pty Ltd., Young, NSW) that contained approximately 10 microgram of boron per gram of feed (see boron analysis).

After assigning the rats into the treatment groups, boron in the form of boric acid (BDH Chemicals, Victoria, Australia) was incorporated into deionized drinking water at three levels: 0.45, 2.86, and 5.72 g of boric acid per litre to provide an estimated daily intake of 2, 12.5, and 25 mg boron/rat/day. The pH of the drinking water that was provided to the animals was adjusted to between 6.5 to 7 with sodium tetraborate (BDH Chemicals).

Study design

Rats were maintained on their respective diets for an experimental period of 6 weeks. After 3 and 6 weeks on the diet, five rats from each group were anaesthesized by using ether, weighed, and blood was drawn by ventral aorta puncture. The blood was transferred into chilled tubes that contained EDTA (1 mg/ml; Disposable Products Pty Ltd., South Australia). Plasma was recovered from blood by centrifugation at 2500 rpm for 10 min at 4°C and divided into the aliquotes, which were then stored at −20°C. A number of organs that included the heart, lungs, liver, kidneys, spleen, and testes were removed, dissected free of extraneous tissues, and stored at −20°C. The testicular wet weight was recorded immediately after the removal of this organ. For comparative purposes, a group of four rats, which were fed their habitual diet, were processed in the same way however, these rats were not included in any statistical analyses but served to provide baseline information.

Analysis of boron

Organ tissues, plasma, and the cellular portion of blood were thawed and prepared for boron analysis within 2 weeks of collection. To avoid contamination, borosilicate glassware, and containers were not used. All samples were acid-digested using the following procedure: to 500 mg of a weighed sample or 0.5 mL of liquid of each tissue in a 5 mL polypropylene tube (Techno-Plas, St. Marys, South Australia), 0.5 mL of analytical grade concentrated sulphuric acid was carefully added and the contents were swirled to mix and allowed to stand for 15 to 30 min. Then, 0.5 mL of RO water was added to the mixture. The acid digestion produced a homogeneous product that was then analyzed using the method of Ikeuchi and Amano[16] with one minor modification in that the last step, 0.75 mL of extract was diluted with 25 mL of 95% ethanol. In our laboratory, the coefficient of variation is less than 2% and the percent recovery of boron has been estimated to be 97%.[13]

The determination of testosterone concentrations in plasma and testes

The plasma testosterone concentrations were determined using a radioimmunoassay (RIA) kit (Diagnostic Product Corp, Los Angeles, CA, USA). The sensitivity of the assay was 0.14 nmol/l (as provided by the manufacturer).

The testis from each rat was decapsulated with forceps, rinsed with ice-cold normal saline, and then 300 mg tissue was homogenised in 2200 μL of Krebs-ringer-bicarbonate buffer with a loose-fitting teflon glass homogeniser (Romer, Carlton, NSW). The sample was centrifuged and the supernatant was diluted 1:10 (v/v) and the testosterone concentration was assayed as described.

The determination of testicular testosterone production in vitro

Testicular homogenates were prepared as described. Androstenedione (Sigma Chemical Co., St. Louis, MO, USA) was added such that the final concentration was 60 μM. The samples were incubated at 37°C in a CO_2 incubator (Flow Laboratories Ltd., UK), under a constant flow of 95% O_2 and 5% CO_2 for 90 min. Aliquotes from each tube were collected at timed intervals (0, 15, 30, 60, and 90 minutes) diluted 1:50 (v/v) with normal saline and assayed for testosterone.

The production of testosterone was quantified as the area under the testosterone/time curve over 90 min by using the Microsoft Excel Version 4 (1985–92 Microsoft Corp. USA).

