The influence of a low-boron diet and boron supplementation on bone, major mineral and sex steroid metabolism in postmenopausal women

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An increase in dietary intake of B from 0·25 to 3·25 mg/d has been reported to increase plasma oestradiol and testosterone and decrease urinary Ca excretion in postmenopausal women. Consequently, it is suggested that the higher level of B intake could reduce bone loss associated with the menopause and cessation of ovarian function. The present study was designed to investigate the effect of a B supplement on bone mineral absorption and excretion, plasma sex steroid levels and urinary excretion of pyridinium crosslink markers of bone turnover in healthy postmenopausal volunteers. The women were accommodated in a metabolic unit, given a low-B diet (LBD; 0·33 mg/d) for 3 weeks and were asked to take a B supplement of 3 mg/d in addition to the LBD for a further 3 weeks. Changing B intake from 0·33 to 3·33 mg/d had no effect on minerals, steroids or crosslinks. However, the LBD appeared to induce hyperabsorption of Ca since positive Ca balances were found in combination with elevated urinary Ca excretion. This phenomenon may have inhibited or obscured any effect of B.

Boron: Calcium: Bone turnover

B is an essential element in many plant species (Jackson & Chapman, 1975), but there is no firm evidence that it is essential in animals and man. Recent reports suggest that B may affect bone and Ca metabolism (Nielsen *et al.* 1988; Hunt, 1989; Beattie & Macdonald, 1991; Hegsted *et al.* 1991) and there is evidence that supplementation of a low-B diet with 3 mg B/d (a total daily B intake of one to two times the average intake in the UK and USA) can influence the metabolism of major minerals and bone-related hormones in human volunteers, particularly in conditions of Mg deficiency (Nielsen *et al.* 1987, 1990). In the former study, Nielsen *et al.* (1987) gave postmenopausal women a diet low in B (0·25 mg/d) for 119 d whereupon they received the same diet but with a supplement of 3 mg B as $Na_2B_4O_7$/d for 48 d. The increase in B intake from 0·25 to 3·25 mg/d increased plasma oestradiol and testosterone concentrations and decreased urinary Ca excretion. A role for B in the aetiology of osteoporosis was, therefore, proposed and supplements containing B are now sold as remedies for osteoporosis. The findings of Nielsen *et al.* (1987) are of considerable significance since they imply that hormonal adjustment can be made by changing diet, thus reducing dependence on hormone-replacement therapy, which is unsuitable for some subjects, has side effects and is expensive. However, these proposed implications have yet to be substantiated.

The aim of the present work was, therefore, to assess the effect of changing B intake, by supplementing a low-B diet with 3 mg B/8 MJ energy intake, on plasma sex steroid levels and bone mineral excretion in healthy postmenopausal women. As there is no evidence that B affects human bone and direct measurements are impracticable, changes in bone turnover were assessed by measurement of urinary pyridinium crosslinks.

METHODS AND MATERIALS
Volunteers

Six volunteers were recruited from over 200 applicants within Scotland. The selection criteria for volunteers was as follows: (1) no current or previous health problems, (2) no prolonged use of drugs for medicinal purposes, particularly steroids, (3) no history of alcohol or drug abuse, (4) no more than 20 years since the menopause, (5) body mass index (weight/height^2; BMI) close to the average for the 200 volunteer applicants (average BMI 25). Plasma concentrations of oestradiol, progesterone and follicle-stimulating hormone in the selected volunteers confirmed their postmenopausal hormonal status. The physical characteristics of these volunteers are presented in Table 1. All volunteers agreed to the conditions of the study which was approved by the Grampian Health Board Ethical Committee, Aberdeen, Scotland.

The study duration was 6 weeks and the women were accommodated, two at any one time, in the Human Nutrition Unit of the Rowett Research Institute between the months of March and August. The movement of volunteers was restricted to an area surrounding the Institute, although supervised visits outwith this area were organized at weekends. Prolonged exercise was discouraged.

Study protocol

On arrival at the Unit the volunteers selected food which was representative of their normal diet. The nutritional composition of the six different diets, as calculated from food composition tables (Paul & Southgate, 1978), was compared with the information obtained from dietary history questionnaires completed during an initial visit to the Unit. The volunteers were allowed this self-selected diet for the first 2 d, which is referred to as the acclimation-period diet (APD). They were then given a diet low in B for the following 3 weeks and the same diet with a daily supplement of 3 mg B/8 MJ energy intake for a further 3 weeks.

Urine samples (24 h) were collected daily throughout the study in clean plastic bottles containing 1·5 g ascorbic acid and were stored in a refrigerator. On completion of each 24 h period the urine volume was measured, the sample was mixed and portions were retained and stored at $-20°$. Stool collections were sealed in autoclavable plastic bags and stored at $-20°$. Fasted blood samples (25 ml) were obtained in heparinized tubes on Monday, Wednesday and Friday of each week and 1 ml samples of plasma were stored at $-20°$.

