The effect of dietary calcium intake in weanling rats on the efficiency of calcium absorption

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Rats were weaned onto high (HCa, 14·6 g/kg)- or low (LCa, 3·88 g/kg)-Ca diets for 12 d and the efficiency of absorption of Ca measured from 20 mg Ca (as $CaCl_2$, labelled with ^{47}Ca) by means of whole-body counting. The LCa group absorbed 74 % of the test dose and the HCa group 60 % of the test dose ($P < 0.001$). All animals were then given the LCa diet for 6 weeks and the absorption test repeated. This time there was no difference in efficiency of absorption (HCa 54 %, LCa 57 %). The femur dry weight was significantly lower in the group initially fed on the LCa diet, but the Ca concentration was similar to that of the HCa group. The results do not lend support to the suggestion that early dietary exposure to Ca manifests itself as a difference in Ca metabolism in later life. They do, however, highlight the importance of adequate Ca intake during critical periods of growth.

Calcium: Absorption efficiency

There is considerable interest at present in the involvement of Ca in the bone disorder osteoporosis. Its aetiology is complex and incompletely understood, and nutrition is only one of the risk factors (Fehily, 1989). However, Ca intake is generally recognized as playing a significant role in the development of osteoporosis, notably in the attainment of peak bone mass, whereby lower intakes during periods of bone growth and consolidation are believed to increase the risk of osteoporosis in later life (Matkovic *et al.* 1990).

Milk products are the major source of dietary Ca, and in the UK they account for approximately half the daily intake of Ca (Gregory *et al.* 1990). Yet there is no firm evidence that non-dairying populations, with a lower Ca intake, have a greater incidence of osteoporosis (World Health Organization, 1990). Various explanations have been offered including differences in the non-nutritional risk factors such as smoking habits. In addition, developing countries generally have lower intakes of NaCl and animal protein, both of which have been shown to enhance Ca loss (Marsh *et al.* 1988; Goulding, 1990). One other difference worthy of consideration is the effect of level of exposure to dietary Ca very early in life; non-dairying countries wean infants onto a relatively-low-Ca diet compared with Western societies where milk makes a major contribution to the diet of infants and young children for many years. This begs the question as to whether or not there is an adaptive response to early dietary exposure to Ca, manifesting itself as a difference in Ca metabolism in later life.

The efficiency of Ca absorption can be modified in three ways: (1) by increasing or decreasing the amount of Ca available for absorption, (2) by altering active Ca transport, (3) by changing passive Ca transport (Bronner, 1988). Changes in Ca intake have two

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effects which tend to work in opposite directions. Active transport is high under conditions of low Ca and is down-regulated when intake increases, whereas transport varies directly with intake. However, given adequate vitamin D status, the main mechanism whereby individuals maintain Ca homeostasis is by altering their absorptive efficiency. The hypothesis being tested in the present study is that exposure to high levels of Ca at weaning influences the subsequent development of the Ca transport mechanism, resulting in a reduced ability to adapt to low-Ca diets in adult life by means of an increased efficiency of absorption.

MATERIALS AND METHODS
Animals

Thirty virgin female Wistar rats (250 g) were caged in pairs in polypropylene and stainless-steel solid-bottomed cages. In every alternate cage a castrated male rat was placed in order to synchronize oestrus (via male pheromones). Vaginal smears were taken from the females after 1 week to identify the stage of oestrus cycle and confirm synchronization, the castrated males removed and fifteen adult males (one per cage) placed with the females. For the next 3 d, vaginal smears were examined each morning for the presence of sperm, indicating that mating had occurred. The males were removed and the females weighed. After 1 week the females were re-weighed and the non-pregnant rats were killed. The pregnant females were provided with nesting material, and at birth all litters were brought to six pups by culling and fostering, keeping as many male rats as possible.

Diets

The dams were provided with a pellet diet until the pups were 2 weeks old, at which stage they were capable of nibbling the mother's diet. In order to prevent rats from consuming a diet high in Ca, the pellets were replaced at this time by a semi-synthetic low-Ca diet (LCa) which provided 75 % of the recommended requirement for the rat (American Institute of Nutrition, 1977). A high-Ca diet (HCa) was also prepared which provided 300 % of the recommended requirement (Table 1). All diets were made up in 5 kg batches and samples from each batch taken for subsequent analysis for Ca.

