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0163–4984/97/5603–0273 $11.50

The Effect of Boron Supplementation on Its Urinary Excretion and Selected Cardiovascular Risk Factors in Healthy Male Subjects

M. R. NAGHII AND S. SAMMAN*

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

Received September 20, 1995; Accepted December 20, 1995

ABSTRACT

Boron (B) is an essential trace element for plants and its interrelationship with mineral and bone metabolism and endocrine function in humans has been proposed. Relatively little is known about the occurrence of B in the food chain and hence a biomarker which reflects its intake is required. Two studies were carried out to quantify the urinary B concentration of subjects consuming their habitual diet and the effect of supplementation. In addition, the effect of supplementation on plasma lipoprotein cholesterol concentrations and susceptibility to oxidation and plasma steroid hormones were determined. Boron excretion, obtained on two different occasions from 18 healthy male subjects, was found to be in the range 0.35–3.53 mg/day, with no significant difference between the two occasions. Supplementation with 10 mg B/d for 4 wk resulted in 84% of the supplemented dose being recovered in the urine. Plasma estradiol concentrations increased significantly as a result of supplementation (51.9 ± 21.4 to 73.9 ± 22.2 pmol/L; $p < 0.004$) and there was a trend for plasma testosterone levels to be increased. However, there was no difference in plasma lipids or the oxidizability of low-density lipoprotein. Our studies suggest that the absorption efficiency of B is very high and estimation of the urinary B concentration may provide a useful reflection of B intake. In addition, the elevation of endogenous estrogen as a result of supplementation suggests a protective role for B in atherosclerosis.

Index Entries: Boron; steroid hormones; lipids; urinary excretion; calcium; humans; variability.

*Author to whom all correspondence and reprint requests should be addressed.

INTRODUCTION

The role of micronutrients in human nutrition is well-established and trace elements have been implicated with a number of chronic diseases. Boron is a nonmetal that possesses properties that are intermediate between metals and nonmetals. In 1923, B was accepted as an essential nutrient for plants and some recent evidence suggests that it may be an essential nutrient for animals *(1,2)*. The B content of some foods has been published from a number of countries *(3–7)* and, consistently, foods of plant origin are rich sources of B. In addition, wine (39.1 μg/g; *8*), raisins (25 μg/g; *6,8*) dried parsley (26.8 μg/g; *5*) and caviar (5430 μg/g; *9*) are particularly rich sources. Intake of B varies widely *(5)*, which may be explained, in part, by differences in agricultural methods and harvesting times.

Boron complexes with organic compounds containing hydroxyl groups and those with more than two hydroxyl groups react more strongly *(1)*. It is established that the simplest hydrogen compounds of B act as mild reducing agents, readily reducing aldehydes, ketones, and acid chlorides *(10)*, some of which are derived from the process of lipid peroxidation *(11)*. Elevated indices of lipid peroxidation in plasma or serum are associated with a wide range of pathological conditions, including atherosclerosis *(12–13)*, and interventions with agents that have antioxidant properties are hypothesized to hinder atherogenesis by preventing or retarding the oxidation of LDL *(14)*, possibly without lowering serum LDL levels.

Boron appears to have a widespread role in biochemistry and nutrition *(15)*. However, one of the current difficulties in advancing knowledge about its role is the lack of a comprehensive table of the B content of foods. As the natural variation in the food content of B influences its intake, a biomarker may provide meaningful information regarding the intake of free-living subjects. To date, relatively little information is available that supports the use of any specific marker.

Boron may influence risk factors for coronary heart disease, such as steroid hormones. Postmenopausal women supplemented with approx. 3 mg of B/d have a significant increase in 17-β estradiol and the concentration of testosterone, the precursor of estradiol, also increased *(16)*. In rats, large doses of B increase the concentration of plasma testosterone in a dose-dependant manner *(17)*. The impact of B supplementation on plasma steroid hormones and the potential effect on cardiovascular risk factors in males has not been determined.

We report the results of two studies carried out in a free-living group. In the first survey, the urinary B excretion of male subjects was measured on two different occasions to determine the basal levels of B excretion and its biological variability. In the second trial, male subjects were supplemented with 10 mg B/d and 24 h urine was collected to determine the extent of B excretion upon supplementation. Given

the postulated involvement of B in the metabolism of steroid hormones and the prevention of lipid peroxidation, the supplementation trial was also aimed at determining the effect of B supplementation on plasma steroid hormones, lipids, and LDL oxidizability in healthy subjects.

