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Short-term effects of dietary sodium intake on bone metabolism in postmenopausal women measured using urinary deoxypyridinoline excretion

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The influence of Na load on bone metabolism was investigated in postmenopausal women using urinary deoxypyridinoline (DPD) as a marker of bone resorption. In a cross-over study, fourteen postmenopausal women were divided into two groups of seven. A fixed diet providing 816 mg Ca/d with either 60 or 170 mmol Na/d was consumed. At the end of an 8 d period the groups switched diets for a further 8 d period. Urine was collected daily for the last 4 d of each period. There was no significant difference in DPD excretion between high-Na and low-Na diets (129 nmol/d *v.* 132 nmol/d; $P = 0{\cdot}18$). There was, however, a significant relationship ($P = 0{\cdot}02$) between the changes in DPD excretion and urinary Ca. Plasma Mg fell from 0·83 to 0·81 mmol/l on the high Na intake ($P < 0{\cdot}001$), but there was no significant effect on plasma Ca or intact parathyroid hormone levels. It is concluded that varying dietary Na intake may affect Ca and Mg metabolism, but we were unable to demonstrate an effect on bone resorption at the levels of intake used.

Deoxypyridinoline: Sodium: Bone: Postmenopausal women

A strong positive relationship between urinary Na and Ca excretion exists in free-living normal men (McCarron *et al.* 1981; Breslau *et al.* 1982; Castenmiller *et al.* 1985; Shortt *et al.* 1988; Chan *et al.* 1992), premenopausal women (Breslau *et al.* 1982; Shortt *et al.* 1988) and postmenopausal women (Breslau *et al.* 1985; Nordin & Polley, 1987; McParland *et al.* 1989; Zarkadas *et al.* 1989).

To examine the influence of Na on bone turnover, some studies used only urinary Ca excretion (Shortt *et al.* 1988; Zarkadas *et al.* 1989), whereas others used urinary hydroxyproline as a marker for bone resorption (Castenmiller *et al.* 1985; McParland *et al.* 1989; Chan *et al.* 1992). However, hydroxyproline is not specific for bone and is, therefore, an insensitive marker for bone resorption. Large amounts of hydroxyproline are metabolized in the liver, and the amount excreted is dependent on the dietary intake of collagen (Robins, 1982; Delmas, 1992). The pyridinium cross-links of collagen, pyridinoline (PYD) and deoxypyridinoline (DPD), have been shown to be more specific urinary markers of bone resorption, both in Paget's disease of bone and in primary hyperparathyroidism (Robins *et al.* 1991; Eyre, 1992). Furthermore, the cross-link markers have the advantage that they are unaffected by dietary sources of collagen (Colwell *et al.* 1990). PYD is found in the collagen of cartilage, bone and in smaller amounts in other connective tissues. In contrast, DPD has only been found in significant amounts in type I

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collagen of bone and is, therefore, a promising marker for the assessment of bone resorption (Delmas, 1992).

The aim of the present study was to examine the influence of Na intake on bone resorption, as measured by urinary DPD excretion.

METHODS

Design

Fourteen postmenopausal women (mean age 62·3 (range 50–70) years) were selected for the study. Exclusion criteria were: smoking, chronic illness or drugs known to affect bone metabolism, any fracture since the menopause, not less than 2 years past the menopause. Characteristics of the subjects are shown in Table 1.

The study was designed in two periods, each of 8 d, in which NaCl was given with the diet either in a low or high concentration, the amounts being 60 and 170 mmol Na/d respectively. In this cross-over study volunteers were divided into two groups of seven. At the end of the first period, the groups switched diets for the second period (Fig. 1). Subjects were instructed to collect 24 h urine for the duration of the study. Only urine samples collected in the last 4 d of each treatment period were analysed, to allow a washout period as previously suggested (Shortt & Flynn, 1990). After an overnight fast, blood samples were taken at 08.00 hours and blood pressure and weight were measured on days 1, 5, 9, 13 and 17 (Fig. 1).

Ethical permission was obtained from the Joint Ethical Committee of the Grampian Health Board and the University of Aberdeen.

