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The effect of high salt and high protein intake on calcium metabolism, bone composition and bone resorption in the rat

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The effects of salt (NaCl) supplementation of rat diets (50 g/kg diet), with normal (200 g/kg) or high (500 g/kg) dietary casein content, were studied in 3-week-old male rats over a 3-week period. Weight gain was reduced by dietary salt but was unaffected by dietary casein. Salt-supplemented rats exhibited a two-and three-fold increase in urinary Mg and Ca excretion respectively, irrespective of dietary casein content. Dietary casein had no effect on urinary Ca or Mg. Salt reduced femoral mass but not femoral mass expressed relative to body weight, but neither variable was affected by dietary casein. Femoral Mg and P contents and concentrations were unaffected by dietary salt or casein. While femoral Ca concentration was unaffected by dietary salt, the Ca content was reduced by salt supplementation, irrespective of dietary casein content. Neither the content nor concentration of Ca in femora was affected by dietary casein. Urinary pyridinoline and deoxypyridinoline levels were increased by salt supplementation, irrespective of dietary casein content, but were unaffected by casein. Net Ca absorption was unaffected by dietary salt or casein. In conclusion, these results show that salt supplementation over the short-term increased the rate of bone resorption in rats. This was as a consequence of Na-induced calciuria. On the other hand, a high dietary protein intake had no effect on Ca metabolism, bone composition or bone resorption, nor did it augment the Na-induced calciuria or increased rate of bone resorption.

Salt: Protein: Calcium: Rat bone

There is evidence that high dietary Na and protein intakes may have adverse effects on bone metabolism and may be risk factors for osteoporosis. Increasing Na intake is associated with increased urinary Ca loss (calciuria) in experimental animals (Goulding & Campbell, 1984; Whiting & Cole, 1986; Greger *et al*. 1987; Shortt & Flynn, 1991; Chan & Swaminathan, 1994) and man (Shortt *et al*. 1988; Shortt & Flynn, 1990; Itoh & Suyama, 1996; Leitz *et al*. 1997; Ginty *et al*. 1998). This dependence of urinary Ca excretion on urinary Na excretion has been attributed to the existence of linked or common reabsorption pathways for both ions in the convoluted portion of the proximal tubule and thick ascending loop of Henle (Shortt & Flynn, 1990).

A calciuric effect is also seen with high-protein diets (in human subjects: Allen *et al*. 1979*a*, *b*; Hegsted, 1981; Mahalko *et al*. 1983; Kerstetter *et al*. 1997; Pannemans *et al*. 1997; in rats: Allen & Hall, 1978; Whiting & Draper, 1980; Howe & Beecher, 1981; Chan & Swaminathan, 1994). However, in this case, the calciuria has been attributed to an increased glomerular filtration rate and a decreased tubular Ca reabsorption caused by catabolism of S-containing amino acids native to the protein (Kim & Linkswiler, 1979; Schuette *et al*. 1980). It has been estimated that a doubling of dietary protein intake increases urinary Ca by approximately 50 % (Walker & Linkswiler, 1972; Heaney, 1993). Furthermore, there is some evidence that the calciuric effects of high Na and high protein intakes may be additive (Goulding & Campbell, 1984; Chan & Swaminathan, 1994). However, it is not known to what extent Na- (and/or protein-) induced calciuria is compensated for by increased absorption of dietary Ca and/or reduced endogenous Ca losses or to what extent this Ca is derived from resorption of bone (Shortt & Flynn, 1990; Itoh *et al*. 1998).

There is evidence that very high Na intakes adversely affects Ca homeostasis and reduces bone mass and bone Ca content in rats (for reviews see Shortt & Flynn, 1990; Massey & Whiting, 1996). There is also evidence that urinary hydroxyproline, a marker of bone resorption, is increased with increasing Na intake in man (Goulding, 1981; Goulding & Lim, 1993; Nordin & Polley, 1987; McParland *et al*. 1989; Need *et al*. 1991; Itoh & Suyama, 1996) and animals (Goulding & Campbell, 1983; Goulding & Gold, 1986, 1988; Chan *et al*. 1993; Chan & Swaminathan, 1994, 1998). Likewise, increasing dietary protein intake is associated

Abbreviations: Dpyr, deoxypyridinoline; PTH, parathyroid hormone; Pyr, pyridinoline.

