Plasma Boron and the Effects of Boron Supplementation in Males

Nancy R. Green¹ and Arny A. Ferrando²

¹Department of Nutrition and Food Science, Auburn University, Auburn, Alabama; ²National Aeronautics and Space Administration, Johnson Space Center, Houston, Texas

Recently, a proliferation of athletic supplements has been marketed touting boron as an ergogenic aid capable of increasing testosterone. The effect of boron supplementation was investigated in male bodybuilders. Ten male bodybuilders (aged 20 to 26) were given a 2.5-mg boron supplement, while nine male bodybuilders (aged 21 to 27) were given a placebo for 7 weeks. Plasma total and free testosterone, plasma boron, lean body mass, and strength measurements were determined on day 1 and day 49 of the study. A microwave digestion procedure followed by inductively coupled argon plasma spectroscopy was used for boron determination. Twelve subjects had boron values at or above the detection limit with median value of 25 ng/ml (16 ng/ml lower quartile and 33 ng/ml upper quartile). Of the ten subjects receiving boron supplements, six had an increase in their plasma boron. Analysis of variance indicated no significant effect of boron supplementation on any of the other dependent variables. Both groups demonstrated significant increases in total testosterone (p<0.01), lean body mass (p<0.01), and one repetition maximum (RM) squat (p<0.001) and one RM bench press (p<0.01). The findings suggest that 7 weeks of bodybuilding can increase total testosterone, lean body mass, and strength in lesser-trained bodybuilders, but boron supplementation affects these variables not at all. — Environ Health Perspect 102(Suppl 7): 73–77 (1994)

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Introduction

Boron content in almost all human tissue and body fluids is poorly documented. Early investigations using colorimetry suffered from contamination and other methodologic problems and high values were reported. Forbes et al. (1) and Johnstone et al. (2) dry-ashed blood samples, then analyzed for boron colorimetrically and reported 0.141 µg/ml and 0.4 µg/ml, respectively. Hill et al. (3) used a wet-tissue digestion prior to analysis for boron and found 0.12 µg/ml blood.

Then in 1963, Imbus et al. (4) published blood boron values for the largest sample (n = 147) found in the literature to date. They dry-ashed samples and followed with spectrographic analysis and reported a median value of 90 ng/g and mean of 114 ng/g with a range 39 to 365 ng/g (Table 1).

More recent studies using neutron activation with mass spectrometer, wet-digestion with inductively coupled plasma-mass spectrometry (ICP-MS), and wet-digestion with inductively coupled plasma emission spectrometer (ICP) have all reported lower values. Ward (5), using prompt gamma neutron activation, reported whole blood boron range of 0.14 to 0.74 µg/ml dry weight or approximately 28 to 148 ng/g. Also in 1987, Clarke et al. (6) reported values for whole blood analyzed by a mass-spectrometric assay of ³⁵He produced by thermal neutron reaction with ¹⁰B. They found a mean boron concentration of 30.8 ng/g with a range of 15.3 to 79.5 ng/g. Abou-Shakra et al. (7) reported whole blood boron on a population of 50 analyzed by wet digestion prior to ICP-MS. They found a median boron value of 56.7 ng/ml, lower quartile 49.2 and upper quartile 72.0, with a range from 8.4 to 170.4 (Table 1).

Our interest in boron was stimulated in 1987 when Nielsen et al. (8) reported its effects on mineral metabolism in 12 postmenopausal women. Boron supplementation of 3 mg/day was found to reduce urinary calcium in a low-magnesium diet, and to significantly increase serum estrogen and testosterone.

Athletes involved in weight training for increased strength or muscular size have long sought to enhance their natural testosterone levels. The use of exogenous testosterone, or anabolic steroids, has been the means of choice; however, the dangers and side effects of these steroids are well documented (9), and the felonious nature of their possession has prompted many athletes to seek safer and more “natural” means of enhancing their testosterone levels. Recently, a proliferation of athletic supplements has been marketed, which tout boron as an ergogenic aid capable of increasing testosterone. The supposed effi-
cacy of boron as an ergogenic aid is based exclusively on the increased testosterone values found in Nielsen's study (8) on postmenopausal women.

Manufacturers of athletic supplements often market products as anabolic or ergogenic aids based on incomplete scientific investigation. The effect of boron supplementation demonstrated in a small population of postmenopausal women simply cannot be extrapolated to a population of young athletes. Therefore, we conducted a study to examine the effect of a commercially-produced boron supplement in healthy, male weightlifters on plasma free and total testosterone, plasma boron, lean body mass (LBM), and strength values (10).