MATERIALS AND METHODS

Subjects and Experimental Design

Study 1

24-h urine samples were obtained on two different occasions at three weekly intervals from 18 healthy male subjects (Table 1). These samples were obtained as baseline samples from a zinc supplementation trial *(18)*. All plastic bottles used for collecting 24 h urine samples contained 15 mL of 5*M* hydrochloric acid as a preservative. After sample collection, the total volume for each sample was recorded and aliquots were centrifuged at 3000g for 15 min and the supernatants were stored at –20°C until analysis.

Study 2

Eight male volunteers were recruited for the study (Table 1). Subjects were asked to participate in a single-blind cross-over trial lasting 8 wk. Each subject acted as his own control in the experiment and was requested to consume two tablets, each containing 5 mg of B as sodium tetraborate, every day with breakfast and dinner for 4 wk, followed by a placebo tablet of identical appearance for 4 wk, or vice versa. The B supplements and placebo tablets used in this study were prepared from sodium tetraborate and lactose (Sigma, St. Louis, MO) at the School of Pharmacy, University of Sydney. Placebo tablets were identical in appearance, and contained lactose only.

Blood samples were collected prior to supplementation and after 4 wk. Venous blood (20 mL) was collected from the antecubital vein from subjects in the sitting position and placed in tubes that contained EDTA (1 mg/mL). Collection was always carried out between 8 and 9 AM with subjects in the fasted state. Plasma was separated by centrifugation at 1000*g* for 10 min at 4°C and stored at –80°C until analysis. Twenty-four hour urine samples were collected before and after the supplementation periods.

All subjects were nonsmokers and did not consume any medications or nutritional supplements, particularly antioxidants (vitamins C or E or β-carotene). They were requested to maintain their habitual diet and exercise throughout the study. The experimental protocols were approved by the University of Sydney Human Ethical Review Committee and written consent was obtained from each subject prior to commencement of the study.

Table 1
Subject Characteristics (Mean ± SD)

	Study 1	Study 2
Age (years)	26.0 ± 8.0	28.0 ± 7.6
Weight (kg)	75 ± 10.7	75 ± 6.6
BMI	24.0 ± 3.0	24.7 ± 1.7
n	18	8

Urinary Analyses

Boron

Urine samples (0.5 mL) were digested in capped polypropylene tubes (Techno-Plas, St. Marys, SA, Australia) at 95°C for 1.0 h. After cooling, 10% (v/v) 2-ethyl-1,3-hexanediol in chloroform was added and shaken vigorously, then centrifuged at 2000*g*. The extract was mixed with curcumin, followed by concentrated sulfuric acid. The mixtures were allowed to cool and then made up to 25 mL with 95% ethanol. The absorbancy was read at 554 nm in a UV-visible spectrophotometer (model UV-160A, Shimadzu, Tokyo, Japan). The method was based on that of Ikeuchi and Amano *(19)*. A urine sample was used as a control for each analytical run and the concentration was found to be consistent (coefficient of variation less than 2%, $n = 5$). The percent recovery of B was determined by the addition of 0.5 mg/mL to six urine samples and estimated to be 96.7 ± 3.4%.

Calcium

Urinary calcium (Ca) concentrations were determined by flame atomic absorption spectrophotometry (Varian Instruments, Melbourne, Australia).

Creatinine

Aliquots of urine were diluted 1:100 (v/v) with deionized water and creatinine concentrations were measured by using a commercially available kit (Roche Diagnostic, Basel) and an autoanalyzer (Cobas Fara, Roche Diagnostica, Switzerland) as described previously *(18)*. Creatinine concentrations were expressed as mmol creatinine/24 h.

Plasma Lipids and Hormone Analysis

Plasma total cholesterol and triacylglycerol concentrations were assayed enzymatically (Cholesterol CHOD-PAP Kit, Boehringer Mannheim, Australia, and Triglyceride PAP Unimate 5, Roche, Australia, respectively) by using an autoanalyzer (Cobas Fara II; Roche Diagnostica). Total high density lipoprotein (HDL) was assayed following dextran sulphate (DS)-magnesium chloride precipitation *(20)*. Briefly, 0.2 mL of a solution con-

taining 10 g DS (Sigma) and 0.5 mol $MgCl_2$ (BDH Chemical) per liter was added to 2 mL plasma, with thorough mixing. The plasma was then centrifuged at 1500*g* for 30 min at 5°C. An aliquot of the resulting supernatant was assayed for total HDL cholesterol, and LDL cholesterol was calculated using the SI version *(21)* of the Friedewald equation *(22)*.