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with increased urinary excretion of hydroxyproline in human subjects (Schuette *et al*. 1981) and rats (Goulding & Campbell, 1984; Chan & Swaminathan, 1994). Chan & Swaminathan (1994) examined the effect of feeding a diet high in protein and salt for 4 months on the urinary excretion of hydroxyproline in rats. They found significant effects of dietary protein and dietary salt on hydroxyproline excretion, but no interaction between the two dietary factors. However, urinary hydroxyproline has been shown to lack sensitivity and specificity as an indicator of bone resorption and urinary pyridinium crosslinks of collagen are considered to be more reliable biochemical markers of bone resorption in human subjects (Delmas, 1992; Ginty *et al*. 1998; Cashman & Flynn, 1999) and rats (Black *et al*. 1989; Egger *et al*. 1994). To date, there has been no study of the effect of high dietary Na and protein intake on the rate of bone resorption in human subjects or rats, as assessed by these more specific and sensitive urinary markers.

Thus, the aim of the present study was to investigate the effect of a high-Na, high-protein diet on Ca metabolism, bone composition and the urinary pyridinium crosslinks of collagen in rats.

Materials and methods

Preparation of rat diets

The AIN-76 purified diet (that recommended for rats by the American Institute of Nutrition, 1977) was used in the present study (Table 1).

Table 1. Composition of the AIN-76 basal diet (American Institute of Nutrition, 1977)

Ingredient*	Content (g/kg)
Casein	200·0†
DL-Methionine	3·0
Maize starch	150·0
Sucrose	484·9
Fibre	50·0
NaCl	2·6†
Maize oil	50·0
AIN mineral mix‡	35·0
AIN vitamin mix§	10·0
Calcium carbonate	12·5
Choline bitartrate	2·0

* Sources of ingredients: casein (sodium caseinate, Kerrymore Milk Products Ltd., Listowel, Co. Kerry, Ireland); DL-methionine (Rhone Poulenc, Animal Nutrition, Commentry, France); maize starch (Cagill Bergen op, Zoom, The Netherlands); sucrose (Irish Sugar plc, Sugar Division, Athy Road, Carlow, Ireland); fibre (Avicel microcrystalline cellulose, N.F., FMC International Food and Pharmaceutical Products Division, Little Island, Cork, Ireland); maize oil (St Bernard's brand, Dunnes Stores Ltd, 67 Stephen Street, Upper Dublin 8, Ireland); choline bitartrate (Brown and Gilmore, Carrigaline East, Co. Cork, Ireland).

† Representing diets containing (per kg): 200 and 2·6 g casein and salt respectively (basal diet); level of addition of protein and/or salt was increased appropriately (in replacement of sucrose) for the high-protein, high-salt and high-protein–high-salt diets.

‡ Contained (g/kg): potassium dihydrogen phosphate 376, dipotassium hydrogen phosphate 160, magnesium oxide 24, manganous carbonate 3·5, ferric citrate 6, zinc carbonate 1·6, cupric carbonate 0·3, potassium iodate 0·01, sodium selenite 0·01, chromium potassium sulfate 0·55, sucrose 428.

§ Contained (/kg): nicotinic acid 3 g, calcium pantothenate 1·6 g, riboflavin 600 mg, thiamin-HCl 600 mg, pyridoxine-HCl 700 mg, pteroylmonoglutamic acid 200 mg, biotin 20 mg, cyanocobalamin 1 mg, cholecalciferol 2·5 mg, menaquinone 5·0 mg, retinyl palmitate 120 mg, DL-α tocopheryl acetate 5000 mg.