Calcium absorption

At 3 weeks of age the pups were removed from their mothers and weaned. All thirty-three male pups were kept plus five females, and allocated to two groups of nineteen (one group with two females and one with three). One group was given the LCa and one the HCa diet *ad lib*. They were housed in groups of three and four in solid-bottomed cages, ear-tagged and weighed daily for 7 d to check that both groups were growing at a similar rate, and then housed singly in wire-bottomed cages. After 5 d, following an overnight fast, each rat was orally dosed with 1 ml of a solution containing 37 kBq^{47}Ca as $CaCl_2$ (Amersham International) and 20 mg Ca, also as $CaCl_2$. The animals were weighed and the radioactivity counted in a small-animal whole-body gamma counter (Fairweather-Tait & Wright, 1985). All the rats were given *ad lib*. access to the LCa diet 3 h post dosing and for the remainder of the study.

After 7 d the rats were reweighed and the radioactivity remeasured. The first count gave the initial intake of ^{47}Ca, the second the amount of radioactivity remaining in the animals once all the unabsorbed ^{47}Ca had been excreted. The difference between the two measurements, taking into account the natural decay and variations in background, gives the apparent absorption of Ca. No correction was made for endogenous excretion of absorbed ^{47}Ca. However, previous experiments show that this loss is very small in rats under the given experimental conditions (S. J. Fairweather-Tait, unpublished results), and,

Table 1. *Composition of low (LCa)- and high (HCa)-calcium diets (g/kg)*

	LCa	HCa
Casein	201	196
Starch	312	303
Sucrose	312	303
Cellulose	40	39
Mineral mix*	32	59
Vitamin mix†	20	20
Maize oil	80	78
Methionine	3	3

* Contained (g/kg diet): LCa: $CaHPO_4$ 13, Na_2HPO_4 7·4, KCl 7·03, $MgSO_4$ 4, trace premix 0·448; HCa: $CaHPO_4$ 13, $NaHPO_4$ 7·4, $CaCO_3$ 28·5, KCl 7·03, $MgSO_4$ 4, trace premix 0·448. Trace premix contained (g/kg diet): $ZnCO_3$ 0·1, $FeSO_4 . 7H_2O$ 0·144, $CuSO_4 . 5H_2O$ 0·023, KIO_3 0·001, $MnSO_4 . H_2O$ 0·18.

† Contained (mg/kg diet): nicotinic acid 60, cyanocobalamin (vitamin B_{12}) in mannitol (Glaxo) 50, calcium-D-pantothenate 40, thiamin hydrochloride 10, riboflavin 10, pyridoxine 10, pteroylmonoglutamic acid 10, D-biotin 1, vitamin K_1 2, Rovimix E-50 (containing 75 mg as DL-α-tocopheryl acetate; Roche) 150, Rovimix A-500 (containing 3·75 mg as retinol; Roche) 25, Rovimix D_3-500 (containing 188 μg as cholecalciferol; Roche) 15, choline bitartrate 1800, starch (bulking agent) 17817.

therefore, apparent absorption from the ^{47}Ca dose is a good approximation of true absorption.

At 6 weeks following the first dose of ^{47}Ca the experiment was repeated with an additional background count before the second dose. After 1 week the radioactivity in the rats was remeasured, the rats were killed and the right femur removed for Ca analysis.

Atomic absorption spectroscopy (AAS)

Femurs were dried overnight in a fan oven at 80° and the dry weight recorded. The bones were heated in silica crucibles at 480° for 48 h in a muffle oven, the ash dissolved in 1–2 ml concentrated HCl (AR) and made up to an appropriate volume with freshly prepared glass-distilled water (to provide an expected value 1–6 μg Ca/ml). Each batch of diet was subsampled in duplicate and 2 g portions charred in a silica crucible on a hot plate, heated at 480° for 48 h, dissolved in HCl and made up to volume with distilled water. The solutions were analysed for Ca by flame AAS (PU9000; Pye Unicam, Cambridge), adding a solution of $LaCl_3$ (100 g/l; 10 ml/l sample) to reduce interference. Certified reference materials were analysed to check the accuracy of the method (National Bureau of Standards, Gaithersburg, USA).

Statistical analysis

Differences between the two groups were evaluated by using two-tailed Student's *t* tests for the independent means.

RESULTS

There was no difference in mean body weight between the two groups at any stage of the study. The mean body weight at weaning was 60 g, and weights at the time of the first and the second oral dose were 170 and 500 g respectively (Table 2). Consumption of diets of different Ca concentration had no effect on growth. The mean Ca content of the LCa and HCa was 3·88 (SD 0·21) and 14·6 (SD 0·4) g Ca/kg diet respectively.