Diets

The low-B diet (LBD) consisted of a 3 d rotating menu (Table 2) and was designed from food tables (Paul & Southgate, 1978; Koivistoinen, 1980) using a computer dietary analysis program (Microdiet; Salford University, Salford). The energy requirement of each volunteer was calculated from an estimate of basal metabolic rate using weight and height values and a factor of 1·56 to account for physical activity. In certain individuals this calculation underestimated energy requirements, and volunteers who showed a weight loss of more than 20 g/kg initial body weight were given a daily supplement of the high-energy-carbohydrate supplement (Maxijul; Scientific Hospital Supplies, Liverpool).

The Ca content of the diet was designed to be the same as the average Ca intake before the study, which was estimated from the dietary history questionnaires. The nutritional compositions of APD and LBD are compared in Table 3. As the LBD diet was deficient in several minerals and vitamins, supplements in gelatine capsules were taken with each

Table 1. *Physical characteristics of the postmenopausal women*

Subject no.	Height (m)	Wt (kg)	BMI	Age (years)	Years after menopause
EM	1·65	70·6	26·0	60	15
AO	1·53	61·1	26·0	65	17
MH	1·56	64·0	26·2	58	8
TM	1·62	66·8	25·4	53	5
PR	1·52	51·2	22·1	57	10
MV	1·54	65·4	27·8	63	11
Mean	1·57	63·2	25·6	59	11

BMI, body mass index (weight/height^2).

Table 2. *The three daily menus of the low-boron diet*

(The menus were rotated throughout the study and the quantities shown are for an 8 MJ intake)

Day...	1		2		3	
Food		Wt (g)	Food	Wt (g)	Food	Wt (g)
Cornflakes		25	Cornflakes	25	Cornflakes	25
Sugar		10	Sugar	15	Sugar	10
Marmalade		10	Marmalade	10	Marmalade	10
White bread		120	White bread	120	White bread	120
Boiled ham		40	Roast pork	30	Liver sausage	30
Cod steak		120	Edam cheese	45	Edam cheese	40
White flour		7	Cream crackers	40	Cream crackers	55
Margarine		34	Jelly	100	Roast beef	70
Whole milk		250	Chicken, cooked	50	Instant potato, dry	10
Edam cheese		45	Chicken soup, can	100	Gravy granules	4
Carrot		10	Peas	10	Jelly	80
White rice, boiled		100	Shortbread	55	Sponge	50
Sponge		50	Margarine	37	Margarine	30
Custard creams		40	Lemonade	600	Custard creams	20
Jelly		50	Whole milk	250	Whole milk	250
Lemonade		500			Lemonade	500
Two teabags			Two teabags		Two teabags	

meal to meet UK recommended daily allowances (RDA). The USA RDA was used where no recommended level has been set in the UK.

The diet was also low in fibre and the women were given a commercial sterculia-gum-fibre supplement (Normacol; Norgine Ltd, Headington, Oxford). The daily total fibre intake was, therefore, 24 g/8 MJ energy intake. The fibre supplement was found to contain calcium and magnesium silicates which contributed an additional 148 mg Ca and 280 mg Mg/d; however, in this form Ca and Mg are insoluble and probably unavailable. Radio-opaque markers enclosed in gelatine capsules were provided with each meal to give a total marker intake of 40/d. The marker shape was changed each week so that 7 d faecal output could be identified.

All foods were accurately dispensed into clean plastic containers and were covered and stored at −20° until required. Microwave ovens were used for heating meals in their

Table 3. *Daily energy and nutrient intake of postmenopausal women on the acclimation-period diet (APD) and the low-boron diet (LBD) as calculated from food composition tables (Paul & Southgate, 1978)**

	APD		LBD			
Nutrient	Mean	Range	Diet mean	Supplement mean	Total mean	Total range
Energy (MJ)	7·69	4·87–9·12	7·95	‡	7·95	7·20–8·50
Protein (g)	62	39–86	66	—	66	59–70
Fat (g)	85	51–123	84	—	84	76–89
Carbohydrate (g)	208	108–303	232	‡	232	210–248
Dietary fibre (g)	21·7	11·3–29·1	11·0	12·8†	23·8	21·6–25·5
Ca (mg)	836	510–1124	927	—	927	840–991
Mg (mg)	299	185–419	130	168	298	270–319
P (mg)	1141	785–1408	980	—	980	887–1048
K (mg)	3015	1913–4507	1149	—	1149	1040–1228
S (mg)	483	215–675	465	223	688	619–731
B (mg)	ID		0·33	2·98§	3·31§	3·00–3·54
Fe (mg)	11·3	7·0–36·0	6·7	3·3	9·9	9·0–10·6
Zn (mg)	7·3	4·9–10·2	7·0	2·9	9·9	9·0–10·6
Cu (mg)	1·25	0·86–1·71	0·67	1·82	2·49	2·25–2·66
Ascorbic acid (mg)	87·8	9·4–286	5·6	53·8	59·4	54·0–63·8
Folic acid (mg)	166	98–288	0·8	0·2	1·0	0·91–1·07

ID, insufficient information.