Experimental design

Thirty-two male rats, 3 weeks old, Wistar strain (average weight 57 g), obtained from the Biological Services Unit, University College, Cork, were randomized by weight into four groups of eight rats each. One group was fed *ad libitum* on a semipurified basal diet (AIN-76) as outlined in Table 1 while a second group was fed a diet (high-protein) similar to the basal diet in every respect except that 300 g/kg of the sucrose component was replaced by dietary casein. The third and fourth groups were fed *ad libitum* on the basal and high-protein diets supplemented with NaCl (50 g/kg diet; high-salt and high-protein–high-salt diets respectively). Rats were housed individually and feed was provided at 17.00 hours each day. All animals were given distilled water *ad libitum* for the duration of the study. Rats were weighed weekly and examined daily for general condition. During the last week of the study, all rats were placed in individual metabolism cages with a grid-floor and a facility for separate collection of faeces and urine. To acclimate the animals to the new environment, rats were placed in these cages 2 d before the beginning of a 4 d metabolic period for determination of net dietary Ca absorption and urinary pyridinium crosslink excretion. *Ad libitum* intake of AIN-76 diets was measured during a 2 d period to determine average food intake per group. To assure complete and equivalent consumption of all food offered during the 4 d metabolic period, an equalized feeding paradigm was used. In the present study, the amount of food offered to all rats during the 4 d balance period was limited to 90 % of the *ad libitum* food intake of the group that ate the least amount of food during the previous 2 d. Feed was provided at 09.00 hours each day over the period and any remaining feed at 09.00 hours the following day was weighed. Quantitative collections of faeces were made over the 4 d period and these were pooled for each rat and stored at 4°C until required for analysis.

Urine samples (24 h) were also collected for each animal during the metabolic period in vessels covered with Al foil to prevent degradation by light of the pyridinium crosslinks. The urine samples for each animal were pooled and the volumes recorded. Portions of the pooled urine samples were acidified with 12 M-HCl (225 μl/100 ml urine) and stored at −20°C until required for analysis.

Net Ca absorption (%) was calculated as the difference between Ca intake from the diet and Ca recovered in the faeces during the 4 d period, while Ca retention was calculated as the difference between dietary Ca intake and Ca excretion in faeces and urine.

After 21 d on the respective diets, all animals were killed by over-exposure to diethyl ether and final body weights were recorded. Femora were harvested, cleaned of adhering soft tissue, dried overnight at 110°C, weighed and stored in sealed containers until required for mineral analysis.

Experimental techniques

Urinary pyridinoline and deoxypyridinoline. Pooled urine samples for each animal were analysed in duplicate using a three-step procedure. Urine was first hydrolysed with an equal volume of 12 M-HCl at 110°C for 18 h, the crosslinks

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were then extracted by CF1 cellulose chromatography with the use of an internal standard (acetylated pyridinoline (Pyr), MetraBiosystems Ltd, Wheatley, Oxon., UK) and were measured using a reversed-phase HPLC method with fluorescence detection (Colwell *et al*. 1993). The acetylated Pyr was used in accordance with the method as described by Calabresi *et al*. (1994) and Robins *et al*. (1994). The crosslinks contents of urines were quantitated by external standardization using a commercially available Pyr–deoxypyridinoline (Dpyr) HPLC calibrator (MetraBiosystems Ltd). The intra-assay CV for Pyr and Dpyr measured as the variation between ten chromatograms obtained between column regenerations as described by Colwell *et al*. (1993) were 6 % and 9 % respectively. The inter-assay CV for Pyr and Dpyr were 7 and 8 % respectively.

Femoral phosphorus and femoral and urinary calcium and magnesium levels. Weighed femora (dried) were digested in 10 ml 16 M-HNO_3–12 M-$HClO_4$ (2 : 1, v/v) on a hot plate (S & J Juniper & Co., Essex, UK) until the sample colour resembled that of the reagent blank. Ca and Mg were analysed in duplicate in femoral digests and urine by atomic absorption spectrophotometry (Pye-Unicam Atomic Absorption Spectrophotometer, Model SP9; Cambridge, Cambs., UK) after appropriate dilution with $LaCl_3$ solution (5 g/l, BDH Ltd, Poole, Dorset, UK). A range of Ca and Mg standards were used to obtain Ca and Mg calibration curves. The intra- and inter-assay CV for Ca were 2·8 % and 7·8 %, and for Mg were 3·2 % and 8·8 % respectively. P was determined in the femoral digests by the method of Weissman & Pileggi (1974). The intra- and inter-assay CV for P were 4·2 % and 6·1 % respectively.

