

## Effect of dietary lactose on salt-mediated changes in mineral metabolism and bone composition in the rat

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The effects of salt (sodium chloride) supplementation of rat diets (80 g/kg diet), with or without lactose (150 g/kg), were studied in weanling rats over 14 d. Dietary salt increased water intake and reduced weight gain and food conversion efficiency, but these variables were unaffected by lactose. Salt-supplemented rats exhibited a three- to fivefold increase in urinary calcium excretion and a small increase in urinary magnesium and phosphorus excretion, irrespective of dietary lactose content. In addition, salt supplementation reduced plasma alkaline phosphatase (EC 3.1.3.1) activity. Lactose increased urinary Ca and Mg excretion and plasma Ca and P concentrations. Salt reduced tibia mass but not tibia mass expressed relative to body-weight, but neither variable was affected by lactose. Both tibia Mg content and concentration were reduced by salt but unaffected by lactose, and neither tibia P content nor concentration was affected by salt or lactose. Tibia Ca content was reduced by salt but this was prevented by lactose. Tibia Ca concentration was unaffected by salt or lactose, although there was a reduction (not significant) in tibia Ca concentration in animals fed on the lactose-free diet. These results show that lactose had no independent effect on bone and that reduced accretion of bone mass and mineral content in rats fed on the high-salt diets was due, at least in part, to reduced growth. Failure to offset sodium-induced hypercalciuria by a compensatory increase in net Ca absorption may have contributed to reduced bone Ca accretion. The protective effect of lactose against reduced bone Ca accretion may be due to increased Ca absorption.

**Salt: Lactose: Calcium: Bone: Rat**

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There is a close relationship between urinary sodium and calcium excretion, and increased ingestion of sodium chloride increases urinary Ca excretion in experimental animals (Whiting & Cole, 1986; Greger *et al.* 1987) and man (Law *et al.* 1988; Shortt *et al.* 1988). Na-induced hypercalciuria leads to reduced serum ionized Ca concentration and stimulates parathyroid hormone activity (Coe *et al.* 1975; Pernot *et al.* 1979; Breslau *et al.* 1982), which, in turn, may lead to increased bone resorption (Goulding & Gold, 1988). In man, consumption of a high-Na diet is accompanied by increased serum 1,25-dihydroxycholecalciferol and enhanced Ca absorption (Breslau *et al.* 1982) which may protect against bone resorption. However, studies on rats have demonstrated that salt supplementation adversely affects Ca homeostasis and reduces bone Ca content (Goulding & Gold, 1986; Goulding & McIntosh, 1986).

Dietary lactose has been reported to increase serum Ca concentration in rats (Au & Raisz, 1967; Miller *et al.* 1988) and to improve Ca retention in rats (Schaafsma & Visser, 1980; Schaafsma *et al.* 1988) and in man (Condon *et al.* 1970), apparently by increasing the intestinal absorption of Ca by the non-vitamin D-dependent pathway. In addition, consumption of lactose-containing diets has been shown to inhibit bone resorption in mice (Marie & Travers, 1983) through a Ca-induced suppression of parathyroid hormone secretion. Thus, the present study was conducted to determine whether dietary lactose

Table 1. *Composition of the basal diet*

Ingredient	Dietary content (g/kg)
Casein*	250
Sucrose	605
Maize oil †	50
Mineral mix ‡	35
Vitamin mix §	10
Cellulose	50

\* Acid casein (870 g protein/kg); Kerry Co-Op, Listowel, Co. Kerry, Republic of Ireland.

† 'Mazola' Cooking Oil, CPC (UK) Ltd, Surrey, UK.

‡ Based on American Institute of Nutrition (1977), supplied (g/kg diet): CaHPO<sub>4</sub> 17.5, NaCl 2.59, K<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>·H<sub>2</sub>O 7.7, K<sub>2</sub>SO<sub>4</sub> 1.82, MgO 0.84, MnCO<sub>3</sub> 0.122, C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>Fe·5H<sub>2</sub>O 0.21, ZnCO<sub>3</sub> 0.056, CuCO<sub>3</sub> 0.0105, KIO<sub>3</sub> 0.00035, Na<sub>2</sub>SeO<sub>3</sub> 0.00035, CrK(SO<sub>4</sub>)<sub>2</sub>·12H<sub>2</sub>O 0.01925, sucrose 4.13.