Methods

Subjects and Training

Nineteen male weightlifters volunteered to participate in this study. All the subjects had been weight training for their own conditioning purposes at least 1 year prior to the study. Each subject outlined his training regimen in a presudy questionnaire and was instructed not to change his existing regimen for the study. The subjects weight trained 4 days a week throughout the 7 weeks of this study using their own bodybuilding protocols. No subject had used anabolic steroids within a 6-month period prior to the start of the experiment.

Subjects were randomly placed in a double-blind fashion into one of two groups. The experimental (boron) group (n = 10) received a commercial boron supplement and the control group (n = 9) a placebo (milk protein). Both groups were given a total of 49 tablets and instructed to take one tablet per day. Compliance for both groups was monitored through supplement counts at the end of the study period.

All boron and placebo tablets were from the same manufactured lots. Both were analyzed for boron content.

Experimental Procedures

A 3-day dietary record was collected over the first 3 days and last 3 days of the study to assess nutritional intake of the subjects. Diets were analyzed for nutrient composition using the Nutritionist III food/nutrient data base. Subjects were instructed to continue their normal dietary habits but were told to refrain from taking any vitamin–mineral supplements or athletic supplements 3 weeks prior to initiation of the study.

Initially and following the supplementation period, subjects were weighed hydrostatically for determination of lean body mass. Blood was drawn on days 1 and 49 between 0830 and 0930 from the antecubital vein for determination of plasma boron and testosterone. Blood was drawn on day 49 within 15 min of the time blood was drawn on day 1. Venous blood was collected into nonborosilicate vacutainers with EDTA tubes for plasma analysis of free and total testosterone. Blood was collected into sodium-heparinized tubes for blood boron analysis. All blood was centrifuged at 3500g for 15 min. Plasma was pipetted into acid-washed (HNO₃) polypropylene tubes, capped and frozen at −50°C until analysis. Free and total testosterone were determined by commercial RIA Coat-A-Count method (Diagnostic Products Corporation, Los Angeles, CA). Each sample was analyzed in duplicate.

Strength values were determined at a local gym. Strength scores were determined by the one repetition maximum (1 RM) in the bench press and back squat.

A microwave digestion technique was developed for sample digestion prior to plasma boron analysis (11). The microwave digestion system (CEM Corp., Matthews, NC) was equipped with a 600-W magnetron power source that is adjustable in 1% increments. The system included a rotating turntable, a fluoropolymer cased heating chamber, and high-volume exhaust blower. Time and power source were controlled by a programmable microprocessor. Digestion was carried out in 120-ml Teflon PFA vessels with relief valves. The Teflon PFA vessels were sealed, using a capping station, to 12 ft lb torque for proper valve operation.

Acids used for digestion and ICP rinse solution were double-distilled from vycor ultrapure nitric (15.8 N) or hydrochloric (6 M). Water used for rinse and dilution was deionized and triple-filtered, 17.8 MΩ/cm, and boron content below detection limit.

For blood digestion, the digestion vessels were weighed to four decimal places and approximately 4 ml (g) of plasma sample was transferred directly to the vessel by Eppendorf pipette with metal-free disposable tips. Two ml of concentrated (15.8 N) nitric acid was then added to the vessel. The safety valve and cap were placed on the vessel and tightened using the capping station. Vessels were placed into the rotating turntable of the microwave.

Four cycles of digestion in the microwave were required. The first cycle was programmed for 4 min at 75% power, followed by 8 min at 50% power, and finally 5 min at 0% power for cooling. Cycles 2 to 4 entailed 4 min at 100% power, followed by 8 min at 50% power, and finally 5 min at 0% power for cooling. The four cycles were required to insure the complete digestion of analyte to a clear yellowish fluid. Vessels were vented after each cycle to prevent "blowing" of the pressure relief disc and the concomitant sample loss.

Following the fourth digestion cycle, the vessels were removed, vented and allowed to cool to room temperature for 30 min, and then opened before weighing. The difference between total-vessel weight and digestion-vessel weight was used to adjust the total weight of the solution. This procedure minimized sources of contamination. Digested samples were transferred directly to acid-washed polypropylene syringes and micron filtered directly into the ICP autosampler.

Boron content of samples was determined with a Plasma II Inductively Coupled Plasma Emission Spectrometer (ICP). Prior to analysis, an optimization was accomplished [using a 1 ppm (μg/ml) boron standard as described previously (11)] for power setting, nebulizer flow rate, and viewing height above plasma.