Feed and faecal calcium levels. Pooled faecal samples (4 d) from each animal were dried overnight at 110°C and mixed thoroughly. Aliquots of the dried faecal samples and of the AIN-76 diets were digested in 10 ml of 16 M-HNO_3–12 M-$HClO_4$ (2 : 1, v/v) on a hot plate (S & J Juniper & Co.) until the sample colour resembled that of the reagent blank. Ca was analysed in duplicate in faecal and feed digests by atomic absorption spectrophotometry (Pye-Unicam Atomic Absorption Spectrophotometer) after appropriate dilution with $LaCl_3$ solution (5 g/l, BDH Ltd). A range of Ca standards was used to obtain Ca calibration curves. The intra- and inter-assay CV for Ca were 2·8 % and 7·8 % respectively.

Statistical methods

All data were subjected to two-way ANOVA, with variation attributed to dietary protein (casein) and salt (Snedecor & Cochran, 1967). Means and their pooled standard errors are tabulated for each measurement with significant differences for dietary casein and salt and casein × salt interaction.

Results

While not quantified, water consumption was noted to be much greater in the salt-supplemented animals compared with the non-supplemented animals.

Mean body weight gain was lower in the salt-supplemented than in the non-supplemented groups, irrespective of dietary protein (casein) content, but was unaffected by dietary casein (Table 2).

Salt-supplemented animals excreted significantly more Ca and Mg in urine than non-supplemented animals (Table 2). Dietary casein had no effect on urinary Ca or Mg. There was no significant effect of dietary salt or casein on faecal Ca or on net Ca absorption or retention.

Femoral dry weight was lower in salt-supplemented than non-supplemented animals, but when femoral mass was expressed relative to body weight, dietary salt had no effect and dietary casein did not affect either of these variables (Table 3). Neither the content (mg/bone) nor concentration (mg/g bone) of Mg or P in femora was affected by dietary salt or casein. While femoral Ca concentration was unaffected by dietary salt, the Ca content was significantly reduced by salt supplementation, irrespective of dietary casein content. Neither the content nor concentration of Ca in femora was affected by dietary casein.

Urinary Pyr and Dpyr levels were increased by salt supplementation, irrespective of dietary casein content, but were unaffected by casein (Table 3).

Discussion

The results of the present study indicate that short-term intakes of salt supplements (50 g/kg diet) significantly increased urinary Ca and Mg excretion in rats irrespective of the protein (casein) content of the diet. The effect of salt

Table 2. The effect of dietary salt and protein on weight gain, calcium absorption and retention and urinary calcium and magnesium in young male rats*

(Mean values and pooled standard errors of the mean)

Dietary treatment …	Low-salt		High-salt		Pooled SEM	Statistical significance of variance ratio (*P*), effects of:		
	Low casein (*n* 8)	High-casein (*n* 8)	Low-casein (*n* 8)	High-casein (*n* 8)		Salt	Casein	Salt × casein
Weight gain (g/21 d)	73·0	71·2	55·7	50·1	4·0	< 0·001	–	–
Urinary Ca (mg/4 d)	1·5	1·6	4·8	5·3	0·4	< 0·0001	–	–
Urinary Mg (mg/4 d)	2·4	2·6	4·5	4·8	0·2	< 0·0001	–	–
Faecal Ca (mg)	101	97	100	102	4	–	–	–
Ca retention (mg)	242	232	239	235	4	–	–	–
Net Ca absorption:†								
(mg)	244	233	243	240	3	–	–	–
(%)	70·7	71·6	70·9	70·3	1·1	–	–	–

* For details of diets and procedures, see Table 1 and pp. 50–51.
† Calculated of the last 4 d of the 3-week study period.