The determination of gonadotrophin hormones concentrations

Plasma follicle stimulating hormone (FSH) was measured using a commercially available kit suitable for the analysis of FSH in rats (RPA 550, Amersham International, UK). The standard was calibrated against the reference standard (FSH preparation NIH-RP2). There was negligible cross-reaction with r-LH, r-TSH, r-GH, r-PRL and r-ACTH and the sensitivity of the assay was 0.9 ng/mL; (information provided by the manufacturer).

Plasma luteinizing hormone (LH) was measured using a commercially available kit suitable for the analysis of LH in rats (RPA 552, Amersham International, UK). The standard was calibrated against the reference standard (LH preparation NIH-RP2). There was negligible cross-reaction with r-FSH, r-TSH, r-GH, r-PRL, and r-ACTH and the sensitivity of the assay was 0.8 ng/mL (information provided by the manufacturer).

Statistical analysis

To assess the effects of time, dose of boron, and their interaction, two-way analysis of variance (ANOVA) was carried out (SAS Institute Inc., Cary, NC, USA). Differences among treatment means were evaluated by use of Duncan's multiple range test. Changes in body weights and data from the in vitro study were analyzed with one way analysis of variance followed by Scheffe F-test. All effects, differences, and correlations were considered significant at $P < 0.05$.

Results

The effect of diet on whole body and testicular weight

Body weight increased significantly ($P < 0.0001$) at the lower doses (2, and 12.5 mg) in comparison with those fed the high dose of boron for either 3 or 6 weeks (*Table 1*).

Compared with animals fed the 2 mg, a significant increase in the testicular weight of the rats receiving 12.5 mg boron and a significant decrease at the 25 mg boron after 6 weeks was detected (*Figure 1*). The duration of treatment on testicular weight was significant for all doses ($P < 0.0001$) and the interaction between dose and time was highly significant ($P < 0.0001$).

The effect of diet on tissue distribution of boron

The boron concentrations of plasma, the cellular portion of blood, and tissues from the control and treated rats are presented in *Table 2*. The boron concentration of tissues in animals fed their habitual diet were below detection in blood components and varied between 0.2 and 0.4 ppm in tissues. After supplementation, the boron concentration of all tissues increased. In those fed 12.5 mg, the tissue boron concentrations at 3 weeks were higher than those fed 2 mg and appeared to increase further with time. In contrast, those receiving 25 mg showed a decrease in the boron concentration after 6 weeks compared with 3 weeks. Overall, the boron concentration in tissues increased with dose and among the organs examined the kidney showed the highest concentration of boron.

The effect of boron on plasma testosterone, testicular testosterone in vivo and testosterone production in vitro

Changes in plasma testosterone concentrations in response to increasing amounts of boron in the drinking water are illustrated in *Figure 2*. The plasma testosterone decreased significantly ($P < 0.002$) as the amount of boron consumed was increased. The response tended to be greater after 6 weeks compared with 3 weeks but this did not reach statistical significance. The decrease in the plasma testosterone concentration with increasing amounts of boron is paralleled by the testosterone concentrations in the testes (*Figure 3*). In addition, the changes in body weight tended to be inversely correlated by dose of treatments and positively correlated to the changes in plasma testosterone (*Figure 4*).

Table 1 The effect of three doses of boron for 3 or 6 weeks on body weight (g) and body weight gain of adult male rats. The means ± SD are obtained from n = 5 in each of the treatment groups and *n* = 4 in a reference group

Time	Reference Group	Treatment groups (mg boron) 2	12.5	25
Week 3				
Day 1	292 ± 17	232 ± 21	275 ± 20	290 ± 14
Day 21		366 ± 33	356 ± 26	306 ± 16
% wt gain/wk		19	10	2
P-value		0.0001	0.0001	NS
Week 6				
Day 1		236 ± 25	292 ± 13	330 ± 10
Day 42		419 ± 38	422 ± 23	377 ± 16
% wt gain/wk		13	7	2.3
P-value		0.0001	0.0001	0.0001

NS, not significant.

