

Effect of dietary calcium intake and meal calcium content on calcium absorption in the rat

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Fifty-four male and forty-five female 7-week-old rats, Wistar strain, average weights 190 g and 140 g respectively, were randomized by weight into three groups of eighteen rats each (males) and three groups of fifteen rats each (females) and fed on a semi-purified diet containing (per kg) 2 (low), 5 (normal) or 20 g (high) Ca as CaCO₃ for 2 weeks. Each group was then further randomized into three groups of six rats each (males) and five rats each (females) and given a meal (10 g of the same diet) containing either 2, 5 or 20 g Ca as ⁴⁷CaCO₃. ⁴⁷Ca was determined in quantitative daily collections of faeces over 7 d and fractional absorption of ⁴⁷Ca estimated by extrapolating the linear portion (days 3–7) of the plot of log ⁴⁷Ca retention v. time back to the time of isotope administration. Absorption of meal Ca was higher in males than in females and was affected similarly in males and females by previous dietary Ca intake and meal Ca content. Fractional absorption of meal Ca decreased with increasing previous dietary Ca intake and with increasing meal Ca content, and the combined effect of these two variables caused fractional Ca absorption to vary from 11–89%. Absolute absorption of meal Ca decreased with increasing previous dietary Ca intake and increased with increasing meal Ca content. The influence on Ca absorption of variations in meal Ca content (load effect) was greater than that of variations in previous dietary Ca intake (adaptive effect). These results show that previous dietary Ca intake and meal Ca content are both major determinants of Ca absorption from meals in intact rats fed in the normal way and that the rat responds to these factors in a manner similar to that reported for humans. This study provides further evidence of similarities between rats and humans in dietary Ca absorption.

Calcium absorption: Calcium intake

The influence of dietary and physiological factors on the absorption of dietary Ca is poorly understood (Miller, 1989). While advances in methodology for the study of Ca absorption in humans using radioisotopes (Heaney *et al.* 1988) or stable isotopes (Fairweather-Tait *et al.* 1989) are improving our understanding of Ca bioavailability in foods, the wider application of these methods is limited by factors such as safety (radioisotopes) and cost (stable isotopes). Thus, there continues to be a need for suitable animal models to complement studies in humans, particularly when more detailed or mechanistic investigations are required, or to assist in the design of human studies.

The rat is the most widely used animal model for studies on Ca absorption and there is evidence that Ca absorption is affected similarly in rats and humans by a number of physiological and dietary factors. For example, Ca absorption in humans is increased by pregnancy (Cross *et al.* 1995), lactation (Heaney & Skillman, 1971) and 1,25-dihydroxy cholecalciferol (Hall *et al.* 1969; Kaplan *et al.* 1977) and is also increased by these factors in rats (Dostal & Toverud, 1984; Brommage, 1989; Brommage *et al.* 1990). Ageing reduces Ca absorption in humans (Heaney *et al.* 1989) and in rats (Buchowski & Miller, 1991). Dietary factors which have been reported to reduce Ca absorption in humans include oxalic

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acid (Heaney *et al.* 1988, 1990) and phytate (Heaney *et al.* 1991) and these have also been shown to reduce Ca absorption in rats (Weaver *et al.* 1987; Lönnerdal *et al.* 1989; Cashman & Flynn, 1993). However, there is evidence that Ca absorption is affected differently in rats and humans by some dietary factors. For example, lactose has been reported to increase Ca absorption in rats (Miller *et al.* 1988; Buchowski & Miller, 1991) and in human infants (Ziegler & Fomon, 1983) but not in human adults (Tremaine *et al.* 1986), while dietary phosphate has been reported to inhibit Ca absorption in rats (Mahoney & Hendricks, 1978) but either to have no effect on (Hegsted *et al.* 1981) or to enhance slightly (Zemel & Linkswiler, 1981) Ca absorption in humans. This indicates that the rat may not be a uniformly good model for studies on factors affecting Ca absorption and there is a need to define better the conditions under which the rat is suitable as a model. Previous dietary Ca intake and meal Ca content (Ca load) are important determinants of Ca absorption in humans. Although the influence of these factors on Ca absorption in rats has been clearly demonstrated for Ca solutions using the *in situ* ligated intestinal loop method (Pansu *et al.* 1981), the relative influence of these factors on Ca absorption from food, particularly under normal conditions of eating, is poorly defined.