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Table 3. The effect of dietary salt and protein on urinary pyridinoline (Pyr) and deoxypyridinoline (Dpyr) concentrations and on femur dry weight and concentrations of calcium, magnesium and phosphorus in young male rats*

(Mean values and pooled standard errors of the mean)

Dietary treatment …	Low-salt		High-salt		Pooled SEM	Statistical significance of variance ratio (*P*), effects of:		
	Low casein (*n* 8)	High-casein (*n* 8)	Low-casein (*n* 8)	High-casein (*n* 8)		Salt	Casein	Salt × casein
Femur								
Dry wt:								
(mg)	192	198	153	171	4·2	0·038	–	–
(g/kg body wt)	1·46	1·52	1·46	1·48	0·05	–	–	–
Ca (mg/bone)	37·2	35·5	32·0	29·8	1·9	0·017	–	–
Mg (mg/bone)	0·71	0·69	0·67	0·69	0·04	–	–	–
P (mg/bone)	21·4	19·3	18·0	22·5	1·9	–	–	–
Ca (mg/g dry wt)	196	197	188	182	5	–	–	–
Mg (mg/g dry wt)	3·99	3·86	3·58	3·62	0·22	–	–	–
P (mg/g dry wt)	112	116	105	118	5	–	–	–
Urine								
Pyr (nmol/d)	10·8	9·7	12·4	13·4	0·7	0·008	–	–
Dpyr (nmol/d)	9·6	9·5	12·0	12·4	0·6	0·006	–	–

* For details of diets and procedures, see Table 1 and pp. 50–51.

on urinary mineral excretion was most marked in the case of Ca, with a three-fold increase in animals given salt-supplemented compared with non-supplemented diets. These findings are consistent with the results of previous studies (Goulding & Campbell, 1984; Goulding & Gold, 1986; Shortt & Flynn, 1991; Chan & Swaminathan, 1994). The dependence of urinary Ca excretion on urinary Na excretion has been attributed to the existence of linked or common reabsorption pathways for the ions in the convoluted portion of the proximal tubule and in the loop of Henle (Shortt & Flynn, 1990, 1991).

In the present study, increasing the content of dietary protein (casein) in the diet had no effect on urinary Ca or Mg excretion. Previous reports have indicated that dietary protein increases the urinary excretion of Ca in rats (Bell *et al*. 1975; Allen & Hall, 1978; Whiting & Draper, 1980; Howe & Beecher, 1981; Goulding & Campbell, 1984; Chan & Swaminathan, 1994). However, the magnitude and duration of this hypercalciuria have varied significantly among studies. For example, 8-month-old male rats, Sprague-Dawley strain, fed 400 g protein/kg diet, as a mixture of gelatin, lactalbumin and casein, exhibited a 10-fold increase in urinary Ca, lasting at least 100 d, relative to controls fed 100 g casein/kg (Bell *et al*. 1975). In contrast, 28-d-old male rats, Wistar strain, fed 360 g casein/kg diet have been reported to exhibit only a slight calciuria which disappeared by 28 d, relative to controls fed 180 g casein/kg diet (Allen & Hall, 1978). Casein is a phosphorylated protein and, as such, increasing its level of inclusion in the diet in the present study from 200 to 500 g/kg would have raised the P content of the diet by about 2 g P/kg diet (Brommage *et al*. 1991). Since dietary P reduces urinary Ca excretion (Anderson & Draper, 1972; Howe & Beecher, 1981), this might explain, at least in part, why the increased casein content in the diet did not increase urinary Ca levels. However, Goulding & Campbell (1984) found that young growing male albino rats fed a high-casein diet (600 g casein/kg diet) excreted significantly more Ca in urine than rats fed a moderate-casein diet (250 g casein/kg diet) over a 14 d period. Whiting & Draper (1980) suggest that the degree of hypercalciuria seen with high-protein feeding in the rat depends on the length of time the diet is fed and on the type and level of protein used. Kerstetter & Allen (1994) on reviewing this topic concluded that while the acute response of rats to a high-protein diet appears to be similar to that of human subjects, the hypercalciuretic response disappears with time. On the other hand, the calciuretic response of human subjects to dietary protein does not diminish with time (Kerstetter & Allen, 1994).