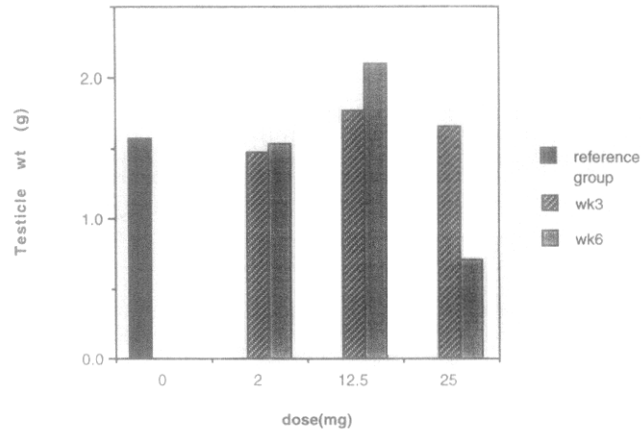

Figure 1 The effect of three doses of boron in drinking water in adult male rats on testicle weight. Each bar represents mean ± SD, *n* = 5 per treatment group and *n* = 4 in reference group. At week 6, rats fed 12.5 mg and 25 mg were significantly different from other treatment groups, as determined by two-way ANOVA followed by Duncan's multiple range test, $P < 0.05$.

The testicular concentrations of testosterone increased markedly in animals fed the 2 mg dose compared to those fed the 12.5 and 25 mg doses (*Figure 3*) such that dose ($P < 0.003$) and time ($P < 0.0001$) effects were significant. The plasma testosterone concentration was significantly correlated with the testicular testosterone concentrations (*Figure 5*) and boron intake at the lowest dose tested (2 mg) was associated with the highest average testosterone concentration.

The incubation of testicular homogenates with androstenedione led to a significant production of testosterone in tissues obtained from rats fed boron at 25 mg for 3 weeks and all other groups fed for 6 weeks ($P < 0.0001$) as compared with tissues from unsupplemented animals fed their habitual diet (*Figure 6*). The response appeared to be unrelated to the boron concentration of tissues.

The effect of treatment on gonadotrophin hormones

The mean plasma FSH concentration increased significantly ($P < 0.05$) in rats fed 25 mg boron compared to all other groups at both 3 and 6 weeks, with a greater increase at 6 weeks and the increase was time and dose dependent (*Figure 7*).

In contrast, the mean plasma LH concentration was significantly higher in animals fed the 2-mg dose and the increase was dose dependent (*Figure 8*). Plasma LH was highly correlated with testosterone production and consequently with the mean of plasma testosterone concentrations (*Figure 9*).

Discussion

Body weight

In this study, adult male Sprague-Dawley rats received 2, 12.5, and 25 mg boron/rat/day in their drinking water for 3 and 6 weeks. Although, no weight loss was seen in this study, the rate of body weight gain was reduced as the intake of boron was increased. Loss of body weight has

Table 2 Tissue concentration of boron (ppm) in male rats receiving boron in their drinking water (2, 12.5, and 25 mg/rat/day) for 3 and 6 weeks. The reference group shows the tissue concentrations on entry