* For details of dietary regimen, see pp. 872–873.

† Fibre supplement was found to contain calcium and magnesium silicates which contributed an additional 148 mg Ca and 280 mg Mg/d; however, in this form Ca and Mg are insoluble and probably unavailable.

‡ Carbohydrate supplement, Maxijul (Scientific Hospital Supplies, Liverpool), supplied to three volunteers to prevent weight loss.

§ Supplement provided during the second period of 3 weeks only (for details, see p. 872).

original containers and clean plastic cutlery and cups were provided. The volunteers were asked to clean the food containers with bread from the diet to ensure complete ingestion of each meal. Distilled water was freely available and multiple use of the tea bags was allowed.

Analyses

Diet. The foods in each LBD daily menu were pooled and homogenized with distilled water. The homogenates included infusions of the two tea bags with hot distilled water. Samples of the homogenized foods were freeze-dried and ground to a powder. Ca, Mg and P concentrations were determined by X-ray fluorescence spectroscopy using compressed pellets of the food powder.

For the measurement of B in LBD or its constituent foods, 200 mg samples of freeze-dried powder were digested in HNO_3 (800 ml/l) in a microwave digestion vessel (Parr Instrument Co., Moline, IL, USA) using a domestic 750 W microwave oven. These vessels do not vent under normal operating conditions and are recommended by the manufacturer for digestion of samples where volatile elements such as B are to be measured. B was analysed by inductively coupled plasma optical emission spectroscopy (ICP/OES).

Faeces. The sealed bags of faeces were autoclaved and X-rayed to identify the number and shape of radio-opaque markers in each collection. Recovery of markers ranged from 96–101%. Those collections containing markers of two different shapes representing the transition between two 7 d periods were pooled. Similarly, the faecal collections with only

one type of marker present were pooled. The faeces were homogenized with deionized water and portions were freeze-dried and ground to a fine powder. Ca, Mg and P concentrations were analysed directly by X-ray fluorescence spectroscopy.

Urine. The concentrations of Ca and Mg were measured colorimetrically (Gitelman *et al.* 1966; Gitelman, 1967) and P was determined by the method of Fiske & Subbarow (1925). The samples were also analysed for B using ICP/OES.

For the measurement of pyridinium crosslinks, total pyridinoline and deoxypyridinoline were extracted from urine and separated using the method of Black *et al.* (1989). In brief, samples of each 24 h urine collection were hydrolysed in 5·5 M-HCl for 18 h at 107°. The pyridinium crosslinks were extracted from the hydrolysates by partition chromatography on cellulose CF1 and then separated by HPLC on a C_{18} column using a linear gradient of 20 mM-NH_4Cl, pH 3·5 and acetonitrile containing 5 mM-octanesulphonic acid (Black *et al.* 1989). The eluting crosslinks were detected by fluorescence spectroscopy with excitation at 295 nm and emission at 400 nm.

Creatinine was determined by the Jaffe reaction (Bosnes & Taussky, 1945).

Plasma. Plasma total testosterone was measured by radioimmunoassay (RIA) using a commercial kit (Serono Diagnostic Ltd, Woking, Surrey). A commercial RIA kit (RSL direct ^{125}I-[estradiol-17β]-RIA kit; ICN Biomedicals Inc., Carson, CA, USA) was also used for the determination of plasma total oestradiol but, because of the low concentrations, the calibration curve included additional standards over the range 5–30 pg/ml. This direct RIA kit requires 50 μl plasma or serum, although for validation of the results (see p. 880), volumes of 50, 63, 75, 88 and 100 μl were used for each plasma sample in the present study.

Statistics

A two-way analysis of variance (ANOVA; subject and sample time) was used to test for differences in urinary bone mineral excretion between successive 7 d periods throughout the study. The values for the 7 d periods for plasma testosterone and urinary pyridinium crosslinks and also the 7 d bone mineral balance values were analysed in the same way. All computations were made using Minitab statistical software. Comparison of mean data was made by calculating *t* values from the ANOVA error mean square.

RESULTS

Boron in food

As shown in Table 4, B levels in the foods selected for LBD were generally higher than those reported for Finnish foods (Koivistoinen, 1980). The mean daily B content of the 3 d rotating menu was 0·33 mg/8 MJ energy intake.