In the present study the influence of previous dietary Ca intake and meal Ca content on Ca absorption from a meal was determined in rats under normal conditions of eating.

MATERIALS AND METHODS

Preparation of rat diets

The AIN-76 purified diet (American Institute of Nutrition, 1977) was used in the present study. The mineral mix was modified by replacing CaHPO_4 with CaCO_3 as the sole source of Ca and by including KH_2PO_4 and K_2HPO_4 to supply the P requirement (Table 1).

Preparation of ^{47}Ca -labelled meals

Labelled CaCO_3 was prepared by mixing ^{47}Ca (as $^{47}\text{CaCl}_2$ in NaCl (9 g/l), specific activity 7.9 GBq/g; Forskningscenter Riso, 4000 Roskilde, Denmark) with 2 M- CaCl_2 , addition of a slight molar excess of Na_2CO_3 to precipitate CaCO_3 , and washing the precipitate on a filter, followed by drying at 100°. ^{47}Ca -labelled meals containing 2, 5 or 20 g Ca/kg were prepared by substituting ^{47}Ca -labelled CaCO_3 for CaCO_3 in the AIN-76 diets outlined in Table 1.

Calcium absorption study

Rats (7 weeks old; fifty-four male, forty-five female), Wistar strain, average weights 190 g and 140 g for males and females respectively, were randomized by weight into three groups of eighteen rats each (males) and three groups of fifteen rats each (females) and fed *ad libitum* on a purified diet (AIN-76) containing (per kg) 2.0 (low), 5.0 (normal) or 20.0 g (high) Ca as CaCO_3 and 4.0 g P and given distilled water *ad libitum* for 14 d. Feed was provided at 17.00 hours each day. On the fifteenth day each group was then further randomized into three groups of six rats each (males; average weight 265 g) or five rats each (females; average weight 180 g). Rats were placed in individual cages with a grid-floor and a facility for separate collection of faeces and urine. After fasting for 10 h (09.00–19.00 hours), animals were given overnight (19.00–09.00) a meal (10 g), containing (per kg) either 2.0, 5.0 or 20.0 g Ca as ^{47}Ca -labelled CaCO_3 (0.037 MBq ^{47}Ca /10 g meal). On the following morning the radiolabelled meal was removed and any remaining feed was weighed, and after 4 h, the rats were replaced on the diets to which they had been adapted.

Ca absorption was determined by a single-tracer faecal ^{47}Ca recovery method. Quantitative collections of faeces were made daily for 7 d after administration of the

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

Ingredient*	Content (g/kg)
Casein	200.0
DL-Methionine	3.0
Maize starch	150.0
Sucrose	495.0, (487.5), (450.0)†
Fibre	50.0
Maize oil	50.0
AIN mineral mix‡	35.0
AIN vitamin mix§	10.0
Calcium carbonate	5.0, (12.5), (50.0)†
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) 2.0 (low), 5.0 (normal) or 20.0 g (high) Ca.

‡ Contained (g/kg): potassium dihydrogen phosphate 376, dipotassium hydrogen phosphate 160, sodium chloride 74, 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 sulphate 0.55, sucrose 354.

§ 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.

labelled meal. ^{47}Ca in the meal samples and in daily faecal collections was determined in a well γ counter (Compugamma, LKB Wallac, LKB Instruments Ltd, South Croydon, Surrey) using an energy range of 1144–1295 keV. Net Ca absorption was calculated as the difference between ingested and faecal ^{47}Ca . Fractional absorption of ^{47}Ca was estimated by extrapolating the linear portion (days 3–7) of the plot of log net ^{47}Ca absorption *v.* time back to the time of isotope administration.