Tissue	Reference group	2 mg		12.5 mg		25 mg	
		3 weeks	6 weeks	3 weeks	6 weeks	3 weeks	6 weeks
Heart	0.23 ± 0.05	1.22 ± 0.28	1.15 ± 0.17	8.18 ± 1.85	11.30 ± 1.06	28.68 ± 4.16	20.28 ± 3.17
Lung	0.28 ± 0.06	1.10 ± 0.18	1.14 ± 0.15	8.36 ± 1.81	10.91 ± 0.72	28.00 ± 3.53	21.43 ± 4.5
Liver	0.30 ± 0.07	1.12 ± 0.20	1.13 ± 0.19	8.68 ± 2.07	11.40 ± 1.65	27.60 ± 4.23	19.36 ± 2.88
Kidney	0.38 ± 0.04	3.37 ± 1.02	3.04 ± 0.60	15.80 ± 3.87	20.20 ± 3.64	44.20 ± 6.36	32.32 ± 7.18
Spleen	0.40 ± 0.11	1.31 ± 0.13	1.20 ± 0.16	9.36 ± 1.91	10.90 ± 0.90	29.55 ± 7.25	23.63 ± 4.80
Testes	0.26 ± 0.02	1.12 ± 0.13	1.07 ± 0.20	9.70 ± 2.10	11.27 ± 1.13	30.14 ± 2.78	23.02 ± 2.70
Plasma	ND	0.95 ± 0.20	1.05 ± 0.11	8.30 ± 2.51	11.19 ± 0.83	24.06 ± 3.02	19.80 ± 2.54
C.P.B*	ND	1.01 ± 0.20	1.14 ± 0.13	9.60 ± 2.62	12.47 ± 2.17	23.99 ± 4.83	22.93 ± 4.25

All values are expressed in ppm as mean ± SD for four rats in the reference group and five rats in all other groups.
Concentrations shown for tissues obtained from animals fed the 2-mg dose are significant different from those fed the 12.5 and 25 mg, and 12.5 mg dose from 25 mg by two-way of ANOVA followed by Duncan's multiple range test, $P < 0.05$.
*Cellular portion of blood.
ND, not determined.

been reported in studies utilizing high doses of boron and has been postulated to be the result of intestinal malabsorption[17,18] and/or reduced nutrient intake.[3] In this study, the higher body weight gain observed at the lowest level of boron intake (2 mg boron/day) was associated with higher testicular and plasma testosterone concentrations relative to animals fed the higher doses.

Concentration of boron in tissues

Rats receiving 12.5 and 25 mg boron/rat/day in their drinking water showed a gradual increase in blood and tissue boron concentrations which continued to increase up to 6 weeks of the study. All the tissues examined appeared to show a homogeneous distribution similar to the plasma concentration of boron in each dose except for the kidney, which attained the greatest concentration of boron. This may have occurred because of the contamination of the kidney with urine as the tissue was not perfused. Boron accumulation in the tissues increased in the rats fed 25 mg boron/day at week 3, but there was a reduction at week 6. Although the mechanism of this effect was not tested in this study, the decrease in tissues concentrations of boron with time may be the result of reduced food intake and/or lower rate of intestinal absorption. Consistent with previous studies,[19] there was no sign of lesions or gross abnormalities in the organs, except the notable testicular atrophy in the animals fed the highest dose.

Effect of boron on reproductive hormones and testicular function

Boron intake had a marked effect on the plasma testosterone concentration. Increasing the intake of boron from 2 mg to 12.5 and 25 mg resulted in lower plasma testosterone concentrations which tended to increase (or partially rebound) by 6 weeks of treatment.

The effect of boron on the plasma testosterone concen-

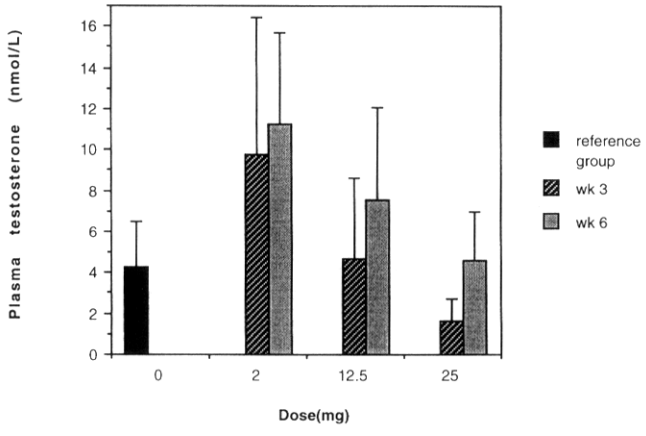

Figure 2 The effect of three doses of boron in drinking water in adult male rats on the concentration of plasma testosterone. Each bar represents mean ± SD, $n = 5$ per treatment group and $n = 4$ in the reference group. The plasma testosterone concentration in animals fed the 2-mg dose is significantly higher than in those fed 12.5 and 25 mg, as determined by two-way ANOVA followed by Duncan's multiple range test, $P < 0.05$.