The Plasma II ICP was allowed to achieve a stable plasma by operating for 1 hr prior to analysis. Because of the tenacity of boron and the high acid content of the plasma samples, all nebulizer and peristaltic tubing was replaced every 8 hr. A new quartz autosampler probe was installed prior to the boron analysis.

Rinse solution was placed in a plastic nitric acid-wash beaker. Solution height in the rinse beaker was maintained above sample height to alleviate sample-to-sample contamination.

The limit of detection was determined according to the test method of the U.S. Environmental Protection Agency. Seven consecutive measurements were obtained on a 0.05 μg/ml boron standard. A detection limit of 12 ng/ml was determined by multiplying the standard deviation of these seven measurements by 3.

All analyses were conducted utilizing boron-spiked acid blanks as control values. No reference materials are certified for boron concentration. National Institute of Science and Technology Standard Reference Material (NIST SRM) 1572, citrus leaves, was used as a test material to develop and validate the digestion procedure, because it had been used by others for boron analysis and allowed comparison.
of values. NIST SRM 1567a, wheat flour, was selected as a second test material because it contains a lower boron content close to blood values. Standards of 1.0 μg/ml of boron were analyzed with citrus leaves and wheat flour SRM.

In the analysis of plasma boron, pool control plasma was spiked with 0.5 μg/ml inorganic boron. All blanks were spiked with 0.5 and 0.05 μg/ml boron and analyzed with each plasma analysis. Also, an unspiked pool of the pooled plasma was analyzed with each run to check run-to-run precision.

Results

Citrus leaf and wheat flour digestate was a clear reddish-brown color. Four samples of SRM 1572, citrus leaves, were analyzed on two separate occasions. Citrus leaf boron values ranged from 49.3 to 54.9 μg/ml, with a mean of 51.8 ± 2.53 μg/ml (mean ± SD) and a CV of 4.9%. The mean obtained for the citrus leaves with the present method, 52 μg/ml, compares well with the value obtained by Hunt and Shuler (12), 50 μg/ml, and Clarke et al. (6), 63 μg/ml.

Analysis of four samples of SRM 1567a, wheat flour, resulted in range of 0.31 to 0.55 μg/ml, with a mean of 0.45 ± 0.10 μg/ml. The mean obtained with the present method, 0.45 μg/ml, compares favorably with the value reported by Clarke et al. (6), 0.58 μg/ml.

Ten boron tablets were analyzed on five separate occasions, while six placebo tablets were analyzed on three separate occasions for boron content. The boron supplement contained 2.49 ± 0.12 mg of boron and the placebo 0.002 ± 0.001 mg. The boron supplement was marketed to contain 5 mg of boron.

The digestion of plasma samples produced a clear yellow digestate. A pool plasma was analyzed with each plasma determination to check precision. Analysis of control plasma spiked with 0.50 μg/ml level resulted in values ranging from 0.51 to 0.61 μg/ml, with a mean of 0.56 ± 0.08 μg/ml and a CV of 13.7%. The mean recovery was 110%. Blank spikes of 50 ng/ml resulted in 92.7% and 98% recovery, this indicates minimal volatilization of boron by these procedures.

Six determinations of the pooled plasma on different days ranged from 16 to 26 ng/ml with an average of 22 ng/ml. Of the 19 subjects tested, 12 had detectable concentrations of boron ranging from 14 ng/ml to 39 ng/ml, with a median of 25 ng/ml. Table 2 shows a comparison of these values to serum values reported by Abou-Shakra et al. (7).

Subjects

Group means of subjects’ age, height, weight and training experience are listed with dietary data in Table 3. There was a significant difference in training history (p<0.005). The experimental group had been weight training for 7.3 ± 4.7 (mean ± SD) years, while the control group had 4.1 ± 2.1 years of weight training experience. Analysis of 3 days of dietary records revealed that individual dietary habits and group means did not deviate significantly from pretest to posttest. However, total kcal and carbohydrate intakes were significantly (p<0.05) different between groups (z-test) with the control group consuming more total calories (3804 ± 1063 kcal versus 3556 ± 1323 kcal) but less carbohydrate (48.5 ± 9.8% vs 59.8 ± 14.6%) than the experimental group.

Changes in the dependent variables are consolidated in Table 4. ANOVA revealed a significant increase (p<0.01) in lean body mass (LBM) for all subjects as a result of 7 weeks of weight training; however, there was no significant difference resulting from boron supplementation. Body fat percentages decreased slightly in both groups but the difference was not statistically significant.