Values for mean Ca absorption from the two test doses are given in Table 2. At 33 d of age, after 12 d of consuming diets of different Ca concentration, there was a highly significant difference in the efficiency of Ca absorption from a 20 mg dose; apparent

Table 2. *Mean calcium absorption (%) from 20 mg calcium in rats weaned onto low (LCa)- and high (HCa)-calcium diets for a period of 12 d at 12 d and 6 weeks post-weaning**

(Mean values with their standard errors)

Initial diet...	Ca absorption (% of dose)				Statistical significance of difference: P
	LCa		HCa		
	Mean	SEM	Mean	SEM	
First test (12 d post-weaning)	74·1	1·4	60·2	2·0	< 0·001
Second test (6 weeks post-weaning)	56·8	2·4	53·5	1·7	NS

NS, not significant.
* For details of diets and procedures, see Table 1 and pp. 528–529.

Table 3. *Bone calcium content in 10-week-old rats weaned on to low (LCa)- or high (HCa)-calcium diet for 12 d**

(Mean values with their standard errors)

Initial diet...	LCa		HCa		Statistical significance of difference: P
	Mean	SEM	Mean	SEM	
Dry wt femur (g)	0·729	0·021	0·793	0·019	< 0·05
Total Ca (mg)	16·09	0·50	16·87	0·40	< 0·1
Ca concentration (mg/g)	22·09	0·31	21·33	0·22	< 0·1

* For details of diets and procedures, see Table 1 and pp. 528–529.

absorption was 74·1 % in the LCa group and 60·2 % in the HCa group ($P < 0·001$). The animals were all given the LCa diet for 6 weeks and when tested again there was no difference between the two groups. However, the efficiency of absorption was reduced; this was more pronounced in the LCa ($P < 0·001$) than in the HCa group ($P < 0·05$) (paired t test).

Femur weight and Ca content are shown in Table 3. Rats weaned onto the HCa diet for 12 d had a significantly higher dry bone weight at 10 weeks of age than the LCa group ($P < 0·05$). The LCa group had a lower total Ca and higher Ca concentration in the femur, but these values did not reach statistical significance ($P < 0·1$).

DISCUSSION

There is a large disparity in Ca intake throughout the world, but this has little apparent effect on health. The possible reasons are discussed by Prentice (1991) who concludes that differences in physiological requirements are unlikely, leaving adaptation as the main contender. Published reports suggest that Ca balance is a function of previous diet history, but such studies do not provide detailed information about the mechanisms involved, that is, whether changes have occurred in the efficiency of absorption or endogenous losses, or both.

Rats are born without an active Ca transport system (Pansu et al. 1983) and without

functional Ca-binding protein (CaBP) in their intestine. At about 5 d of age CaBP becomes detectable (Ueng *et al.* 1979), increases until the animals are about 30 d old, and, thereafter, declines gradually. Active Ca transport parallels these changes. In humans, however, little is known about the potential for CaBP induction in the intestine and whether high habitual Ca intakes may blunt any CaBP response to changes in intake. The developmental time-course of the non-saturable route is quite different. Initially virtually all the Ca is absorbed by this pathway, but when rats are 30 d old only about 17 % of the luminal Ca is absorbed by passive transport and this remains constant through adult life (Pansu *et al.* 1983). The absorption tests in the present study were performed when the rats were 33 d old, that is when the passive absorption pathway had stabilized and the active pathway was gradually declining; this is illustrated by the results of the group fed on the LCa diet throughout the study where the efficiency of absorption fell from 74·1 to 56·8 %. The age-dependent decline in active Ca absorption in rats has also been described by Armbrecht *et al.* (1980).

The rats fed on the LCa diet for the first week of weaning had a lower dry bone weight which implies that less substrate was available for the bony matrix to be laid down at this time. However, the concentration of Ca in the femur at 10 weeks of age was not significantly different from that of rats fed on the HCa diet (if anything, it was higher), indicating that bone density was not reduced by feeding the LCa diet, merely bone size; body weight was unaffected. In contrast, more severe Ca deprivation studies in rats show significant growth retardation (El-Maraghi *et al.* 1965) which is related to a lower voluntary food intake, thereby exerting a 'sparing' action on the bones.