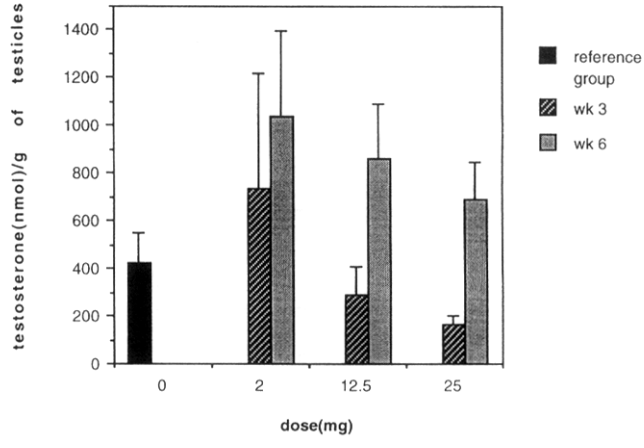

Figure 3 The effect of three doses of boron in drinking water in adult male rats on the testicular testosterone concentration. Each bar represents mean ± SD, $n = 5$ per treatment group and $n = 4$ in the reference group. The testicular testosterone concentration of animals fed the 2-mg dose is significantly higher than in those fed 12.5 and 25 mg, as determined by two-way ANOVA followed by Duncan's multiple range test, $P < 0.05$.

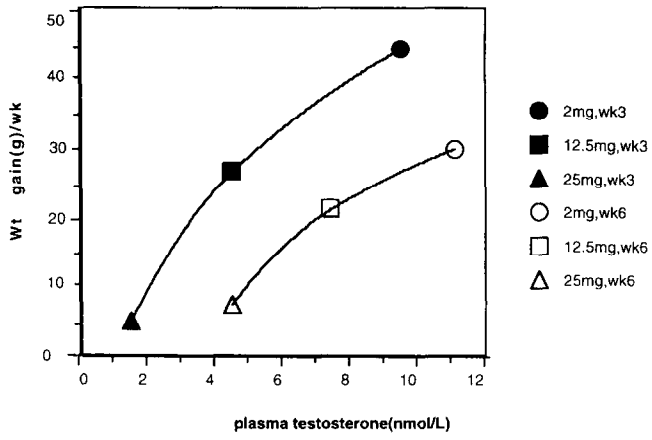

Figure 4 The relationship between the mean of plasma testosterone (nmol/L) and the mean of body weight gain per week (g) with three different doses for week 3 and 6.

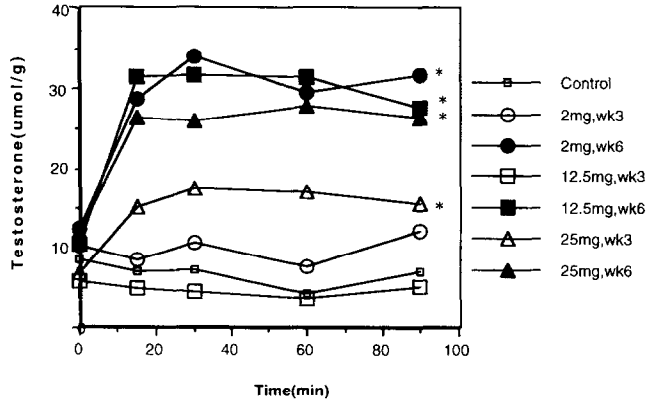

Figure 6 Testosterone production from androstenedione by testicle tissues obtained from animals fed 3 different levels of dietary boron. *Significantly higher than control, as determined by one-way ANOVA, $P < 0.0001$. Values were calculated from the areas under the curves.