Diet

The diet consisted of a 2 d rotation with amounts of energy and nutrients kept constant. Nutrient intakes, other than NaCl, were identical for each 8 d period. For each subject, total energy expenditure was calculated using BMR equations and the physical activity level of 1·4 (Department of Health, 1991). The exact amounts for the major nutrients are shown in Table 2. Diet prescription sheets were used to convert the 8·3 MJ standard diet into the calculated energy requirements. Amounts of energy, protein, carbohydrate and Ca in the diet were based on the report of national consumption of household food in Scotland 1990 (Ministry of Agriculture, Fisheries and Food, 1990). Dietary fibre was close to that reported by the Scottish Heart Health Study (Bolton-Smith *et al.* 1991). The amount of Na in the

Table 1. *Characteristics of the subjects participating in the study*

(Mean values with their standard errors and ranges)

Subjects	Mean	SEM	Range
Age (years)	62·3	1·5	50–70
Wt (kg)	66·4	2·3	50·1–80·2
BMI (kg/m^2)	26·1	0·8	20·9–30·3
Age at menopause (years)	48·2	0·9	42–54
No. of years since menopause	14·5	2·2	2–25
Blood pressure (mm Hg):			
Systolic	129	4	104–152
Diastolic	81	2	70–94

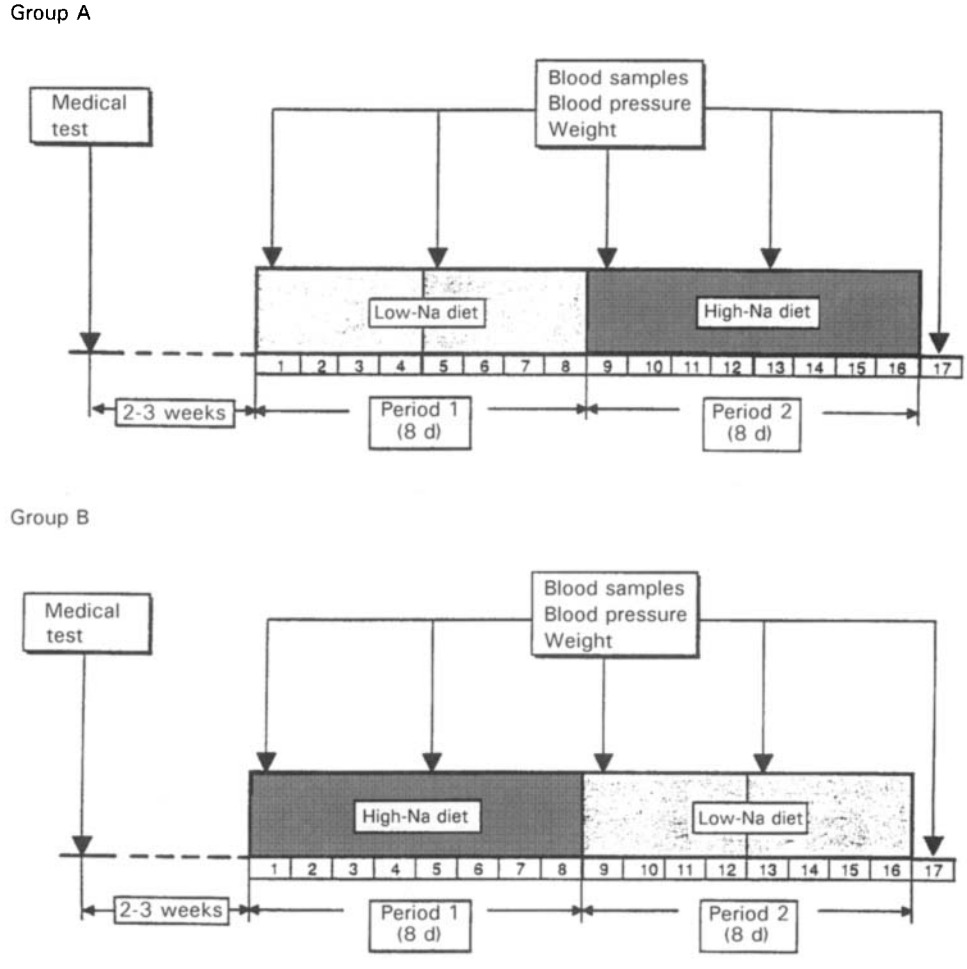

Fig. 1. Schedule of study design.