§ Based on American Institute of Nutrition (1977), supplied (mg/kg diet): thiamine hydrochloride 6, riboflavin 6, pyridoxine hydrochloride 7, nicotinic acid 30, calcium pantothenate 16, folic acid 2, D-biotin 0.2, cyanocobalamin 0.01, vitamin K 0.05, vitamin A 1.5 (as retinol), vitamin D 0.025, vitamin E 50, and cellulose 9.8 g/kg diet.

|| Commercial grade; FMC International, Cork, Republic of Ireland.

would counteract the effects of salt supplementation on Ca metabolism and bone composition in weanling rats.

## MATERIALS AND METHODS

### *Animals and diets*

Male weanling Wistar rats (approximately 95 g), obtained from the Biological Services Unit, University College, Cork, were randomly divided into four groups of eight rats each. One group was given a basal diet (diet 1) as outlined in Table 1 while a second group was given a diet (diet 2) similar to the basal diet in every respect except that 150 g/kg of the sucrose component was replaced by lactose. The third and fourth groups were fed on diets 1 and 2 supplemented with NaCl (80 g/kg diet; diets 3 and 4 respectively).

Diets were prepared in a solidified form by incorporation (1:1, w/w) into 30 g agar/l solutions (Baker *et al.* 1979), stored in sealed trays at 4° and cut as required for feeding. The Ca and phosphorus contents of the basal diet (by analysis, see p. 75) were 5.5 and 4.0 g/kg dry weight respectively.

### *Experimental methods*

A 14 d balance study was carried out, with rats housed individually in wire-bottom cages in which the trays were covered with stainless-steel wire mesh to separate faeces and left-over food from urine. Throughout the study food intake was controlled so that the energy intakes of all groups were similar. Fresh diet was provided daily and left-over food was recorded. Distilled drinking water was available *ad lib.* and consumption was monitored throughout the study. Urine was collected daily in containers primed with thymol and concentrated hydrochloric acid, made up to a known volume with distilled water, and stored at 4° until mineral analyses were carried out. Faeces were collected daily (from day 2 to day 15 inclusive), pooled for each animal, and stored at -20° in air-tight containers.

On day 15, rats were anaesthetized after an overnight fast by intraperitoneal injection of sodium pentobarbital solution (1 ml/kg body-weight; Sagatal, May & Baker Ltd, Dagenham, Essex). The abdomen was opened and a blood sample was obtained from the posterior vena cava and transferred to a heparinized vial. Plasma was obtained by

centrifugation and stored frozen at  $-20^{\circ}$ . The tibia was removed from the right side of each animal, cleaned of adhering tissue, dried overnight at  $100^{\circ}$ , weighed and stored in sealed containers.

#### *Analytical methods*

Weighed diet samples and bones were digested in 10 ml of a 2:1 (v/v) mixture of concentrated nitric and perchloric acids on a hot plate (S. & J. Juniper & Co., Essex) until the sample colour resembled that of the reagent blank. Pooled faeces, for each animal, were dried overnight at  $105^{\circ}$ , mixed thoroughly and weighed portions (about 4 g) were ashed at  $550^{\circ}$  in a muffle furnace for 12 h. The ash was dissolved in a mixture of concentrated hydrochloric and nitric acids (1:2, v/v) and diluted appropriately for mineral analysis.

Dietary Na was determined by atomic absorption spectrophotometry (Model SP9; Pye Unicam Ltd, Cambridge). Ca in plasma, urine, faeces, diets and bones and magnesium in urine and bones were determined by atomic absorption spectrophotometry using lanthanum chloride (1 g/l; BDH Ltd, Poole, Dorset) as diluent. P was determined in serum, urine, bones and diet by the method of Weissman & Pileggi (1974). Plasma alkaline phosphatase (EC 3.1.3.1) activity was determined according to the method of Bessey *et al.* (1946). Net intestinal absorption of Ca was calculated as the difference between dietary intake and faecal output, while retention was calculated as the difference between dietary intake and excretion in faeces and urine.