Urinary calcium, magnesium and phosphorous

Urinary Ca excretion increased rapidly following the transition from APD to LBD and in several cases more than doubled. This high level of excretion was maintained throughout the 6-week study and was unaffected by B supplementation (Table 5). To verify these results, which indicated diet-induced hypercalciuria, the urine samples were treated with $LaCl_3$ and re-analysed for Ca using atomic absorption spectrophotometry. The values obtained by this method (results not shown) were similar to those obtained with the colorimetric assay.

Urinary Mg excretion followed a similar pattern to Ca except that the initial elevation

Table 4. *The boron content of selected foods: comparison of food composition table values* with present analyses*

Food	Analysed (mg B/kg)	Published* (mg B/kg)
Peas, frozen	3·1	1·8
Carrots	4·6	3·2
Wholemeal bread	1·0	0·5 (estimation)
Brussel sprouts	3·8	No value
Edam cheese	0·5	< 0·3
Cornflakes	0·6	0·7
Cod, frozen	0·4	0·2
Milk	0·3	0·2
White bread	0·6	0·5
Broccoli	7·2	4·6
Potato powder	4·2	3·7
Banana	2·6	1·7

* Values calculated from the food composition tables of Koivistoinen (1980).

Table 5. *Urinary mineral excretion of postmenopausal women on the day before starting the low-boron diet (baseline) and throughout the study†*

(Mean values and standard deviations for daily collections, except for baseline values)

Week	Ca (mg/d)		Mg (mg/d)		P (mg/d)	
	Mean	SD	Mean	SD	Mean	SD
Baseline‡	132·5	47·6	81·0	25·5	749·5	245·6
1	239·1*	79·7	108·3	27·1	577·7	103·4
2	258·7	77·0	115·4	26·6	541·5	79·9
3	263·2	74·4	115·6	24·8	544·1	76·7
4	260·9	74·7	112·3	21·9	549·4	70·6
5	265·0	76·1	115·6	20·6	544·6	70·8
6	264·1	66·9	117·1	26·3	549·6	88·3

* Mean value was significantly different from baseline value: $P < 0.005$.
† For details of diets and procedures, see Table 2 and pp. 872–873.
‡ Baseline values are not included in ANOVA.

was less marked. Urinary excretion of P, however, decreased slightly during the initial 3 d period and then stabilized; it was not affected by B (Table 5).

Plasma sex steroids

Plasma testosterone concentrations varied markedly between subjects but were unaffected by B supplementation (Table 6).

Plasma oestradiol proved difficult to measure by any commercial or 'in-house' RIA method due to the low circulating levels of this steroid in postmenopausal women. Nielsen *et al.* (1987) used a direct commercial assay kit to measure oestradiol in postmenopausal plasma and so the same kit has been used in the present study. In accordance with the procedure adopted by Nielsen and colleagues (S. K. Gallagher, personal communication), a calibration curve was constructed to extend the normal range of the assay below 10 pg/ml. This was accomplished by diluting the supplied oestradiol standards with the zero standard. All samples and standards were dispensed in duplicate using electronic pipettes in multidispense mode to minimize pipetting inaccuracy. A linear standard curve

Table 6. *Plasma sex steroid levels of postmenopausal women determined immediately before starting the low-boron diet (baseline) and at the end of each week throughout the study**

B intake (mg/d)...		0·33			3·33		
Subject	Baseline	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Testosterone (ng/ml)							
EM	0·780	0·997	0·658	0·737	0·745	0·800	0·822
TM	0·273	0·330	0·278	0·291	0·330	0·267	0·306
AO	0·132	0·177	0·208	0·185	0·142	0·154	0·128
PR	0·396	0·434	0·438	0·483	0·507	0·443	0·451
MH	0·114	0·054	0·034	0·040	0·103	0·095	0·023
MV	0·264	0·261	0·253	0·316	0·290	0·219	0·270
Mean	0·330	0·376	0·312	0·342	0·353	0·330	0·333
SD	0·242	0·331	0·214	0·243	0·240	0·259	0·281
Oestradiol (pg/ml)†							
EM	10·3	7·2	7·1	7·0	5	7·3	12·3
TM	2	2	< 1	4	< 1	3	3
AO	7·6	5·3	7·3	5	4	6·4	8·2
PR	6·1	3	4	5	2	< 1	< 1
MH	11·0	5·1	10·1	9·4	6·0	10·1	8·8
MV	10·1	3	1	3	6·7	2	2

* For details of diets and procedures, see Table 2 and pp. 872–875.

† Values for oestradiol which were below the lowest standard (5 pg/ml) are shown as the nearest whole number.