Plasma testosterone and 17 β estradiol concentrations were measured by radioimmunoassay kits (Spectria, Orion Diagnostica, Finland). The estradiol kit uses a specific estradiol antibody, with negligible cross-reaction with estriol and estrone. The tracer is $[^{125}I]$ estradiol. The sensitivity of the assay is 20 pmol/L and within assay coefficient of variation was quoted as 9.7%. To minimize technical variation, samples from the same individual were measured as a batch.

LDL Isolation

LDL was isolated from plasma by using a single spin ultracentrifugation procedure as described previously *(23)*. Briefly, 3 mL plasma was adjusted to density (d) 1.21 g/mL with solid KBr (BDH Chemicals) and layered under 7 mL saline (d = 1.006 g/mL) containing 0.1% EDTA (AJAX Chemicals, Australia) in Quickseal tubes (Beckman Instruments, Palo Alto, CA). The tubes were filled with density solution (1.006 g/mL), then centrifuged at 70,000 rpm, for 3.5 h at 4°C in a 70.1 Ti rotor in a Beckman L8-M ultracentrifuge. The separated yellow LDL band was removed by aspiration (using needle and syringe) through the side of the tube and then dialyzed for 18 h at 4°C with six changes of buffered saline (0.9% NaCl, pH 7.4) made with Milli-Q purified water (Millipore, Australia). The protein concentration of dialyzed LDL was determined by a modified Lowry method *(24)*.

In Vitro LDL Oxidation

LDL obtained from the volunteers during the placebo and treatment periods was subjected to oxidation. Oxidation of LDL was determined as the production of conjugated dienes, which was monitored by continuous measurement of the change in absorbance at 234 nm *(25)*. Using buffered saline as diluent, freshly prepared LDL (equivalent to 200 μg LDL protein) was incubated with $CuSO_4$ (final concentration 5 μmol/L) for 1.5 h at 37°C in a UV-visible spectrophotometer (model UV-160 A; Shimadzu) with an attached electronic temperature regulator (CPS-Controller, Shimadzu). Control incubations were conducted in the absence of $CuSO_4$. Absorbance at 234 nm was automatically recorded at 5 min intervals and lag phase was determined *(25)*.

Malondialdehyde (MDA) Determination

Following the in vitro oxidation of LDL, butylated hydroxy toluene (BHT), a chain-breaking antioxidant, was added to the incubation mix-

ture to prevent amplification of lipid peroxidation. To measure the extent of LDL lipid peroxidation, a thiobarbituric acid (TBA) assay was employed. Briefly, the test sample is heated with 2-TBA at low pH and a pink chromagen, the [TBA]2-MDA adduct, is measured by fluorescence at 553 nm *(26)* and samples were compared to appropriate standards formed from 1,1,3,3-tetraethoxypropane (Sigma). The true [TBA]2-MDA compound was separated from other chromogens absorbing at this wavelength by HPLC. A C18 column (250 mm × 4.6 mm; particle size 5 μm) (Supelco, Bellefonte, PA) was used to separate the assay products in conjunction with an integrator (Chromatopac C-R3A, Shimadzu) and a fluorescence detector (RF-535, Shimadzu).

Determination of Compliance

Compliance of the subjects with supplementation was determined in two ways. First, B tablets were provided in 2-wk lots, with a number of surplus tablets. The subjects were asked to return the leftovers to allow us to gauge the number of tablets apparently consumed. From the number of tablets given and the number of tablets returned, the degree of apparent compliance could be estimated. Secondly, 24 h urine was collected into bottles containing 15 mL HCl (5*M*) as preservative. Boron was extracted and its concentration determined as described above.

Statistical Analysis

The paired Student's t test was used to determine the statistical significance between mean values. Differences were considered significant at $p < 0.05$.

RESULTS

The results obtained in Study 1 show that in subjects maintaining their habitual diet, the mean concentrations of urinary B in samples obtained on two different occasions were 1.87 ± 0.15 (mean ± SE) and 1.90 ± 0.23 (range: 0.35–3.53 mg B/d; Table 2). The difference between the two occasions was not statistically significant. Figure 1 shows the individual distribution of the excreted urinary B in two periods.