In the present study, salt supplementation increased the excretion of pyridinium crosslinks of collagen in urine, suggesting that the rate of bone resorption was increased. This is the first study to our knowledge that has examined the effect of increased Na and/or protein intake on urinary pyridinium crosslink excretion in the rat. Three recent studies have investigated the effects of dietary Na on urinary pyridinium crosslinks of collagen in human subjects. Evans *et al*. (1997) reported that urinary Dpyr in postmenopausal women was increased by a high Na intake, while Lietz *et al*. (1997) found no effect. With regard to premenopausal women, both Evans *et al*. (1997) and Ginty *et al*. (1998) reported that urinary Dpyr was unaffected by dietary Na intake. Earlier studies in human subjects and experimental rats have reported that higher Na intakes are associated with increased urinary excretion of hydroxyproline (in man: Goulding & Lim, 1983; Goulding *et al*. 1986; Goulding & MacDonald, 1986; Chan *et al*. 1992; Itoh & Suyama, 1996; in rats: Goulding & Campbell, 1983; Goulding & Gold, 1986, 1988; Chan & Swaminathan, 1994). However, the suitability of urinary hydroxyproline as a marker of bone resorption has been questioned due to its lack of specificity and sensitivity and the changes in hydroxyproline excretion may not necessarily reflect changes in bone metabolism, but rather may be due to contributions from other sources, e.g. connective tissues or from an alteration in the liver catabolism of hydroxyproline (Leitz *et al*. 1997). Additionally, in a number of these studies, dietary hydroxyproline was not controlled and it has been well established that dietary

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hydroxyproline may affect urinary hydroxyproline excretion (Kivirikko, 1970; Massey & Whiting, 1996).

The impact of dietary protein on bone resorption is controversial. In the present study, increasing the casein content of the diet had no effect on the urinary excretion of pyridinium crosslinks of collagen. This is in agreement with the findings of Shapses *et al*. (1995) in human subjects who reported that increasing the levels of dietary protein had no effect on bone resorption in young adult subjects, as measured by the pyridinium crosslinks levels in urine. More recently, Kerstetter *et al*. (1999) found that while switching healthy young adult women from a low (45 g/d) to a medium dietary protein intake (63 g/d) increased the urinary excretion of *N*-telopeptides, another sensitive and specific marker of bone resorption, changing from the medium to a high level of protein intake (129 g/d) had no effect. On the other hand, Schuette *et al*. (1981) and both Goulding & Campbell (1984) and Chan & Swaminathan (1994) reported that urinary hydroxyproline excretion increased with increasing levels of dietary protein intake in humans and rats respectively. However, as pointed out above, the suitability of urinary hydroxyproline as a marker of bone resorption has been questioned. The lack of effect of high dietary protein intakes on the rate of bone resorption (as measured by the more specific and sensitive biomakers) relative to medium, or recommended, levels may be due to the fact that, despite causing calciuria, protein loads appear not to elevate circulating parathyroid hormone (PTH) levels, a potent stimulator of bone resorption (Adams *et al*. 1979; Kim & Linkswiler, 1979; Kerstetter *et al*. 1997).

In the present study, femoral mass was reduced by salt supplementation but was unaffected by dietary casein. However, when expressed on a body weight basis, there was no effect of salt on femoral mass, suggesting that the effect of salt was due, at least in part, to lower body weight gain in salt-supplemented animals. Shortt & Flynn (1991) have reported similar effects of salt supplementation on tibia mass in rats. Femoral Ca, but not P or Mg, content was reduced by dietary salt. However, salt had no effect on femoral Ca, Mg or P concentration. Therefore, in spite of the hypercalciuria and increased rate of bone resorption in salt-supplemented rats, their bone mass, when adjusted for body weight, was unaffected. This is in agreement with the findings of other studies in rats that found that salt supplementation (80 g/kg diet) for 10 d–8 weeks had no effect on bone mass despite the presence of hypercalciuria and increased bone resorption, as measured by urinary hydroxyproline (Goulding & Gold, 1988; Chan *et al*. 1993). Thus, it may be that, as suggested by Goulding & Gold (1988), the duration of the present study was too short to elicit significant osteopenia. There is, for example, considerable evidence that salt supplementation (80 g/kg diet) for longer periods (2–4 months) reduces bone mass and Ca content in young and adult rats consuming diets either deficient (Goulding & Campbell, 1982, 1983; Goulding & Gold, 1986; Chan *et al*. 1993) or adequate (Goulding & Campbell, 1984; Chan *et al*. 1993; Chan & Swaminathan, 1994, 1998) in Ca, although some studies have failed to show this (Goulding, 1980). In addition, Chan *et al*. (1993) and Chan & Swaminathan (1998) found that chronic (i.e. 12 months) high Na intake reduced bone mineral content in rats consuming diets adequate in Ca. Alternatively, the lack of effect of salt supplementation on femur mass in the present study, in spite of the evident hypercalciuria and increased bone resorption, may have been due to Ca mobilization from some part of the skeleton other than the femur. The lack of effect of dietary casein on bone mass or macro-mineral composition in the present study is in agreement with several studies in rats which failed to find an effect of excess dietary protein on these variables (Allen & Hall, 1978; Whiting & Draper, 1981; Goulding & Campbell, 1984).