Table 7. *Urinary pyridinium crosslink excretion (nmol/mmol creatinine) of postmenopausal women measured in 24 h urine samples collected 1 d before starting the low-boron diet (baseline), on the first day of the study (D1) and at the end of each week throughout the study**

B intake (mg/d)...			0·33			3·33		
Subject	Baseline	D1	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Pyridinoline								
EM	28·8	19·6	24·2	16·6	40·0	21·0	27·0	21·8
AO	18·2	17·2	21·3	23·6	32·6	19·9	14·5	18·8
MH	43·3	31·0	41·8	28·1	28·5	39·2	28·4	38·2
TM	52·0	48·6	44·1	45·1	36·8	31·1	35·3	28·2
PR	27·6	26·4	26·9	23·2	28·3	27·9	22·9	27·6
MV	30·3	28·5	36·4	32·5	36·7	31·6	37·4	37·3
Mean	33·4	28·6	32·5	28·2	33·8	28·5	27·6	28·7
SD	12·2	11·1	9·6	9·8	4·8	7·2	8·4	7·9
Deoxypyridinoline								
EM	11·6	4·7	5·0	3·5	10·6	3·9	6·1	5·1
AO	4·7	3·7	4·2	4·7	11·3	5·3	5·0	5·4
MH	8·2	11·3	14·2	8·3	8·7	8·8	7·1	9·8
TM	18·1	11·3	12·5	13·6	10·6	9·1	10·8	8·0
PR	13·9	14·6	12·5	10·2	12·1	15·5	20·3	11·6
MV	13·0	11·0	15·3	12·1	12·9	10·6	9·3	13·5
Mean	11·6	9·4	10·6	8·7	11·0	8·8	9·8	8·9
SD	4·7	4·3	4·8	4·0	1·5	4·1	5·6	3·4

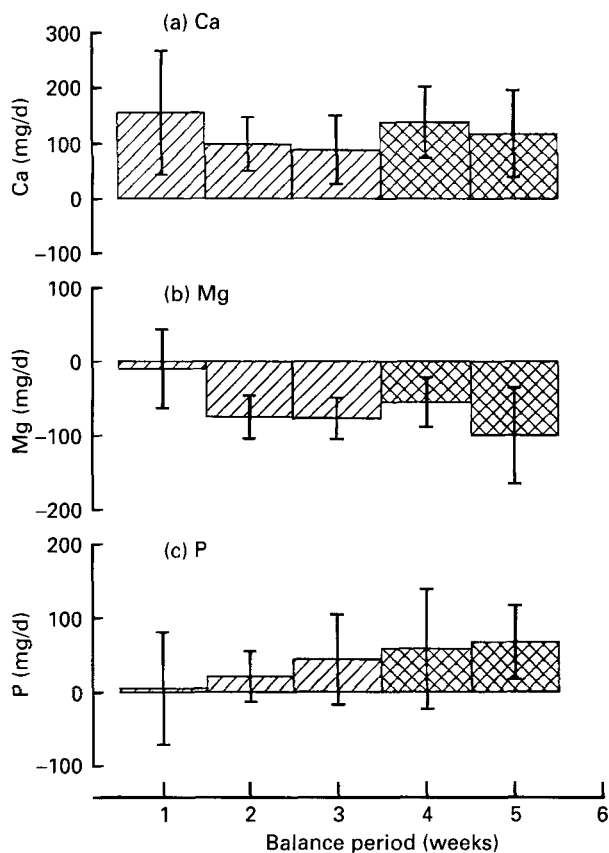* For details of diets and procedures, see Table 2 and pp. 872–873.

Fig. 1. Successive 7 d mineral balances for healthy postmenopausal women on a low boron diet (▨) and subsequently on the same diet with a B supplement (▩). Values are the means and standard deviation represented by vertical bars for six volunteers. For details of diets and procedures, see Table 2 and pp. 872–875.

Table 8. *Detailed mineral balance values for postmenopausal volunteer TM (mg/d)**

Mineral	Week	Mineral intake				Mineral excretion			Balance
		Food	Normacol	Supplement	Total	Urine	Faeces	Total	
Ca	1	1054	148	—	1202	204	840	1044	+158
	2	1064	148	—	1212	224	855	1079	+133
	3	1051	148	—	1199	236	927	1163	+36
	4	1054	148	—	1202	234	872	1106	+96
	5	1064	148	—	1212	240	902	1142	+70
Mg	1	128	280	169	577	124	466	590	−13
	2	127	280	169	576	146	502	648	−72
	3	127	280	169	576	135	531	666	−90
	4	128	280	169	577	128	505	633	−56
	5	127	280	169	576	130	562	692	−166
P	1	983	—	—	983	600	461	1061	−78
	2	973	—	—	973	548	441	989	−16
	3	970	—	—	970	515	464	979	−9
	4	983	—	—	983	510	447	957	+26
	5	973	—	—	973	505	460	965	+8