The subjects supplemented with B (Study 2) demonstrated a high degree of compliance, as determined by tablet counts and by measuring urinary B excretion before and after supplementation. The mean concentration of urinary B increased from 1.64 ± 0.30 to 10.16 ± 0.92 mg/d (Table 3). The difference occurred in every individual and was therefore highly significant ($P < 0.0001$). Given the previously reported reduction in Ca excretion in postmenopausal women supplemented with B *(16)*, the concentration of excreted urinary Ca in men was determined. Calcium excre-

Table 2
Urinary Boron and Creatinine Excretion Determined on Two Different Occasions in Free-Living Male Subjects. (Values Shown as Mean ± SE, n = 18)

	Occasion 1	Occasion 2
Urinary B (mg/d)	1.87 ± 0.15	1.90 ± 0.23
Creatinine (mmol/d)	14.6 ± 0.7	15.4 ± 1.2
B/creatinine (mg/mmol)	0.12 ± 0.01	0.12 ± 0.01

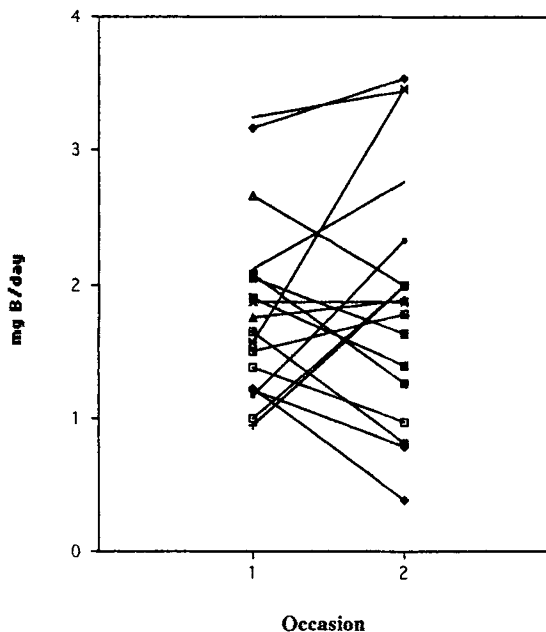

Fig. 1. Distribution of urinary B obtained from subjects on two different occasions.

tion tended to be reduced following supplementation (from 185 ± 14 to 158 ± 19.3 mg/d), which represents an increase in Ca retention of 17%.

Plasma Lipid Concentration and Oxidation of LDL

Supplementation with 10 mg B/d for 4 wk did not alter plasma total cholesterol or triglyceride concentrations or the distribution among the lipoprotein fractions, LDL and HDL (Table 4). The oxidizability of isolated LDL was unaffected by 4 wk supplementation with B. The lag phase and production of MDA were similar in subjects before and after supplementation (Table 4).

Table 3
Urinary Boron and Creatinine Excretion in Subjects Supplemented with 10 mg Boron per day for 4 wk. (Values Shown as Mean ± SE, $n = 8$)

	Presupplement	Postsupplement
Urinary boron (mg/d)	1.64 ± 0.3	10.16 ± 0.92*
Creatinine (mmol/d)	12.09 ± 4.56	11.17 ± 3.24
Boron/creatinine (mg/mmol)	0.16 ± 0.05	0.98 ± 0.13*

*Significantly different ($p < 0.0001$) from presupplement values using the Student's paired *t test*.

Table 4
Effect of Supplementation with 10 mg B/d for 4 Wk on Plasma Lipids and Parameters of LDL Oxidation in Healthy Volunteers. Data Shown as Mean ± SD, $n = 8$

	Boron supplementation	
	Before	After
Total cholesterol (mmol/L)	4.24 ± 1.02	4.39 ± 1.12
Triglycerides (mmol/L)	1.38 ± 0.94	1.18 ± 0.52
HDL-cholesterol (mmol/L)	1.18 ± 0.53	1.25 ± 0.67
LDL-cholesterol (mmol/L)	2.44 ± 0.82	2.48 ± 1.17
Lag time (min)	43.0 ± 7.0	42.8 ± 7.0
MDA (μ*M*/50 μg of protein)	6.61 ± 0.38	6.80 ± 1.16

Plasma Estradiol and Testosterone Concentrations

Plasma testosterone concentrations for all subjects were within the normal range of 8–34 nmol/L. The mean total testosterone concentration (nmol/L) increased from 17.4 ± 3.5 to 19.4 ± 2.1 ($p < 0.06$, using a one-tail *t* test) after 4 wk supplementation, but this increase did not reach statistical significance (Fig. 2). The mean plasma estradiol concentration increased significantly from 51.9 ± 21.4 to 73.9 ± 22.2 pmol/L as a result of supplementation ($p < 0.004$). Data from one subject was excluded because of an abnormally high level at baseline (154 pmol/L, or >3SD of the mean) (Fig. 3). The ratio of estradiol to testosterone increased significantly ($p < 0.01$) after supplementation (3.10 ± 1.2 vs 3.96 ± 1.3, mean ± SD, $n = 7$).