Because of injuries, not all subjects were able to perform posttest 1 RM. Strength values reported are for subjects who completed both the pre- and posttest squat or bench press. In the squat, both the experimental mean (n = 9) and the control group mean (n = 6) increased from pretest to posttest. This increase was significant (p<0.001) in both groups and therefore was probably the result of 7 weeks of training (ANOVA). For the bench press, the experimental group mean (n = 9) and the control group mean (n = 9) increased from pretest to posttest. This increase was again significant (p<0.01) in both groups as a result of 7 weeks of training (ANOVA). There were no statistical differences in one repetition maximum for the squat or the bench press between groups as a result of boron supplementation.

Total testosterone values for all subject values were within the normal range of 2.7 to 10.7 ng/ml. The experimental group’s mean total testosterone increased from pretest to posttest, as did the control group’s. Total plasma testosterone was not significantly different between groups as a result of supplementation (ANOVA), but significantly increased in both groups with 7 weeks of training (p<0.001).

Free testosterone values ranged from 5.44 to 34.67 pg/ml, which is within the most reliable portions of the calibration curve. One value was discarded in the experimental group because of an inaccurate assay value. Plasma-free testosterone was not significantly different between

| Variable          | Boron (n = 10) | Control (n = 9) |
|-------------------|---------------|----------------|
| Lean body mass, kg | 70.2 ± 7.9    | 72.4 ± 5.5     |
| Body fat, %       | 88.1 ± 1.8    | 11.7 ± 2.2     |
| 1RM squat, kg     | 151.2 ± 32.5  | 136.5 ± 36.8   |
| 1RM bench press, kg | 128.6 ± 22.4 | 108.6 ± 24.7   |
| Total testo, ng/ml | 5.4 ± 1.6    | 5.4 ± 2.1      |
| Free testo, pg/ml | 17.3 ± 8.5    | 19.5 ± 7.3     |

RM, repetition maximum. Values are means ± SD. *Boron n = 9; control n = 6. *Boron n = 5; *Boron n = 9. *p<0.01 vs pretest. *p<0.001 vs pretest.

Table 2. Boron in plasma and serum (ng/ml).

| Quartile        | Plasma boron* | Serum boron* |
|-----------------|---------------|--------------|
| Median          | 25            | 22.3         |
| Lower quartile  | 15            | 18.3         |
| Upper quartile  | 33            | 24.6         |
| Minimum         | 14            | 8.3          |
| Maximum         | 39            | 40.1         |

*Microwave digestion and ICP analysis used in current study. n = 12. *Wet digestion and ICP-MS analysis. n = 50 (7).

Table 3. Summary of subject group anthropometric and dietary data.

|                | Experimental (n = 10) | Control (n = 9) |
|----------------|-----------------------|----------------|
| Age, years     | 23.2 ± 3.3            | 23.8 ± 2.9     |
| Height, cm     | 174.7 ± 4.8           | 178.9 ± 6.6   |
| Weight, kg     | 77.2 ± 9.5            | 82.2 ± 7.5    |
| Training, years| 7.3 ± 4.7             | 4.1 ± 2.1     |

Values are means ± SD. *Significant difference between groups (p<.05).

Table 4. Summary of dependent variable changes.

| Variable          | Pretest | Posttest | Pretest | Posttest |
|-------------------|---------|----------|---------|----------|
| Lean body mass, kg | 70.2 ± 7.9 | 71.2 ± 7.3 | 72.4 ± 5.5 | 73.3 ± 5.1 |
| Body fat, %       | 88.1 ± 1.8 | 83.1 ± 1.5 | 11.7 ± 2.2 | 10.8 ± 3.0 |
| 1RM squat, kg     | 151.2 ± 32.5 | 159.9 ± 31.4 | 136.5 ± 36.8 | 146.6 ± 15.0 |
| 1RM bench press, kg | 128.6 ± 22.4 | 130.5 ± 22.9 | 108.6 ± 24.7 | 114.1 ± 23.7 |
| Total testo, ng/ml | 5.4 ± 1.6    | 7.2 ± 2.7   | 5.4 ± 2.1   | 6.5 ± 1.9   |
| Free testo, pg/ml | 17.3 ± 8.5   | 19.8 ± 6.6  | 19.5 ± 7.3  | 20.9 ± 7.2  |
Table 5. Individual data for plasma boron (ng/ml).