* For details, see Table 1.
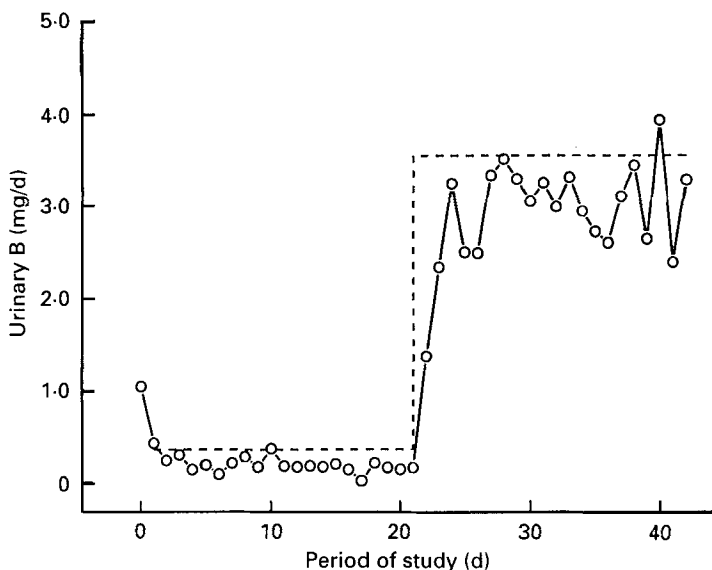
Fig. 2. Urinary boron excretion (O—O) for one healthy postmenopausal volunteer throughout the 6-week study. Time 0 represents B excretion on the day before starting the low-B diet. (– –), B intake.

with a correlation coefficient of 0·9913 was obtained from five standards within the range 5–30 pg/ml. Plasma oestradiol levels were generally within the range 1–12 pg/ml (Table 6). All values below the lower limit of the standard curve are expressed only to the nearest whole number. As discussed later (pp. 880–881), these results are tentative but are included for comparison with the results of Nielsen *et al.* (1987) who used the same method. Oestradiol levels tended to decrease on LBD but did not change consistently after B supplementation.

Urinary pyridinium crosslinks

The urinary excretion of pyridinoline and deoxypyridinoline was relatively constant throughout the study and was unaffected by B supplementation. There were, however, marked differences between volunteers in urinary crosslink concentrations (Table 7).

Bone mineral balances

Faecal mineral excretion for successive 7 d periods was calculated as the sum of total minerals excreted during the clear marker periods (radio-opaque markers of uniform shape) with a proportion of minerals from the mixed-marker periods representing the transition from one balance period to the next. The amount of minerals in the mixed-marker periods was apportioned according to the ratio of marker shapes. Balances were calculated by subtracting the sum of faecal and urinary mineral excretion from the total intake of minerals in food and supplements during the balance period. An example of the contribution of minerals from different sources is shown in Table 8.

All volunteers were in positive Ca balance throughout the study (Fig. 1). The balance for week 6 has been omitted since the faecal collections for this period were incomplete. In addition, the first week's balance in each 3-week dietary period has been regarded as an acclimation period. However, comparison of week 2 with week 5 shows no significant effect of B on Ca balance.

With each successive balance period, P balance became increasingly positive whereas the converse was true for Mg balance (Fig. 1). Supplementation with B had no clear effect on P and Mg balance.

Urinary boron

The urinary excretion of B reflected the dietary intake of the element (Fig. 2). It decreased within 48 h of starting LBD and then stabilized. Similarly, urinary B excretion increased very rapidly after the B supplement was given and stabilized at more than 90 % of total B intake.

DISCUSSION

Nielsen *et al.* (1987) have reported a significant increase in plasma testosterone and oestradiol levels within 8 d of supplementing postmenopausal women with the same level of B used in the present study. The study of Nielsen *et al.* (1987) was conducted on subjects who had been on a low-B diet for 119 d and the effect of B supplementation was enhanced when the diet was deficient in Mg. These authors suggest that B may exert its influence through interaction with some hormones and enzyme reactions. A mechanism for B–oestrogen interactions has been proposed (Beattie & Weersink, 1992) in which borate has its effect by forming a complex with the vicinal hydroxyl groups of catechol oestrogens, thus limiting subsequent methylation and changing the rate or pathway for the catabolism and excretion of the major oestrogens. Such a mechanism, analogous to that proposed by Humphries (see Bremner, 1991), for Mo effects on sex hormone metabolism in cattle could have led to an increase in plasma oestrogen concentrations.