It has been suggested that Na-induced hypercalcuria leads to a reduction in serum ionized-Ca which, in turn, stimulates the secretion of PTH (Goulding *et al*. 1986). PTH helps to restore serum ionized-Ca by increasing bone resorption and/or, via increased 1,25 dihydroxy-vitamin D_3, increasing intestinal absorption of Ca (Shortt & Flynn, 1990). Increased Ca absorption would normalize plasma Ca which, in turn, would reduce the stimulus for PTH secretion and for PTH-stimulated bone resorption. In the present study, however, despite the marked increase in urinary Ca loss, salt supplementation did not increase net Ca absorption. This finding is in agreement with those of similar studies in rats fed on diets deficient (Goulding & Campbell, 1983; Goulding & McIntosh, 1986) or adequate (Goulding & Campbell, 1984; Shortt & Flynn, 1991) in Ca which showed that salt supplementation (80 g/kg diet) did not increase net Ca absorption. In contrast, Goulding & Gold (1986) reported increased net Ca absorption in salt-supplemented rats fed on a Ca-deficient diet for 12 weeks.

In the present study, salt supplementation increased urinary Ca excretion by an amount equivalent to only about 2 % of the total Ca absorbed and retained. While the precision of the balance method does not permit determination of whether Ca absorption or retention were altered by such an amount, it is clear from the elevated urinary pyridinium crosslinks levels and lower femoral Ca content of the animals given salt-supplemented diets that homeostasis was achieved, at least in part, at the expense of bone Ca.

There is some evidence in human subjects to suggest that healthy individuals respond to Na-induced calciuria by a PTH-mediated increase in intestinal Ca absorption (Meyer *et al*. 1976; Breslau *et al*. 1982), although Evans *et al*. (1997) did not observe any effect of increasing dietary Na on Sr absorption (an index of Ca absorption) in premenopausal women. However, this adaptive mechanism does not appear to function in all individuals, e.g. those with impaired parathyroid function (Breslau *et al*. 1982), postmenopausal women (McParland *et al*. 1989); even in those individuals who appear to adapt, the increase in net Ca absorption may not be sufficient to offset the increase in urinary Ca losses (Breslau *et al*. 1982). In addition, the capacity for such adaptation may be limited by low dietary Ca intakes, poor vitamin D status, impaired renal function or poor intestinal Ca absorption (Shortt & Flynn, 1990).

In the present study, dietary protein (casein) had no effect on net Ca absorption. This is in agreement with several studies in rats (Allen & Hall, 1978; Howe & Beecher, 1981; Yuan & Kitts, 1994) and human subjects (Spencer *et al*. 1978; Heaney & Recker, 1982; Schuette & Linkswiler, 1982; Spencer *et al*. 1983; Draper *et al*. 1991) which have failed

to find an effect of high dietary protein intake on Ca absorption.

In conclusion, the salt-induced increase in the rate of bone resorption in rats, as assessed by the urinary pyridinium crosslinks, observed in the present study confirms the findings of previous studies which used urinary hydroxyproline as a marker of bone resorption. This increased rate of bone resorption was as a consequence of Na-induced calciuria. On the other hand, a high dietary protein intake had no effect on Ca metabolism, bone composition or bone resorption. Nor did it augment the Na-induced calciuria or increased rate of bone resorption. However, due to differences in the calciuric response to dietary protein between rats and human subjects, together with evidence from epidemiological studies which show a strong positive correlation between animal protein intake and incidence of fracture of the femoral neck (Feskanich *et al*. 1996; Meyer *et al*. 1997), there is a need for further study of the effect of high protein intake, independently and in combination with high salt intake, on bone metabolism in human subjects.

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