DISCUSSION

In subjects consuming their habitual diets, supplementation with 10 mg B/d resulted in a significant increase in the excretion of urinary B, accounting for 84% of the ingested dose. This suggests that the uri-

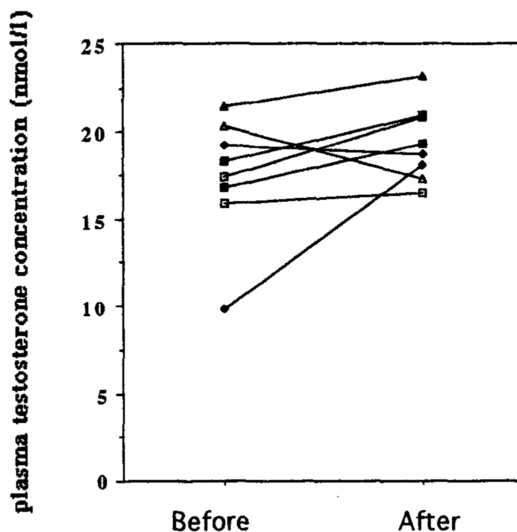

Fig. 2. Effect of B supplementation, 10 mg/d for 4 wk, on plasma testosterone concentrations in individual subjects.

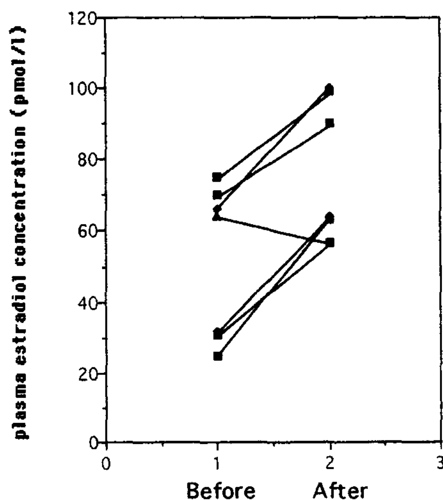

Fig. 3. Effect of B supplementation, 10 mg/d for 4 wk, on plasma estradiol concentration in individual subjects. Data shown for seven subjects. One subject was excluded from the analyses because the initial value was 154 pmol/L, a value >3SD of the mean.

nary excretion of B is a reflection of intake. When the variation within individuals was assessed, the concentration of urinary B in healthy adult males consuming their habitual diet was shown to be in the range 0.3–3.53 mg/d, with no significant difference between the mean concentration of urinary B in samples obtained from the same individuals on two different occasions. This suggests that the dietary intake of B

could be constant in a population with similar or stable pattern of food consumption.

The small variation in urinary B excretion, which was observed within and between some individuals, is an indication of the difference between their daily consumption of B. It has been suggested *(5)* that the daily intake of B varies widely because of the concentration of B in (drinking) water, which fluctuates considerably according to its geographical source; individual food preference; and ingesting B as an ingredient of many personal-care products and food preservatives, for instance, in the form of boric acid in caviar, which may contain up to 5430 ppm B *(9)*.

The excretion of anionic trace elements is through the urine, bile, sweat, and breath *(27)*, and the magnitude of the daily intake is indicated by the 24-hr excretion *(28,29)*. Jansen et al. *(28)* reported that 90% of a pharmacological dose of boric acid (from an ointment and a water solution) was recovered in the urine. The results of our studies, in humans consuming a small dose of B equivalent to that obtained by diet, support this notion.

Dietary B may have a putative regulatory role in mineral metabolism *(16,30)*. Boron deprivation in animals and humans leads to an increase in urinary Ca excretion *(16,31)*. In contrast, supplementing a low B diet with 3 mg/d of B reduced urinary Ca excretion in postmenopausal women *(16)*. However, this study in free-living male subjects, along with another study in postmenopausal women *(32)*, could not confirm this effect. This aspect of B metabolism requires further clarification.

In the supplementation trial carried out, a dose of 10 mg/d was chosen, because this amount would be achievable by dietary means and this magnitude is considered safe *(33)*. Supplementation of healthy males with 10 mg B/d resulted in a significant rise in the plasma estradiol concentration and the rise was within the upper limits of the normal range for males. The increase is consistent with some reports *(16)*, but not others *(32,34)*. The lack of effect in the latter is likely to be caused by confounders such as exercise, a smaller supplementation dose, and/or a short experimental period.