The plasma oestradiol level recorded by Nielsen *et al.* (1987) after giving a B supplement of 3 mg/d with a low-B, Mg-deficient diet was approximately 41 pg/ml compared with 21 pg/ml on the basal diet alone (0·25 mg B/d). This B-related increase in plasma oestradiol was less marked on a Mg-adequate diet but evident nevertheless. In the present study oestradiol values obtained using a commercial direct RIA kit were considerably lower than those reported by Nielsen *et al.* (1987) who used the same assay kit. The present values were also lower than initial results obtained with an 'in-house' RIA (Peace & Beattie, 1991) and a significant proportion were below the standard range of the assay kit. In addition to poor precision and questionable accuracy at these low levels, cross-reactivity of the antibody with other oestrogens, oestrone in particular, and wide fluctuations in plasma levels of oestradiol in postmenopausal women could result in spurious or misleading results. After confirming the linearity of the relationship between assay sample volume and apparent oestrogen level using both pre- and postmenopausal plasma, we routinely assayed five different volumes, namely 50, 63, 75, 88 and 100 μl, of each plasma sample. The regression slope for postmenopausal samples was consistently greater than 1 and the larger volumes gave apparent oestrogen values which were well within the standard range of the assay. Taking advantage of the improved precision of these values we were able to assess, by regression, the accuracy of the analysis with 50 μl (the recommended assay sample volume).

The antibody supplied by ICN Biochemicals Inc. had a specified cross-reactivity with oestrone of 20 %, and this was confirmed by our own tests. Since oestrone is the principal oestrogen in postmenopausal plasma and is present at approximately three times the concentration of oestradiol-17β (Reed & Murray, 1979), then almost 40 % of the oestrogen value obtained with this or similar kits could be attributable to the presence of oestrone. Changes in the levels recorded could, therefore, be interpreted as an effect on oestradiol, oestrone or both oestrogens.

Of concern in the determination of oestradiol levels in postmenopausal plasma are the wide and apparently random fluctuations in the concentrations during a 24 h period. In studies where blood samples were obtained from postmenopausal volunteers every 20 min for 24 h, oestradiol was found to change markedly by as much as a factor of two, even between sequential samples (Hutton *et al.* 1979). Rae *et al.* (1988) have discussed the

problems associated with measuring plasma oestradiol in postmenopausal women and suggest that it is more appropriate to determine urinary oestrogens from 24 h collections.

Conclusions concerning the influence of a B supplement on oestradiol levels in postmenopausal plasma based on the results obtained by commercial direct RIA kits must, therefore, be regarded as tentative. In the present study there was suggestion of a decrease in oestrogen levels when starting LBD, an effect which was abolished towards the end of the study in two of the volunteers. This effect could be due to B but may equally be related to homeostatic adaptation to changes in other components of the diet.

Postmenopausal plasma testosterone concentrations, which are more readily determined using commercial RIA kits, were unaffected by an increase in dietary B intake from 0·33 to 3·33 mg/d. This result conflicts with that obtained by Nielsen et al. (1987), who also used a commercial RIA kit for testosterone analysis and found that the plasma level of this steroid increased from 0·31 to 0·83 ng/ml in response to B supplementation.

The present study was designed in the expectation that the combined monitoring of sex steroids and pyridinium crosslinks might link hormonal effects with changes in bone turnover. Black et al. (1989) have reported that ovariectomized rats have a higher deoxypyridinoline excretion than a sham-operated control group. Also, a decrease in pyridinium crosslink excretion was observed in postmenopausal women given hormone-replacement therapy (Ubelhardt et al. 1991). Other studies (Seibel et al. 1989; Robins et al. 1991) have shown the validity of monitoring deoxypyridinoline excretion as an index of bone turnover. Recent findings show a higher deoxypyridinoline output in osteoporotics (Robins et al. 1990), which supports the concept that the excretion of non-reutilizable crosslinks from type I collagen in bone is a good indicator of normal and pathophysiological modulation of bone turnover. Thus, the constancy of the deoxypyridinoline excretion in the women throughout the present 6-week study strongly suggests a constant rate of bone turnover, although, as Table 7 indicates, there was substantial but consistent inter-individual variation. The maintenance of the sex hormone and bone turnover status of all the subjects at the same levels as those on their normal diet argues strongly for the lack of effect of either the experimental diets or B on these variables.

The findings clearly show a marked and progressive increase in intestinal Ca retention and urinary excretion on the experimental diet which was not immediately explicable by, for example, a proportional increase in dietary Ca intake. These changes stabilized within the first week and the hypercalciuria was, thereafter, sustained in all subjects and remained unaffected by the supplementation with B. In contrast, Nielsen et al. (1987) report that supplementing a low-B diet with 3 mg B/d reduces urinary Ca excretion in postmenopausal women. It is possible that diet-induced perturbations of Ca metabolism found in our volunteers inhibited or masked any effects of B. Increased acid load as a result of diminished vegetable and fruit intake may have contributed to the hypercalciuric effect observed, and removal of dietary inhibitors of Ca absorption such as phytates and oxalates could have enhanced Ca absorption. A study is, therefore, in progress to investigate the effect of several factors in LBD, including acidity, on Ca excretion and urine acidity in postmenopausal women.