high Na period was similar to the mean urinary Na excretion in Scotland (Smith *et al.* 1988), and the amount in the low-Na period was close to the reference nutrient intake (70 mmol Na/d) for adults (Department of Health, 1991). Na was weighed out as NaCl and incorporated into the chosen food items. Drinking water was not restricted, but all water used was demineralized. Because caffeine may increase urinary Ca excretion, only decaffeinated tea or coffee was allowed. Volunteers drank the same amount of tea or coffee every day.

Collection and preparation of samples

The urine samples were stored at 4° until the end of the 24 h collection period. Then they were acidified with 10 ml 6 M-HCl and finally stored at −20° until required. Blood was collected by venipuncture into vacutainer tubes. Plasma was obtained following centrifugation at 7000 rev./min for 10 min and immediately stored at −70° until required.

Table 2. *Total nutrient content of diet compared with Ministry of Agriculture, Fisheries and Food (MAFF; 1991) report and results from the Scottish Heart Health Study (SHHS; Bolton-Smith* et al. *1991)*

Nutrient	Diet*		Nutrient intake from:	
	Day 1	Day 2	MAFF	SHHS
Energy: kcal	1985	1984	1980	
kJ	8343	8337	8300	
Carbohydrate (g)	245·3	244·5	244·0	
Lactose (g)	16·1	16·9		
Sugars (g)	59·5	49·9		
Starch and dextrin (g)	169·6	177·6		
Fibre (Southgate)†(g)	16·4	19·1		18·0–20·5
Protein (N × 6·25) (g)	65·7	66·2	65·8	
Total N (g)	10·7	10·8		
Fat (g)	89·3	89·2	89·0	
P : S quotient	0·29	0·29		0·28–0·31
Ca: mg	816·2	815·6	840	
mmol	20·4	20·3		
Phosphate: mg	1095	1070		
mmol	35·4	34·5		
P (mmol) : Ca (mmol)	1·73	1·69		
Mg (mg)	214	227		
K (mg)	2401	2746		

P : S, polyunsaturated : saturated fat.
* For detailed daily menus, contact author.
† Water-soluble polysaccharides, hemicellulose, cellulose and lignin, according to Southgate (1969).

Experimental techniques

Radioimmunoassay. The Allegro Intact PTH Immunoassay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), used for quantitative determination of human intact parathyroid hormone in serum, is a two-site immunoradiometric assay for the measurement of the biologically-intact eighty-four amino acid peptide. The radioactivity was measured using the Cobra-Gamma counting system (Packard Instrument Ltd, Downers Grove, IL, USA). Every sample was analysed in duplicate in the same run and the intra-assay CV was 3·5 %.

Plasma calcium and magnesium. Both Ca and Mg were analysed in duplicate using the KONE Analyser (Labmedics Ltd, Stockport, Cheshire) according to methods previously described (Bauder, 1981; Pesce & Kaplan, 1987). Intra-assay CV were 2·2 and 2·0 % for Ca and Mg respectively. All samples were measured together in the same run.

Urinary calcium, magnesium, sodium and potassium. Ca was analysed in duplicate as previously described (Ray Sarkar & Chauhan, 1967). Intra- and inter-assay CV were 2·4 and 7·3 % respectively. Mg was analysed in duplicate using the Technicon AutoAnalyzer (Technicon Instruments Co. Ltd, Basingstoke, Hants) as previously described (Gitelman *et al.* 1966). Intra- and inter-assay CV for Mg were found to be 2·6 and 10·5 % respectively. Na and K were analysed in duplicate using the Technicon Flame Photometer (Technicon Instruments Co. Ltd) according to Technicon Instruments Co. Ltd (1971). Intra- and inter-assay CV for Na were 4·2 and 7·1 % and for K 4·2 and 10·3 % respectively.