In order to obtain an estimate of urinary losses of ^{47}Ca , urine collections were made for selected groups of animals for each of the first 3 d after feeding the labelled meal. Cumulative urinary loss over the first 3 d was expressed as a percentage of administered dose and also as a percentage of absorbed dose.

Statistical methods

^{47}Ca absorption data were subjected to both three-way ANOVA, with variation attributed to sex, dietary Ca and meal Ca and two-way ANOVA, with variation attributed to dietary Ca and meal Ca. Urinary ^{47}Ca data were subjected to one-way ANOVA. To follow up the ANOVA, all pairs of means were compared by the method of least significant difference (Snedecor & Cochran, 1967).

RESULTS

The effects of the three factors, i.e. dietary Ca, meal Ca and sex of the rat, on fractional Ca absorption in 9-week-old rats were evaluated by three-way ANOVA. This showed that there was no three-way interaction between dietary Ca, meal Ca and sex. Neither were there any two-way interactions between these factors. However, each factor had a significant effect on fractional Ca absorption. Fractional Ca absorption in males was significantly higher than that in females. Because of this, the effects of dietary Ca intake and meal Ca

Table 2. *Effect of dietary calcium intake and meal calcium content on calcium absorption in 9-week-old rats**

(Mean values with the pooled standard error of the mean)

Dietary Ca (g/kg)	Meal Ca (g/kg)	Ca absorption					
		Male			Female		
		<i>n</i>	(%)	(mg)	<i>n</i>	(%)	(mg)
2.0	2.0	6	89.0	17.8	5	83.7	16.7
	5.0	6	76.0	38.0	5	53.7	26.9
	20.0	6	39.7	79.5	5	37.1	74.2
5.0	2.0	6	69.8	14.0	5	73.7	14.7
	5.0	6	48.5	24.2	5	41.4	20.7
	20.0	6	24.8	49.7	5	17.9	35.8
20.0	2.0	6	65.9	13.2	5	55.2	11.0
	5.0	6	43.6	21.8	5	35.6	17.8
	20.0	6	18.4	36.9	5	17.5	35.0
Pooled SEM			4.3	3.8		3.8	5.2
Significant (<i>P</i>) of variance ratio, effect of:							
Dietary Ca			< 0.001	< 0.001		< 0.001	< 0.001
Meal Ca			< 0.001	< 0.001		< 0.001	< 0.001
Dietary Ca × meal Ca			NS	< 0.001		NS	< 0.010
Least significant difference (<i>P</i> < 0.05):			12.2	11.0		10.8	15.0

* For details of diets and procedures, see Table 1 and pp. 464–465.

content on Ca absorption were assessed separately for males and females using two-way ANOVA.

Two-way ANOVA showed that fractional absorption of meal Ca was significantly affected by previous dietary Ca intake and meal Ca content in both male and female rats (Table 2). Fractional absorption of meal Ca decreased with increasing previous dietary Ca intake and with increasing meal Ca content in both males and females. Fractional Ca absorption in males varied from 89% on the low-Ca diet–low-Ca meal to 18% on the high-Ca diet–high-Ca meal (Table 2) while fractional Ca absorption in females varied from 84% on the low-Ca diet–low-Ca meal to 18% on the high-Ca diet–high-Ca meal (Table 2).

Absolute absorption of meal Ca was significantly affected by previous dietary Ca intake and meal Ca content in both male and female rats (Table 2). Absolute absorption of meal Ca decreased with increasing previous dietary Ca intake but increased with increasing meal Ca content in both males and females. Absolute Ca absorption from a 10 g meal in males varied from 80 mg on the low-Ca diet–high-Ca meal to 13 mg on the high-Ca diet–low-Ca meal (Table 2). Absolute Ca absorption from a 10 g meal in females varied from 74 mg on the low-Ca diet–high-Ca meal to 11 mg on the high-Ca diet–high-Ca meal (Table 2).