Boron compounds act as lewis acids, reducing agents, and participate in hydroboration-reactions. The latter occur without skeletal rearrangement and give rise to *cis*-addition of B to the less-hindered side of a double bond. The B atom can be replaced, with complete retention of configuration, by a hydroxyl group *(35)*. The increase in estradiol levels could be obtained through the same mechanism, that is, an enhancement of hydroxylation of estrogen precursors. In this study, plasma testosterone, an immediate precursor of estrogen *(36)*, tended to increase. Therefore, it is possible that an increase in testosterone production as a result of higher rate of hydroxylation on carbon 17, and its conversion to estradiol or further hydroxylation on carbon 3, would account for the elevation of estradiol. Alternatively, B could reduce the rate of estradiol catabolism by inhibiting methylation of 2-hydroxy estrone to 2-methoxyestrone *(37)*. However, an in vivo study in rats did not support the proposal *(38)*; there-

fore, the hydroxylation reaction theory and estradiol synthesis from testosterone as the precursor seems more appropriate.

The secretion of testosterone, which is regulated by pituitary leutenizing hormone (LH) and is sensitive to negative feedback control *(36)*, may obscure the response to B supplementation. In a study involving men and postmenopausal women *(39)*, serum calcitonin, which is reported as an inhibitor of testosterone and LH secretion *(40)*, was higher during an initial B depletion period.

Regardless of the mechanism, an increase in plasma estradiol has a number of implications for cardiovascular risk factors. In women with coronary heart disease, estradiol-17β induced coronary relaxation and peripheral vasodilation following exercise-induced myocardial ischemia *(41)*, an improvement in risk independent of changes in plasma lipids. In addition, it is believed that estrogen suppresses hepatic lipase activity and lowers the level of apolipoprotein B and LDL cholesterol by increasing its rate of clearance from plasma *(42)*. However, in this study, no effect of B supplementation on lipids was established, despite a significant increase in the plasma estradiol concentration. It is possible that a longer experimental period or a trial in hyperlipidemic subjects was required for this effect to be observed.

The lack of effect of B supplements on the susceptibility of LDL to oxidation does not exclude the possibility that estrogen can ameliorate copper-induced peroxidation of LDL particles and may protect against atherosclerosis *(43–48)*. The lack of effect of B supplementation on LDL oxidizability may be explained partly by the experimental conditions used for the oxidation studies. Boron may be lost during ultracentrifugation (LDL preparation) and overnight dialysis. A study of the incorporation of B compounds into tissues showed that all compounds localized mainly in the cytosolic fraction, however, diffused out of the cells quickly when the cells were exposed to B-free medium, indicating no strong bonds had formed with cellular components *(49)*.

Our study, along with one other *(16)*, shows that the metabolism of endogenous estrogens is influenced by one of the minor dietary factors found in nuts, legumes, fruit, and vegetables. Further studies are required to establish the feasibility of modulating steroid hormones by B supplementation in males and females and, in turn, the possible influence on plasma lipoproteins. A reliable way of estimating B intake may be by its urinary excretion. Such knowledge is increasingly important in view of the evidence suggesting a role for B in metabolism *(15)*.

ACKNOWLEDGMENTS

Presented in abstract form at the annual meeting of the Australasian Clinical Nutrition Society: M. R. Naghii, A. P. Verus, and S. Samman, Boron excretion in healthy subjects: intra- and inter-individual differ-

ences. *Proc. Aust. Clin. Nutr. Soc.*, poster 9 (1994), and the Annual meeting of the Australian Atherosclerosis Society: M. R. Naghii and S. Samman, Boron supplements raise plasma oestrogen without affecting plasma lipoproteins in normolipidaemic men. *Proc. Aust. Atherosclerosis Soc.* **21**, 58 (1995).

M. R. Naghii acknowledges the receipt of a postgraduate scholarship from the Iranian Ministry of Health and Medical Education. The authors thank A. P. Verus for providing the samples used in the first trial, and Z. I. Ahmed for technical assistance.