Urinary pyridinoline and deoxypyridinoline. Samples were analysed in duplicate using ion-paired reversed-phase HPLC directly linked to an automatic sample preparation system with extraction columns (Gilson ASPEC; Anachem, Luton, Beds.) together with the use of an internal standard as previously described (Pratt *et al.* 1992). Intra- and inter-assay CV were respectively, for pyridinoline 3·2 and 6·1 %, and for deoxypyridinoline 6·3 and 5·0 %.

Validity of the urine collection. Urine collections (24 h) were checked for completeness by using creatinine excretion. Creatinine was analysed in duplicate as previously described (Stevens *et al.* 1953). Intra- and inter-assay CV were 2·6 and 5·8 % respectively. The overall mean was found to be 978 mg/d. The intra-individual CV of creatinine averaged 8·1 %. Collections were designated incomplete when their creatinine excretion was outside two standard deviations from their mean. From 223 samples, ten were presumed incomplete.

Statistical analysis

Data are presented as means with their standard errors. Comparison between the periods and between the groups was performed using ANOVA. The statistical package used was Genstat (Lawes Agricultural Trust, 1987).

RESULTS

Urinary Ca excretion was significantly higher during the high-Na treatment period than the low-Na treatment period ($P < 0{\cdot}001$; Table 3). Urinary Mg tended to increase with Na loading, but the difference was not statistically significant ($P = 0{\cdot}08$; Table 3).

There was no significant difference in DPD or PYD excretion on varying the Na load (Table 3). A positive correlation was found between changes in mean urinary Ca and mean DPD excretion across the two diet periods (r 0·62, $P < 0{\cdot}02$; Fig. 2). There was no significant effect of Na loading on urinary volume or creatinine excretion (Table 3).

Plasma Ca did not change across the diet periods, but there was a significant fall in Mg levels with Na loading ($P < 0{\cdot}001$; Table 4). PTH was measured in only three volunteers from each group, and no change with dietary Na was found (Table 4).

The CV for the daily DPD excretion averaged 9·5 %, lower than the value of 24 % obtained previously (McLaren *et al.* 1993), which probably relates to the improved precision of the assay using an internal standard.

There was a small, but not significant, rise in diastolic and systolic blood pressure with Na loading (Table 4).

DISCUSSION

Many dietary factors are thought to influence Ca homeostasis, e.g. vitamin D, P, caffeine, protein, Na and dietary fibre (in part by the action of phytate). It is important, therefore, to keep all other dietary influences on Ca homeostasis constant when studying the influence of Na on bone and Ca metabolism. It was stressed that adaptation of the body to changes in Ca intake can take from 6 to 18 months (Zarkadas *et al.* 1989). The diet in the present study conformed with average Scottish intakes for all major nutrients, and it is hoped that the effects of adaptation were reasonably small. We also chose to examine the effect of changing Na intakes from the current average to a level close to the reference nutrient

Table 3. *The effects of two levels of dietary sodium (as sodium chloride) supplements on the urinary excretion of calcium, magnesium, potassium, creatinine, pyridinoline and deoxypyridinoline averaged from four 24 h urine collections in postmenopausal women**

(Mean values with their standard errors)

	Na content in diet (mmol/d)				Statistical significance of difference:
	60		170		
	Mean	SE	Mean	SE	*P*
Na (mmol/d)	39·1	1·4	130·4	2·0	< 0·001
Ca (mmol/d)	4·0	0·3	4·5	0·4	< 0·001
Mg (mmol/d)	3·2	0·2	3·4	0·3	0·08
K (mmol/d)	40·7	1·7	41·9	1·6	NS
Creatinine (mg/d)	967	26	979	28	NS
Pyridinoline (nmol/d)	476	22	465	25	NS
Deoxypyridinoline (nmol/d)	132	7	129	7	NS
Urine output (g/d)	1971	138	2002	141	NS

* For details of subjects and procedures, see Table 1, Fig. 1 and pp. 74–77.