The significant two-way interaction between dietary Ca and meal Ca for absolute Ca absorption (Table 2) shows that the effect of meal Ca content on absolute Ca absorption varies inversely with previous dietary Ca intake, and that the influence of previous dietary Ca intake on Ca absorption from a meal increases with increasing Ca content of the meal.

The effect of dietary Ca intake and meal Ca content on cumulative urinary ⁴⁷Ca loss in selected groups of male rats can be seen in Table 3. Over the first 3 d, only 2–4% of the administered ⁴⁷Ca dose appeared in the urine. Urinary ⁴⁷Ca excretion, expressed as percentage of the administered dose, was not significantly affected by previous dietary Ca

Table 3. *Effect of dietary calcium intake and meal calcium content on cumulative urinary loss of ⁴⁷Ca in male rats**

(Mean values with their standard errors)

Dietary Ca (g/kg)	Meal Ca (g/kg)	n	Cumulative urinary ⁴⁷ Ca losses							
			Administered dose (%)						Absorbed dose (%)	
			Day 1		Day 2		Day 3		Day 3	
			Mean	SE	Mean	SE	Mean	SE	Mean	SE
2.0	2.0	6	0.6	0.4	1.8	0.4	2.5	0.4	2.8	0.4
5.0	2.0	6	0.7	0.4	2.1	0.3	2.3	0.3	3.3	0.4
	5.0	6	0.7	0.2	2.3	0.5	3.9	0.6	8.0	1.2
20.0	20.0	6	1.3	0.7	3.8	0.5	4.3	0.4	17.3	1.6
	20.0	6	1.3	0.8	2.3	0.5	4.3	0.4	23.4	2.2
ANOVA (one-way), <i>P</i> value			NS		NS		NS		< 0.001	
Least significant difference (<i>P</i> < 0.05)			1.6		1.6		2.7		7.3	

* For details of diets and procedures, see Table 1 and pp. 464–465.

intake or meal Ca content. When urinary ⁴⁷Ca losses were expressed as a percentage of absorbed ⁴⁷Ca dose increasing meal Ca content significantly increased urinary ⁴⁷Ca loss while increasing previous dietary Ca content had no effect.

Endogenous loss of ⁴⁷Ca in faeces, as determined from the slope of the plot of log ⁴⁷Ca retention *v.* time, from day 3 to day 7, was negligible, regardless of previous dietary Ca intake or meal Ca content.

DISCUSSION

In the present study fractional absorption of meal Ca was significantly higher in male rats than in females. This could be due to the higher growth rates of the male rats which would lead to a greater demand for Ca for skeletal mineralization, resulting in a greater efficiency of Ca absorption.

Separate statistical analysis of data for males and females using two-way ANOVA showed that both previous dietary Ca intake and meal Ca content affected fractional Ca absorption from a meal similarly in males and females. Fractional absorption of meal Ca decreased with increasing previous dietary Ca intake and with increasing meal Ca content. The combined effects of these two variables caused fractional absorption of meal Ca to vary in the range of 11–89%, indicating that these variables are major determinants of Ca absorption.

Ca balance studies in rats (Henry & Kon, 1953; Benson *et al.* 1969) fed on solid feeds have previously shown that fractional Ca absorption decreases with increasing dietary Ca intake. However, a limitation of these studies was that the effect of meal Ca content on Ca absorption could not be separated from the effect of previous dietary Ca intake since isotopic tracers were not used. The results of the present study are also in agreement with the findings of Weeks & King (1985) who reported that fractional Ca absorption from cows' milk labelled with ⁴⁷Ca in rats adapted to a low-Ca diet (2 g/kg) was higher than in rats adapted to a normal-Ca diet (5 g/kg). However, these authors found no difference in fractional Ca absorption from 5 ml milk compared with a meal of 10 ml milk.