tration was paralleled by the testicular testosterone concentration (as shown by a significant and strong correlation, $r = 0.86$, $P < 0.0001$). However, the difference between weeks 3 and 6 appeared more marked than that observed for the plasma testosterone concentration and suggests that the leydig cells, which are responsible for the production of testosterone, are intact despite the observed testicular atrophy in rats fed 25 mg. These observations are consistent with previous findings which show testicular histopathology in rats consuming 23 to 30 mg of boron per day for 90 days[17] and evidence of atrophy when the boron concentration in the testes is greater than 20 ppm. Lower concentrations of boron in the testes (5 ppm), which are achieved when rats are fed 6 mg boron for 9 weeks, inhibit spermiation.[7]

Although the mechanism for the effect of boron on plasma testosterone was not tested in these experiments, it is tempting to suggest that the lower concentration of testosterone in the plasma and testes of animals fed 25 mg compared with those fed 2 mg may be an effect of feed back control. The higher concentration of FSH as seen with animals fed the 25 mg dose, inhibits testosterone production, which results in less LH secretion, which leads to a lower plasma testosterone concentration. However, the mechanism is likely to be multifactorial and may include the inhibition of precursor formation with high doses of boron.

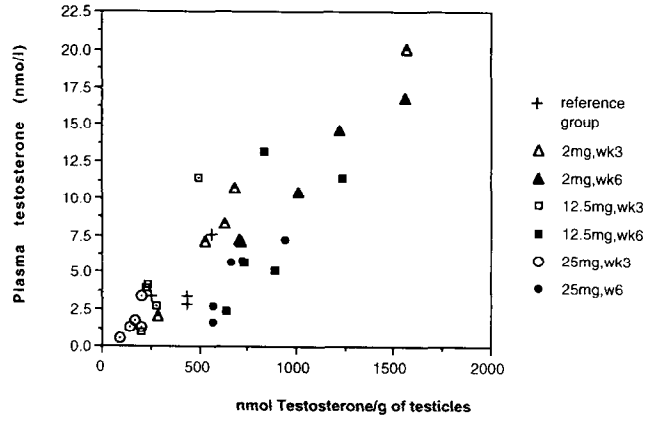

Figure 5 The relationship between the testosterone concentration in testicles (nmol/g) and the plasma testosterone concentration (nmol/L). ($y = 0.011x-0.3$; $R = 0.86$; $P < 0.0001$).

Effect on the biosynthesis of testosterone in vitro

An important consideration regarding the impact of boron on the metabolism of steroid hormones was to determine the extent of steroidogenesis in the absence of a feed back control mechanism. Thus, testicular homogenates from rats fed different levels of boron (as boric acid) were incubated with androstenedione in vitro and were shown to produce higher testosterone concentrations than homogenates obtained from animals fed their habitual (low boron) diet. These observations are in contrast to those of Ku et al.[20] who could not demonstrate any effect of added boron on steroidogenesis. The feature of our study was the physiological incor-

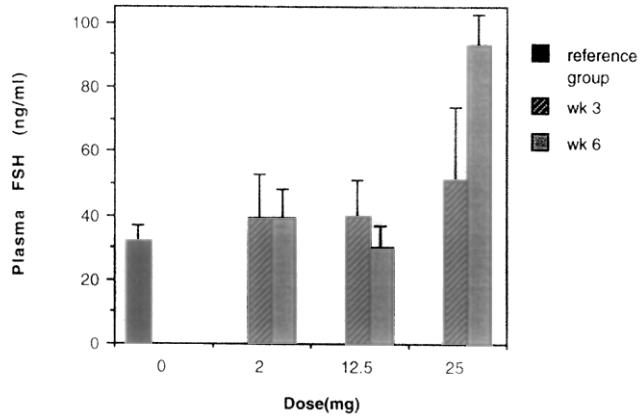

Figure 7 The effect of three doses of boron in drinking water in adult male rats on the plasma FSH concentration. Each bar represents mean ± SD, $n = 5$ per treatment group and $n = 4$ in the reference group. The plasma FSH concentration of animals fed the 25 mg dose for 6 weeks is significantly higher than those fed 12.5 and 25 mg, as determined by two-way ANOVA followed by Duncan's multiple range test, $P < 0.05$.