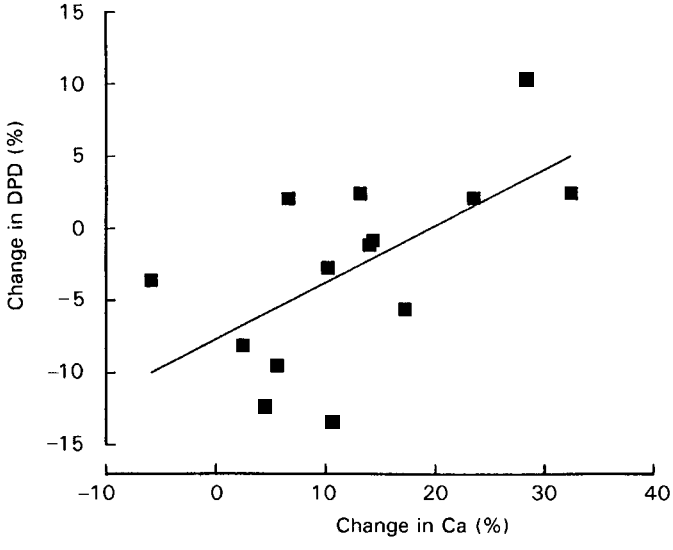

Fig. 2. Correlation between urinary calcium and deoxypyridinoline (DPD) excretion in postmenopausal women for two diet periods of 8 d each in which they received sodium (as sodium chloride) supplements of 60 or 170 mmol/d in a cross-over study. For details of subjects and procedures, see Table 1, Fig. 1 and pp. 74–77. r 0·616; $P < 0·019$.

intake of the Department of Health (1991). Thus, we did not subject our volunteers to extremes of Na intake.

It has been known for a long time that high intakes of Na cause hypercalciuria in healthy and osteoporotic subjects. In our study urinary Ca increased by 0·55 mmol/d per 100 mmol/d increase in urinary Na. Similar results were obtained in postmenopausal women, with an increase in urinary Ca of about 0·58 (McParland *et al.* 1989) and 0·6 (Zarkadas *et al.* 1989) mmol/d per 100 mmol/d increase in urinary Na.

Table 4. *The effects of two levels of dietary sodium (as sodium chloride) supplements on blood pressure, plasma calcium, magnesium and parathyroid hormone (PTH) in postmenopausal women**

(Mean values with their standard errors)

	Na content in diet (mmol/d)				Statistical significance of difference:
	60		170		
	Mean	SE	Mean	SE	*P*
Blood pressure (mm Hg)					
Systolic	128	3	131	4	NS
Diastolic	76	2	79	2	NS
Ca (mmol/l)	2·36	0·01	2·35	0·01	NS
Mg (mmol/l)	0·83	0·01	0·81	0·01	< 0·001
PTH (pg/ml)†	34·6	6·6	34·7	6·4	NS

* For details of subjects and procedures, see Table 1, Fig· 1 and pp. 74–77.
† Measured for six volunteers only.

We found no significant change in DPD excretion on varying Na load. This observation appears to contrast with the results of the study of McParland *et al.* (1989), where hydroxyproline was reported to increase after Na loading. Need *et al.* (1991) also found that Na restriction in postmenopausal women on a gelatin-free diet was associated with a fall in hydroxyproline excretion. On the other hand, studies in younger subjects have found both an increase (Chan *et al.* 1992) and no change (Castenmiller *et al.* 1985) in hydroxyproline excretion with Na loading. It is possible that the lower Na load used in our study is responsible for the lack of change in DPD excretion across the two dietary treatments. Another explanation might be the relatively shorter duration of this study. Even though Na balance was achieved after 3–4 d, bone remodelling did not appear to increase after 8 d. On the other hand, McParland *et al.* (1989) and Chan *et al.* (1992) showed an increase in hydroxyproline after 10 d, and a recent study looking at the effect of short-term starvation on bone turnover (Grinspoon *et al.* 1995) demonstrated a significant decline in bone formation markers after only 4 d. However, hydroxyproline used in previous studies is known to be a much-less-sensitive marker of bone resorption than urinary DPD, and the changes observed in previous studies, therefore, do not necessarily reflect alterations only in skeletal metabolism. Increases in hydroxyproline excretion could occur from many other connective tissues, including degradation of newly-formed collagen, or from an alteration in the liver catabolism of hydroxyproline.