Animals fed on the LCa diet for the first 12 d of weaning had lighter bones in adulthood than those fed on the HCa diet for 12 d, implying that they were unable to compensate during the ensuing 6 weeks. Assuming an efficiency of absorption of 74·1 % in the LCa (3·88 g Ca/kg diet) group and 60·2 % in the HCa (14·6 g Ca/kg diet) group, and estimated 12 d food intakes of 154 g, calculated absorption of Ca would be 0·44 and 1·35 mg respectively. In practice the efficiency of absorption would probably be less from food than from the test dose of $CaCl_2$. However, it is clear from this calculation that the leeway to increase the efficiency of absorption in the LCa group was not great enough to compensate for the lower dietary concentration; the HCa group were absorbing Ca very efficiently, presumably because of their high requirements during the weaning period of rapid growth. The results of the present study also underline the importance of an adequate dietary Ca supply during periods of rapid growth because, in the rat at least, there appears to be no compensatory mechanism to make up for the short-term deficit at weaning. In other words, weaning appears to be a critical time when an inadequate supply of Ca results in less bone being laid down (Widdowson, 1970).

The results of the present study do not lend support to the hypothesis that the level of Ca in the diet at weaning affects the development of the absorptive pathways. Animals weaned on to a high- or low-Ca diet did not appear to have a different efficiency of absorption in later life, but the level of Ca in the diet at weaning had a significant influence on bone weight at maturity.

REFERENCES

American Institute of Nutrition (1977). Report of the American Institute of Nutrition Ad Hoc Committee on Standards of Nutritional Studies. *Journal of Nutrition* **107**, 1340–1348.

Ambrecht, H. J., Zenser, T. V., Gross, C. J. & Davis, B. B. (1980). Adaptation to dietary calcium and phosphorus restriction changes with age in the rat. *American Journal of Physiology* **239**, E322–E327.

Bronner, F. (1988). Gastrointestinal absorption of calcium. In *Calcium in Human Biology*, pp. 91–123 [B. E. C. Nordin, editor]. London: Springer-Verlag.

El-Maraghi, N. R. H., Platt, B. S. & Stewart, R. J. C. (1965). The effect of the interaction of dietary protein and calcium on the growth and maintenance of the bones of young, adult and aged rats. *British Journal of Nutrition* **19**, 491–507.

Fairweather-Tait, S. J. & Wright, A. J. A. (1985). The effect of the 'fibre-filler' (F-plan diet) on iron, zinc and calcium absorption in rats. *British Journal of Nutrition* **54**, 585–592.

Fehily, A. M. (1989). Dietary determinants of bone mass and fracture risk: a review. *Journal of Human Nutrition and Dietetics* **2**, 299–313.

Goulding, A. (1990). Osteoporosis: why consuming less sodium chloride helps to conserve bone. *New Zealand Medical Journal* **103**, 120–122.

Gregory, J., Foster, K., Tyler, H. & Wise, A. M. (1990). *The Dietary and Nutritional Survey of British Adults*. London: H.M. Stationery Office.

Marsh, A. G., Sanchez, T. V., Michelson, O., Chaffee, F. L. & Fagal, S. M. (1988). Vegetarian lifestyle and bone mineral density. *American Journal of Clinical Nutrition* **48**, 837–841.

Matkovic, V., Fonatan, D., Tominac, C., Goel, P. & Chesnut, C. H. III (1990). Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. *American Journal of Clinical Nutrition* **52**, 878–888.

Pansu, D., Bellaton, C. & Bronner, F. (1983). Developmental changes in the mechanisms of duodenal calcium transport in the rat. *American Journal of Physiology* **240**, G20–26.

Prentice, A. (1991). Calcium: the functional significance of trends in consumption. In *Modern Lifestyles, Lower Energy Intake and Micronutrient Studies*, pp. 147–149 [K. Pietrzik, editor]. London: Springer-Verlag.

Ueng, T.-H., Golub, E. E. & Bronner, F. (1979). The effect of age and 1,25-dihydroxyvitamin D_3 treatment on the intestinal calcium-binding of suckling rats. *Archives in Biochemistry and Biophysics* **196**, 624–630.

Widdowson, E. M. (1970). Harmony of growth, *Lancet* **i**, 901–905.

World Health Organization (1990). *Diet, Nutrition, and the Prevention of Chronic Diseases. Technical Report Series* no. 797, pp. 80–83. Geneva: WHO.

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