REFERENCES

1. Health Effects of Boron. Env Health Perspectives Supplements 102, Suppl 7, 1–141 (1994).
2. F. H. Nielsen, Boron—an overlooked element of potential nutritional importance, *Nutr. Today* **23,** 4–7 (1988).
3. E. G. Zook and J. Lehmann, Mineral composition of fruits, *J. Am. Diet. Assoc.* **52,** 225–231 (1968).
4. A. Gormican, Inorganic elements in foods used in hospital menus, *J. Am. Diet. Assoc.* **56,** 397–403 (1970).
5. C. D. Hunt, T. R. Shuler, and L. M. Mullen, Concentration of boron and other elements in human foods and personal-care products, *J. Am. Diet. Assoc.* **91,** 558–568 (1991).
6. P. Koivistoinen, Mineral element composition of Finnish foods, *Acta Agric. Scand.* Suppl **22,** 7–165 (1980).
7. D. L. Anderson, W. C. Cunningham, and T. R. Lindstrom, Concentrations and intakes of H, B, S, K, Cl, and NaCl in foods, *J. Food Comp. Anal.* **7,** 59–82 (1994).
8. L. Edwall, B. Karlen, and A. Rosen, Absorption of boron after mouthwash treatment with Bocosept, *Eur. J. Clin. Pharmacol.* **15,** 417–420 (1979).
9. V. Franco and W. Holac, Preservatives and artificial sweeteners—collaborative study of the determination of boric acid in caviar by emission spectroscopy, *JAOAC* **58,** 293–296 (1975).
10. H. C. Brown, From little acorns to tall oaks: from boranes to organoboranes, *Science* **210,** 485–492 (1980).
11. H. Esterbauer, R. J. Schaur, and H. Zolliner, Chemistry and biochemistry of 4-hydroxynoneal, malonaldehyde and related aldehydes. *Free Rad. Biol. & Med.* **11,** 81–128 (1991).
12. G. G. Duthie, Lipid peroxidation, *Eur. J. Clin. Nutr.* **47,** 759–764 (1993).
13. D. Steinberg, S. Parthasarathy, W. T. Care, J. Khoo, and J. L. Witztum, Beyond cholesterol modification of low density lipoprotein that increase its atherogenicity, *N. Engl. J. Med.* **320,** 915–924 (1989).
14. R. A. Riemersma, Epidemiology and the role of antioxidants in preventing coronary heart disease: a brief overview, *Proc. Nutr. Soc.* **53,** 59–65 (1994).
15. M. R. Naghii and S. Samman, The role of boron in nutrition and metabolism, *Prog. Food Nutr. Sci.* **17,** 331–349 (1993).
16. F. H. Nielsen, C. D. Hunt, L. M. Mullen, and J. R. Hunt, Effect of dietary boron on mineral, estrogen, and testosterone metabolism in postmenopausal women, *FASEB J* **1,** 394–397 (1987).
17. I. P. Lee, R. J. Sherins, and R. L. Dixon, Evidence for induction of germinal aplasia in male rats by environmental exposure to boron, *Toxicol. Appl. Pharmacol.* **45,** 577–590 (1978).
18. A. P. Verus and S. Samman, Urinary zinc as a marker of zinc intake: results of a supplementation trial in free-living men, *Eur. J. Clin. Nutr.* **48,** 219–221 (1994).
19. I. Ikeuchi and T. Amano, A colorimetric determination of boron in biological materials, *Chem. Pharm. Bull.* **26,** 2619–2623 (1978).