It was stressed by Need *et al.* (1991) that subjects with an initial Na : creatinine value of less than 15 mmol/mmol are not likely to show a significant decrease in hydroxyproline excretion on a modest Na-restricted diet. Since participants in the present study had an initial Na : creatinine value of 10 mmol/mmol on day 1 of the study, very little change in DPD excretion might be expected. The one subject in our study who had an initial sodium:creatinine value over 15 mmol/mmol manifested the largest increase in DPD excretion with Na load. Nordin & Polley (1987) found a significant effect of Na restriction on fasting urinary Ca values only when the initial Na : creatinine value was greater than 15 mmol/mmol, which was the case in only 25 % of postmenopausal women.

It is noteworthy that we found a significant relationship betwen Na-induced changes in DPD and Ca concentrations. This suggests that the new bone marker may help to identify those individuals who are susceptible to the detrimental effects of increased Na intake on bone metabolism.

Increased urinary Ca excretion has been suggested to depress plasma Ca, leading to enhanced PTH secretion which seeks to maintain plasma Ca by increasing bone resorption (Shortt & Flynn, 1990). Furthermore, Breslau *et al.* (1982) found that increasing dietary Na enhanced fractional gut Ca absorption in young men and women. This could also help to explain the increase in urinary Ca. Since this response did not occur in two subjects with hypoparathyroidism, they suggested that this response was related to PTH stimulation of 1,25-dihydroxycholecalciferol production. However, we were unable to detect any significant change in PTH in our small subsample, and in a later paper, Breslau *et al.* (1985) also found no increase in PTH in a study with osteoporotic women, and suggested that the PTH response to Na-induced calciuria may be slow. Zarkadas *et al* (1989) also reported no increase in PTH in postmenopausal women. However, when PTH was measured by urinary cAMP in postmenopausal women, an increase after Na loading was detected. Despite apparent increases in PTH, 1,25-dihydroxycholecalciferol and Ca absorption did not increase, suggesting an impaired PTH–vitamin D axis (McParland *et al.* 1989).

We did not measure 25-hydroxy- or 1,25-dihydroxycholecalciferol. The PTH measurements from the six subjects would suggest that they did not have secondary hyperparathyroidism as a consequence of vitamin D deficiency, the study being conducted during the winter. We also could not assess whether changes in 1,25-dihydroxycholecalciferol could have influenced Ca absorption.

The dependence of urinary Ca excretion on urinary Na-excretion has been attributed to the existence of linked or common re-absorption pathways for both ions in the proximal tubule and thick ascending loop of Henle (Shortt & Flynn, 1990). However, more recently, MacGregor & Cappuccio (1993) have suggested that Na-induced calciuria results from expansion of central blood volume, with a slight fall in plasma Ca, stimulating PTH secretion. They suggested that subjects with essential hypertension have an inherited defect in renal clearance of Na, leading to greater Na-induced calciuria and PTH secretion. In our study subjects were normotensive and, thus, could have been less likely to have enhanced PTH secretion and enhanced bone resorption. None of the previous studies on postmenopausal women have evaluated the possible differential response to Na loading in normotensive and hypertensive subjects. Most studies used normotensive subjects (Castenmiller *et al.* 1985; McParland *et al.* 1989; Zarkadas *et al.* 1989; Chan *et al.* 1992), but others did not mention the blood pressure of their subjects (Breslau *et al.* 1985; Need *et al.* 1991).

Plasma Mg fell with Na loading, and there was a tendency for urinary Mg to increase, suggesting that Na may also influence Mg metabolism. Very little is known about the control of Mg homeostasis (Lemann, 1993), but PTH administration has been shown to depress plasma Mg without significant effect on urinary Mg (Slovik *et al.* 1981). Osteoporotics have been found to have lower serum Mg levels that controls (Reginster *et al.* 1989), and the role of Mg in bone metabolism has been neglected (Seelig, 1990).

In summary, the present study, using a recently-developed sensitive marker of bone resorption, has shown that no direct change in bone resorption occurred in postmenopausal women when Na intake changed from 60 to 170 mmol/d. A further study, using DPD as a marker, is needed to test whether postmenopausal women with hypertension or an initial Na : creatinine value greater than 15 mmol/mmol are likely to show an increase in bone turnover under conditions known to induce hypercalciuria. In addition, there is evidence that Na intake may affect Mg metabolism.

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