The metabolic balance of Mg and P was also affected following the transition from APD to LBD. As for Ca, this could not be attributed to changes in dietary intake of these elements (Table 3). The mean P intake decreased by about 14% and yet the women were in P balance at the start of LBD and there was a steady increase in balance throughout the 6 weeks. The values in Table 8, which are representative of those from all volunteers, reveal that the negative Mg balance induced by LBD was due to increased urinary excretion and also to decreased absorption, since faecal Mg increased progressively over the first 3 weeks and the net endogenous excretion of Mg into the intestinal lumen is negligible (Dreosti,

1986). About 50% of the total intake of Mg was contributed by the Normacol fibre supplement but this Mg was present in the form of insoluble silicates. Reduced Mg absorption and increased urinary Mg excretion may have been related to an increase in Ca intake during the study (Lakshmanan *et al.* 1984; Dreosti, 1986).

The lack of any effect of a B supplement on minerals, steroids and pyridinium crosslinks could not be attributed to a poor absorption of B from $Na_2B_4O_7$. Analysis of urine confirmed that most of the B in LBD and in the supplement was rapidly absorbed and excreted in the urine (Fig. 2). A rapid turnover of B following intravenous injection has been demonstrated previously (Jansen *et al.* 1984). On starting the B supplementation in the present study urinary excretion of B was more than 90% of intake, which is in agreement with published findings (Kent & McCance, 1941). This result shows that the absorption efficiency for B is very high and that urine is the main pathway for its excretion. The rapid decrease in urinary B within 1 d of starting LBD was particularly interesting as it suggests that urinary B excretion provides an accurate reflection of B intake over the 24 h period of collection. It also suggests that B does not form strong complexes with large molecules such as proteins in the body and that it moves rapidly across membranes.

Although we did not record an effect of B in the present study, other dietary conditions may favour a response to this element. Nielsen (see Peace & Beattie, 1991) has suggested that the duration of B depletion may be crucial, although to our knowledge the effect of depletion period has never been systematically studied. The duration of the present study was limited to 6 weeks for practical reasons. Whether crucial or not, the period of B depletion may have limited practical relevance, at least in developed countries, as there is no evidence of sustained B depletion in human populations. The paucity of information on the B content of foods limits any detailed assessment of dietary B intake in population groups, but the average intake in the UK has been determined at 2·8 mg B/d (Hamilton & Minski, 1972). Since B is very rapidly absorbed and excreted, the body burden of this element probably fluctuates widely during a 24 h period, particularly in relation to the consumption of some fruit-based products, including preserves and beverages, and also to seasonal changes in diet. The degree of variability in B intake could be assessed, without extensive dietary analysis, by measuring the amount of this element in successive 24 h urine samples, since urinary B appears to reflect accurately recent dietary intake. Unfortunately, this type of information is not available.

Several recent publications (Hunt & Nielsen, 1981, 1988; Nielsen, 1988; Nielsen *et al.* 1988; Hunt, 1989; Hegsted *et al.* 1991) indicate that interactions with Mg, vitamin D and other nutrients are the key to the significance of B as an essential element. In the case of Mg and B, one element is thought to ameliorate the deficiency symptoms associated with the other. However, since B-deficient diets tend also to be deficient in Mg, such an interaction seems unlikely in free-living populations. Supplementation with physiological amounts of B may prove beneficial in cases of combined B and Mg deficiency but then so might supplementation with Mg.

It is also notable that B concentrations are high in foods of high fibre content and recent studies indicate that diets providing a high fibre intake, as is the case with vegetarian diets, significantly reduce plasma oestradiol concentrations in pre- and postmenopausal women (Goldin *et al.* 1982; Barbosa *et al.* 1990). Fibre appears to reduce the entero-hepatic cycling of oestrogens, thus increasing their faecal excretion and reducing plasma levels of active oestrogens (Goldin *et al.* 1982). This evidence would appear to contradict the hypothesis that foods containing relatively large amounts of B, i.e. those of plant origin, would increase plasma oestradiol.

In conclusion, we could detect no effect of a daily 3 mg supplement of B on bone mineral metabolism, bone turnover as indicated by collagen crosslink excretion or plasma sex

steroid levels in postmenopausal women volunteers. However, LBD designed for the present study had a major influence on Ca absorption and excretion in postmenopausal women which may have inhibited or obscured any effect of B.

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