#### *Statistical analysis*

Results were subjected to two-way analysis of variance, variation being attributed to dietary lactose and salt (Snedecor & Cochran, 1967). Means with their pooled standard errors are tabulated for each measurement with significant differences for dietary lactose and salt and lactose  $\times$  salt interaction. Because of unequal variances, values for water intake and urinary Ca, Mg and P were  $\log_{10}$  transformed for statistical analysis and back transformed to geometric means, but pooled standard errors for these variates were left in the log scale.

### RESULTS

As shown in Table 2, the mean food intake of all groups was similar. Mean body-weight gain and the food conversion efficiency were lower in the salt-supplemented than in non-supplemented groups, irrespective of dietary lactose content, but were unaffected by lactose. Water consumption was about three times greater in the salt-supplemented compared with non-supplemented animals.

Salt-supplemented animals excreted significantly more Ca, Mg and P in urine than non-supplemented animals (Table 2). Lactose also increased urinary Ca and Mg, but not P, and the increase in urinary Ca was only observed at the lower salt intake. There was no significant effect of dietary salt or lactose on faecal Ca or on net Ca absorption or retention.

Mean plasma Ca and P concentrations of the basal and salt-supplemented groups (Table 3) were within the normal limits for rats (Mitruka & Rawnsley, 1977) and both were increased by lactose supplementation regardless of salt intake. Plasma alkaline phosphatase activity was reduced by salt supplementation but was unaffected by lactose.

Tibia mass was lower in salt-supplemented than in non-supplemented animals, but when tibia mass was expressed relative to body-weight, dietary salt had no effect and lactose did not affect either of these variables (Table 3). Both content and concentration of Mg in tibia were reduced by salt supplementation irrespective of dietary lactose. A significant salt  $\times$  lactose interaction occurred for tibia Ca content which was reduced by salt supplementation, but only with the lactose-free diet, while lactose had no independent effect on tibia Ca content. Tibia Ca concentration (mg/g bone) was unaffected by dietary

Table 2. *The effect of dietary salt and lactose on weight gain, food and water intake, food conversion efficiency, and calcium balance and urinary magnesium and phosphorus in weanling rats over 14 d\**

Dietary treatment...	Low-salt		High-salt		Pooled SEM	Statistical significance of variance ratio (P), effects of:		
	- Lactose 1	+ Lactose 2	- Lactose 3	+ Lactose 4		Salt	Lactose	Salt x lactose
Diet no...								
Wt gain (g/14 d)	72.9	74.4	63.6	68.5	1.5	< 0.05	—	—
Food intake (g/14 d)	387.8	393.4	389.2	386.4	1.4	—	—	—
Food conversion efficiency†	0.187	0.189	0.164	0.177	0.004	< 0.05	—	—
Water intake (ml/14 d)‡	199	205	622	653	0.28	< 0.001	—	—
Urine								
Ca (mg/14 d)‡	8.2	13.5	39.8	37.5	0.02	< 0.001	< 0.05	< 0.01
Mg (mg/14 d)‡	12.1	17.4	18.6	22.5	0.02	< 0.001	< 0.01	—
P (mg/14 d)‡	269	278	379	434	0.01	< 0.001	—	—
Ca intake (mg)	1068	1081	1068	1062	5	—	—	—
Faecal Ca (mg)	267	269	271	239	6	—	—	—
Ca retention (mg)	792	798	756	785	7	—	—	—
Net Ca absorption (mg)	801	812	797	823	6	—	—	—

\* For details of diets and procedures, see pp. 74-75.

† Weight gain (g)/food intake (g) over 14 d.

‡ Geometric means with pooled SEM in log scale.