The results of the present study show that both previous dietary Ca intake and meal Ca content have a major influence on Ca absorption in intact rats fed on solid feed in the normal way. This is similar to the findings obtained by Pansu *et al.* (1981) in studies on Ca absorption from Ca solutions in rats using *in situ* perfusion of intestinal loops.

The findings of the present study are also in agreement with human studies which found that fractional Ca absorption decreased with increasing previous dietary Ca intake (Ireland & Fordtran, 1973; Heaney *et al.* 1975, 1989; Heaney, 1991) and with increasing meal Ca content (Ireland & Fordtran, 1973; Heaney, 1991). The change in fractional Ca absorption with meal Ca content (Ca load) is due to a 'physico-chemical' effect, resulting in the absorption of a larger fraction of a small load than of a large, while the change in Ca absorption with previous Ca intake represents a calcitriol-mediated adaptation of active transport (Heaney, 1991). Absolute Ca absorption from the meal in both male and female rats decreased with increasing previous dietary Ca, while it increased with increasing meal Ca content. This has also been demonstrated in rats using *in situ* perfusion of intestinal loops with Ca solutions (Pansu *et al.* 1981) and is similar to the findings of Ireland & Fordtran (1973) and Heaney (1991) in human studies.

The effects of the two processes of Ca absorption are illustrated in the present study when a 10-fold change in dietary Ca intake occurs. When rats adapted to a low-Ca diet are changed on to a high-Ca diet, there is an immediate increase in Ca absorption as a result of the meal effect (reflecting increased absorption via both the passive paracellular and the active transcellular pathways), which is then modulated over time by a decrease in absorption due to the adaptive effect (reflecting a reduction in the capacity of the active transcellular pathway). When rats adapted to the high-Ca diet change to a low-Ca diet, there is an immediate reduction in Ca absorption as a result of the meal effect (probably mainly due to a decrease in Ca absorption via the passive paracellular pathway), which is then modulated over time by an increase in absorption due to the adaptive effect (reflecting increased capacity of the active transcellular pathway).

The results of this present study show that the influence of variations in meal Ca content on Ca absorption (load effect) was greater than that of variations in previous dietary Ca intake (adaptive effect), in agreement with findings in humans (Heaney, 1991). In the case of male rats, a 10-fold difference in meal Ca content (2–20 g/kg) resulted in increments of 61.7, 35.7 and 23.7 mg respectively in Ca absorption for animals habituated to low-, normal- and high-Ca diets, while a 10-fold difference in dietary Ca content (2–20 g/kg) resulted in increments of 42.6, 16.2 and 4.6 mg respectively in Ca absorption for the high-, normal- and low-Ca meals. Similar observations were made for females.

Overall, urinary ^{47}Ca loss was small, with only 2–4% of the administered dose lost in urine in the first 3 d after isotope administration. These findings are similar to those of McCredie *et al.* (1984), who reported that only 1.7% of the ^{47}Ca administered to rats as an oral dose of CaCl_2 appeared in the urine within 2 d. When urinary ^{47}Ca loss was expressed as a percentage of the absorbed dose, it was found that increasing meal Ca increased the urinary ^{47}Ca losses significantly. This finding shows that at lower Ca loads ^{47}Ca retention approximates fractional absorption while at higher loads ^{47}Ca retention may be significantly lower than fractional absorption.

The results also suggest that Ca (as CaCO_3) is potentially totally available to rats from a purified diet, subject to Ca need and Ca content of the diet. This confirms the findings of human studies (Sheikh *et al.* 1987; Recker *et al.* 1988) in which CaCO_3 was reported to be a good source of bioavailable Ca, as good as milk and some milk products. The overall findings of the present study show that this rat model responds to two of the key determinants of Ca absorption in a manner similar to that reported for humans and provides further evidence of similarities between rats and humans in dietary Ca absorption.

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