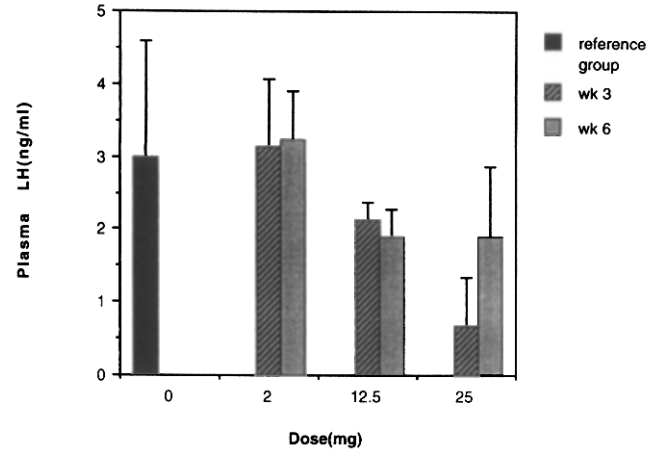

Figure 8 The effect of three doses of boron in drinking water in adult male rats on the plasma LH concentration. Each bar represents mean ± SD, n = 5 per treatment group and n = 4 in the reference group. The plasma LH concentration of animals fed the 2-mg dose is significantly higher than 12.5 and 25 mg, and those fed the 12.5 mg dose demonstrated a significantly higher concentration than their counterparts fed 25 mg, as determined by two-way ANOVA followed by Duncan's multiple range test, $P < 0.05$.

poration of boron into the tissues rather than adding boron in vitro. This suggests that the form of boron has a significant bearing on steroidogenesis in the rat.

Testicular toxicity has been postulated to be the result of certain biological processes that are unique to the testes as a result of boron exposure.[21] It has been hypothesized that these biological processes include perturbations to DNA and energy metabolism in the testes.[20] These suggestions are worthy of further investigation as is the use of lower doses of boron, which are likely to be of nutritional significance. In conclusion, it appears boron influences the hypothalamic-pituitary-gonadal axis in male rats via stimulation of testosterone biosynthesis in the testes.

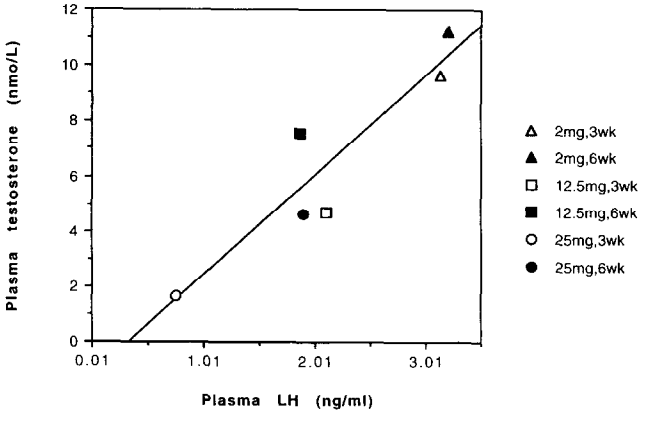

Figure 9 The relationship between mean of plasma LH (ng/ml) and mean of plasma testosterone (nmol/L). (y = 3.622x-1.292; R = 0.93; $P < 0.007$).

Acknowledgments

MR Naghii is the recipient of a postgraduate scholarship from the Ministry of Health and Medical Education of the Islamic Republic of Iran. We thank Mr. M. Neghab for technical assistance.