20. G. R. Warnick, J. Benderson, and J. Albers, Dextran sulfate-Mg^{2+} precipitation procedure for quantitation of high density-lipoprotein cholesterol, *Clin. Chem.* **28,** 1379–1388 (1982).
21. S. Samman and A. S. Truswell, The Friedewald equation for the determination of low-density-lipoprotein cholesterol: a special case, *Am. J. Clin. Nutr.* **58,** 928–929 (1993).
22. W. T. Friedewald, R. I. Levy, and D. S. Fredrickson, Estimation of the concentration of low density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18,** 499–502 (1972).
23. L. M. Gatto and S. Samman, The effect of zinc supplementation on plasma lipids and LDL oxidation in males. *Free Rad. Biol. Med.* **19,** 517–521 (1995).
24. M. A. Markwell, S. M. Haas, L. L. Bieber, and N. E. Tolbert, A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples, *Anal. Biochem.* **87,** 206–210 (1978).
25. H. Esterbauer, G. Striegl, H. Puhl, and M. Rotheneder, Continuous monitoring of in vitro oxidation of human low density lipoprotein, *Free Rad. Res. Comm.* **6,** 67–75 (1989).
26. B. Halliwell and S. Chirico, Lipid peroxidation: its mechanism, measurement and significance, *Am. J. Clin. Nutr.* **57,** 715S–725S (1993).
27. F. H. Nielsen and J. R. Hunt. Trace elements emerging as important in human nutrition, in Proceedings of the fourteenth national databank conference, P. J. Stumbo, ed., Univ. Iowa, Iowa City, June 19–21, 135–143 (1989).
28. J. A. Jansen, J. S. Schou, and B. Aggerbeck, Gastro-intestinal absorption and in vitro release of boric acid from water-emulsifying ointments. *Food Chem. Toxic.* **22,** 49–53 (1984).
29. N. L. Kent and R. A. McCance, The absorption and excretion of minor elements by man. I. Silver, gold, lithium, boron and vanadium. *Biochem. J.* **35,** 837–844 (1941).
30. C. D. Hunt and J. L. Herbel, Effects of dietary boron on calcium and mineral metabolism in the streptozotocin-injected, vitamin D3-deprived rats. *Mag. Trace Elemen.* **10,** 387–408 (1991).
31. C. D. Hunt, Dietary boron modified the effect of magnesium and molybdenum on mineral metabolism in the cholecalciferol-deficient chick. *Biol. Trace Elem. Res.* **22,** 201–220 (1989).
32. J. H. Beattie and H. S. Peace, 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 (1993).
33. F. H. Nielsen, Ultratrace minerals: Boron, in *Modern Nutrition in Health and Disease,* M. E. Shils, J. A. Olsen, and M. Shike, eds. Lea & Febiger, Philadelphia, pp. 272–274 (1994).
34. A. A. Ferrando and N. R. Green, The effect of boron supplementation on lean body mass, plasma testosterone levels, and strength in male bodybuilders, *Int. J. Sport Nutr.* **3,** 140–149 (1993).
35. J. H. Brewster and E. Negishi, Passes through the mountains (1), *Science* **207,** 44–46 (1980).
36. D. K. Granner, Hormones of the gonads, in *Harper's Biochemistry,* R. K. Murray, D. K. Granner, P. A. Mayes, and V. W. Rodwell, Appleton & Lange, Los Altos, CA, pp. 530–546 (1988).
37. J. H. Beattie and E. Weersink, Borate and molybdate inhibitory of catechol estrogen and pyrocatechol methylation by catechol-o-methyltransferase, *J. Inorg. Biochem.* **46,** 153–160 (1992).
38. J. H. Beattie, The metabolic fate of [^{3}H] estradiol in relation to dietary intake of boron in ovariectomized rats, *J. Steroid Biochem. Mol. Biol.* **45,** 549–554 (1993).
39. F. H. Nielsen, Effect of boron depletion and repletion on calcium and copper status indices in humans fed a magnesium-low diet, *FASEB J.* **3,** A760 (1989).
40. P. S. Wang, S. Tsai, G. Hwang, S. Wang, C. Lu, J. Chen, S. Liu, K. Lee, E. J. Chin, C. Chien, H. Lee, C. Lau, and C. Tsai, Calcitonin inhibits testosterone and luteinizing hormone secretion through a mechanism involving an increase in cAMP production in rats, *J. Bone Min. Res.* **9,** 1583–1590 (1994).
41. G. M. C. Rosano, P. M. Sarrel, P. A. Poole-Wilson, and P. Collins, Beneficial effect of oestrogen on exercise-induced myocardial ischaemia in women with coronary artery disease, *Lancet* **342,** 133–136 (1993).

42. A. A. Nabulsi, A. R. Folsom, A. White, W. Patsch, G. Heiss, K. K. Wu, and M. Szklo, Association of hormone-replacement therapy with various cardiovascular risk factors in postmenopausal women, *N. Engl. J. Med.* **328,** 1069–1075 (1993).
43. K. Mukai, K. Daifuku, S. Yokoyama, and M. Nakano, Stopped-flow investigation of antioxidant activity of estrogens in solution, *Biochim. Biophys. Acta* **1035,** 348–352 (1990).
44. C. Maziere, M. Auclair, M. Ronveaux, S. Salmon, R. Santus, and J. Maziere, Estrogens inhibit copper and cell-mediated modification of low density lipoprotein, *Atherosclerosis* **89,** 175–182 (1991).
45. V. A. Rifici, L. Chong, and A. K. Khachadurian, Inhibition of low density lipoprotein oxidation by 17 β estradiol, *Clin. Res.* **40,** 208A (1992).
46. A. D. Mooradian, Antioxidant properties of steroids, *J. Steroid Biochem. Mol. Biol.* **45,** 509–511 (1993).
47. A. Negre-Salvayre, M. Pieraggi, L. Mabil, and R. Salvayre, Protective effect of 17 beta estradiol against the cytotoxicity of minimaly oxidized LDL to cultured bovine aortic endothelial cells, *Atherosclerosis* **99,** 207–217 (1993).
48. M. N. Sack, D. J. Rader, and R. O. Cannon III, Oestrogen and inhibition of oxidation of low-density lipoproteins in postmenopausal women, *Lancet* **343,** 269–270 (1994).
49. T. Nguyen, G. L. Brownell, S. A. Holden, and B. A. Teicher, Intracellular distribution of variouse boron compounds for use in boron neutron capture therapy, *Biochem. Pharm.* **45,** 147–155 (1993).