Table 3. The effect of dietary salt and lactose on plasma calcium, phosphorus and alkaline phosphatase (EC 3.1.3.1) and tibia content and concentration of Ca, magnesium and P in weanling rats over 14 d\*

Dietary treatment ...	Low-salt				High-salt				Pooled SEM	Statistical significance of variance ratio (P), effects of:		
	1	2	3	4	1	2	3	4		Salt	Lactose	Salt x lactose
Diet no ...												
Plasma												
Ca (mg/l)	87.3	90.4	84.8	89.9	0.5	—	—	—	< 0.001	—	—	
P (mg/l)	79.1	87.3	84.0	90.6	1.6	—	—	—	< 0.05	—	—	
Alkaline phosphatase (Sigma† units/l)	66.0	59.3	47.1	54.3	2.2	—	—	—	< 0.01	—	—	
Tibia												
Dry wt:												
(mg)	184	174	162	169	2.5	—	—	—	< 0.05	—	—	
(g/kg body-wt)	1.09	1.03	1.02	1.02	0.01	—	—	—	—	—	—	
Ca (mg/bone)	33.1	31.1	28.1	31.5	0.6	—	—	—	< 0.05	—	—	
Mg (mg/bone)	0.78	0.77	0.64	0.67	0.02	—	—	—	< 0.001	—	< 0.05	
P (mg/bone)	18.6	18.1	18.1	18.3	0.2	—	—	—	—	—	—	
Ca (mg/g dry wt)	180.5	178.2	173.6	178.6	2.2	—	—	—	—	—	—	
Mg (mg/g dry wt)	4.27	4.42	3.97	3.97	0.07	—	—	—	< 0.05	—	—	
P (mg/g dry wt)	101.6	104.2	112.1	108.1	1.7	—	—	—	—	—	—	

\* For details of diets and procedures, see pp. 74-75.

† 1 unit hydrolyses 1.0 μmole of P-nitrophenyl phosphate/min at pH 10.4 (glycine) at 37°.

salt or lactose, although there was a decrease (not significant) in tibia Ca concentration with the lactose-free diet. Neither the content nor concentration of P in tibia was affected by dietary salt or lactose.

#### DISCUSSION

The results of the present study indicate that short-term intakes of salt supplements (80 g/kg diet) significantly increased urinary Ca, Mg and P excretion in rats irrespective of the lactose content of the diet. The effect of salt on urinary mineral excretion was most marked in the case of Ca, with a three- to fivefold 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). The dependence of urinary Ca excretion on urinary Na excretion has been attributed to the existence of linked or common re-absorption pathways for the ions in the convoluted portion of the renal proximal tubule and in the loop of Henle (Antonioni *et al.* 1969; McCarron *et al.* 1981).

Inclusion of lactose in the diet also increased urinary Ca and Mg excretion but had no significant effect on urinary P excretion. The effect of lactose on urinary Ca excretion was only observed at the lower salt intake. Previous reports have indicated that dietary lactose increases the urinary excretion of Ca (Schaafsma & Visser, 1980; Schaafsma *et al.* 1988) and Mg (Marie & Travers, 1983; Schaafsma *et al.* 1988), but has no effect on urinary P excretion (Schaafsma & Visser, 1980).

Plasma Ca concentration was unaffected by dietary salt but was increased by lactose. Dietary lactose has previously been shown to increase serum Ca concentration (Marie & Travers, 1983; Miller *et al.* 1988) and to improve Ca retention (Forbes, 1964; Miller *et al.* 1988; Schaafsma *et al.* 1988) in animals, apparently by increasing the intestinal absorption of Ca. Plasma P concentration was also significantly increased by lactose, in agreement with previous studies which showed that diets containing lactose at 100 (Marie & Travers, 1983) or 300 (Debiec & Lorenc, 1988) g/kg diet increased plasma P concentration in animals.

Salt supplementation reduced plasma alkaline phosphatase activity, which is consistent with a reduced rate of Ca accretion in bone (Klein *et al.* 1964), although plasma alkaline phosphatase activity is not regarded as a sensitive marker of the rate of bone formation (Taylor *et al.* 1988). Dietary lactose had no independent effect on plasma alkaline phosphatase activity in the present study, similar to the findings of Schaafsma & Visser (1980).