References

1 Naghii, M.R. and Samman, S. (1993). Role of boron in nutrition and metabolism. *Prog. Fd. Nutr. Sci.* **17,** 331–339
2 Mastromatteo, E. and Sullivan, F. (1994). Summary: International symposium on the health effects of boron and its compounds. *Environ. Health Perspect.* **102**(Suppl 7), 139–143
3 Krasovskii, G.N., Varshavaskaya, S.P., and Borisov, A.L. (1976). Toxic and gonadotropic effects of cadmium and boron relative to standards for these substances in drinking water. *Environ. Health Perspect.* **13,** 69–75
4 Lee, I.P., Sherins, R.J., and Dixon, R.L. (1978). Evidence for induction of germinal aplasia in male rats by environmental exposure to boron. *Toxicol. Appl. Pharmacol.* **45,** 577–590
5 Seal, B.S. and Weeth, H.J. (1980). Effect of boron in drinking water on the male laboratory rat. Bull. *Environ. Contam. Toxicol.* **25,** 782–789
6 Treeinen, K.A. and Chapin, R.E. (1991). Development of testicular lesions in F344 rats after treatment with boric acid. *Toxicol. Appl. Pharmacol.* **107,** 325–335
7 Ku, W.W., Chapin, R.E., Wine, R.N., and Gladen, B.C. (1993). Testicular toxicity of boric acid (BA): Relationship of dose to lesion development and recovery in the F344 rat. *Reprod. Toxicol.* **7,** 305–319
8 Anderson, S.A., Sauls, H.R., Pearce, S.W., and Fail, P.A. (1992). Endocrine responses after boric acid exposure for 2, 9, or 14 days in cannulated male CD rats. *Biol. Reprod.* **46**(Suppl), 124.
9 Shkolnik, M.Y.A. (1984). Boron. In *Trace Elements in Plants* (M.Y.A. Shkolnik, ed.) pp. 68–109, Elsevier, Amsterdam
10 Haddad, K.S. (1978). Boron, essential plant nutrient, review of the literature. Biological and Chemical Research Institute, Department of Agriculturc (NSW, Australia)
11 Nielsen, F.H. (1988). Boron, an overlooked element of potential nutritional importance. *Nutr. Today.* **23,** 4–7
12 Nielsen, F.H., Hunt, C.D., Mullen, L.M., and Hunt, J.R. (1987). Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women. *FASEB J.* **1,** 394–397
13 Naghii, M.R. and Samman, S. (1996). The effect of boron supplementation on its urinary excretion and selected cardiovascular risk factors in healthy male subjects. *Biol. Trace Element Res.* (in press)
14 Beattie, J.H. and Peace, H.S. (1993). The influence of a low-boron diet and boron supplementation on bone, major mineral and sex steroid metabolism in postmenopausal women. *Br. J. Nutr.* **69,** 871–884
15 Ferrando, A.A. and Green, N.R. (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
16 Ikeuchi, I. and Amano, T. (1978). A colorimetric determination of boron in biological materials. *Chem. Pharm. Bull.* **26,** 2619–2623
17 Weir, R.J. and Fisher, R.S. (1972). Toxicologic studies on borax and boric acid. *Toxicol. Appl. Pharmacol.* **23,** 351–364
18 Green, G.H. and Weeth, H.J. (1977). Responses of heifers ingesting boron in water. *J. Anim. Sci.* **46,** 812–818
19 Lim, B.T. (1989). Some toxic effects of boric acid on rats. *Mardi Res. J.* **17,** 69–74
20 Ku, W.W., Mason Shish, L., and Chapin, R.E. (1993). The effects of boric acid (BA) on testicular cells in culture. *Reprod. Toxicol.* **7,** 321–331
21 Ku, W.W., Chapin, R.E., Moseman, R.F., Brink, R.E., Pierce, K.D., and Adams, K.Y. (1991). Tissue disposition of boron in male Fischer rats. *Toxicol. Appl. Pharmacol.* **111,** 145–151