| Boron (n=10) | Control (n=9) |
|--------------|--------------|
| Pretest      | Posttest     | Pretest | Posttest |
| ≤12 *        | 12           | 30      | ≤12      |
| 24           | 58           | 22      | ≤12      |
| 29           | 36           | ≤12     | 17       |
| ≤12          | 77           | ≤12     | 17       |
| 30           | ≤12          | 27      | ≤12      |
| 15           | ≤12          | 38      | ≤12      |
| 28           | 24           | ≤12     | ≤12      |
| 19           | 23           | ≤12     | ≤12      |
| 14           | 14           | ≤12     | ≤12      |
| 22           | 75           |         |          |

Three replicates for each sample were averaged. *Below detection limit, 12 ppb.

Plasmaboron values for both groups pre- and posttest are shown in Table 5. The experimental group demonstrated a 90% compliance in their supplementation as determined by supplement collection on day 49 of the study. The mean plasma boron concentration of the experimental group increased while the control group mean decreased. Plasma boron values were significantly different on account of group membership (p<0.01). Despite the opposite changes in plasma boron values, there was no statistically significant effect of time on boron values (ANOVA). Post hoc analysis (Fisher Least Significant Differences) revealed no significant difference as a result of supplementation, possibly because of the large standard deviation of the posttest experimental cell mean.

Discussion

The increase of approximately 1 kg of LBM through 7 weeks of weight training found in the present study is consistent with the literature. Resistance training will typically increase LBM. A 1 kg increase in LBM was noted after only 5 weeks (13) and 10 weeks (14) of weight/strength training in 8 and 26 males, respectively.

The absence of change in free testosterone is in agreement with the literature as well (15, 16). The increase in plasma testosterone was evident in both groups in this study, so it cannot be attributed to boron supplementation. The subjects displayed a high level of motivation and training intensity; therefore, the testosterone increase was probably the result of bodybuilding training. A significant increase (p<0.05) in serum total testosterone was noted in subjects of similar training status after 8 weeks of training (17).

Despite the overall increases in both group means of total testosterone and strength parameters as a result of training, there was no correlation between the increase in muscle strength and plasma testosterone values. Therefore, the increase in strength parameters was most likely the result of a concentrated bodybuilding training program.

It is highly probable that boron supplementation increases plasma boron content. A t-test of pre- and posttest values of each group revealed that plasma boron concentrations in the experimental group increased significantly, while the control group values decreased significantly (p<0.05). The ANOVA approached significance (p<0.07) due to boron supplementation despite the large standard deviation in the posttest experimental group values. There was no significant correlation between plasma boron and any dependent variables. Furthermore, there was no consistent trend between changes in plasma boron and changes in testosterone or strength values. For example, certain subjects with a significant increase in plasma boron demonstrated marginal increases in total testosterone and strength, whereas others with a significant decrease in plasma boron demonstrated increases in total testosterone and strength.

There was a wide variation in plasma boron in the experimental group (≤12 ng/ml to 77 ng/ml). Plasma boron values may have fluctuated because of the timing of supplement ingestion prior to blood sampling. Subjects were instructed to take one (2.5 mg) tablet per day, but were not instructed to do so at a specific time of the day. Five subjects displayed plasma boron increases, while three displayed little or no change in plasma boron (Table 5). Because compliance was >90% in the experimental group, and the experimental group displayed a significant increase in plasma boron, it can be concluded that 7 weeks of supplementation is capable of elevating plasma boron.

It is noteworthy that seven members of the control group had plasma boron values at or below the detection limit; only five had pretest values below detection limits. The decline in posttest control values may be the result of one or more factors. Inductively coupled plasma emission spectrometer analysis near the detection limit often resulted in negative values. Each plasma sample was evaluated by three separate readings and a negative value was averaged with the remaining values as a "zero."

Another factor affecting plasma boron may have been a fluctuation in boron intake. A national data base for boron in foods and personal care products has not been established; therefore, it is not possible to quantify boron intake.

Also, plasma boron may have declined because of training. Although subjects were asked not to change their training regimens for this study, it is not certain whether their increased training intensity during the study contributed to the decrease in posttest plasma boron values. Though not employed in the present study, a crossover design would have further clarified the influence of training on plasma boron.

The proposed efficacy of boron as an ergogenic aid is based on its ability to increase testosterone. Nielsen et al. (8) proposed that the increase in serum testosterone and β-estradiol demonstrated in 12 postmenopausal women was the result of an endocrine mechanism. However, boron supplementation did not increase plasma testosterone in young males in the present study.

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