Tibia mass was reduced by salt supplementation but was unaffected by lactose. However, when expressed on a body-weight basis, there was no effect of salt on tibia mass, suggesting that the effect of salt was due, at least in part, to lower body-weight gain in salt-supplemented animals. Tibia Ca and Mg, but not P, contents were reduced by dietary salt. Salt also reduced tibia Mg concentration but not tibia Ca concentration, although there was a reduction (not significant) in bone Ca concentration in rats fed on the lactose-free diet. There is considerable evidence that salt supplementation (80 g/kg diet) for periods of 10 d–12 weeks reduces bone mass and Ca content in young and adult rats consuming diets either deficient (Goulding, 1980*a, b*; Goulding & Campbell, 1982, 1983; Goulding & Gold, 1986) or adequate (Goulding & Campbell, 1984) in Ca, although some studies have failed to show this (Goulding, 1980*b*; Goulding & Gold, 1988).

It has been suggested that Na-induced hypercalciuria leads to a reduction in serum ionized Ca which, in turn, stimulates the secretion of parathyroid hormone (Goulding *et al.* 1986). While serum ionized Ca was not measured in the present study, there was no significant reduction in plasma Ca in the salt-supplemented animals, suggesting that Ca

homeostasis was maintained despite the Na-induced hypercalciuria. An increase in urinary Ca excretion in the absence of a compensatory increase in Ca absorption, or a reduction in endogenous Ca losses, may result in reduced Ca retention and lead to bone loss.

The literature is conflicting in relation to changes in Ca absorption during salt supplementation in rats. Net Ca absorption was not increased in salt-supplemented (80 g/kg diet) rats fed on diets deficient (Goulding & Campbell, 1983; Goulding & McIntosh, 1986) or adequate (Goulding & Campbell, 1984) in Ca. 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 3–4% of the total Ca absorbed and retained over the 2-week study period. 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 lower tibia Ca content of the animals given lactose-free, salt-supplemented diets that homeostasis was achieved, at least in part, at the expense of bone Ca.

Evidence in humans suggests that healthy individuals respond to Na-induced calciuria by a parathyroid-hormone-mediated increase in intestinal Ca absorption (Meyer *et al.* 1976; Breslau *et al.* 1982). However, this adaptive mechanism does not appear to function in all individuals, e.g. those with impaired parathyroid function (Breslau *et al.* 1982), post-menopausal women with osteoporosis (Breslau *et al.* 1985), as well as in some healthy post-menopausal women (McParland *et al.* 1989), and 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). Furthermore, 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). Nordin & Polley (1987) reported that bone density was negatively correlated with urinary Na excretion in post-menopausal women. However, moderate dietary salt restriction did not reduce the rate of bone loss over a 9-month period compared with unrestricted control subjects.

Although lactose had no independent effect on tibia mass or mineral content, a significant salt  $\times$  lactose interaction occurred for tibia Ca content, indicating that salt supplementation did not affect bone Ca content in animals fed on the lactose-containing diets. The finding that lactose protected against the decrease in tibia Ca content, but not against the decrease in body-weight gain or tibia mass or Mg content, indicates that lactose selectively protects salt-supplemented animals against reduced Ca accretion in bone. This protective effect of lactose on bone Ca is consistent with the findings of Marie & Travers (1983) who reported that dietary lactose increased bone calcification rate and inhibited bone resorption in X-linked hypophosphataemic mice, who are particularly prone to bone resorption.

It seems likely that the protection by lactose of bone Ca against the effect of high salt intake may be due to increased Ca absorption as indicated by increased plasma Ca concentration and increased urinary Ca excretion. While no effect of lactose on net Ca absorption was observed in the present study, the precision of the balance method is not sufficient to detect the relatively small changes in Ca absorption which may have occurred. Increased Ca absorption would normalize plasma Ca which, in turn, would reduce the stimulus for parathyroid hormone secretion and for parathyroid-hormone stimulated bone resorption.

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