Eur J Nutr 39: 12–17 (2000)

ORIGINAL CONTRIBUTION

S. Kennefick
K. D. Cashman

Inhibitory effect of wheat fibre extract on calcium absorption in Caco–2 cells: evidence for a role of associated phytate rather than fibre *per se*

Summary *Background:* In spite of the strong evidence for the beneficial heath effects of dietary fibres, one of the potential nutritional disadvantages of high fibre diets is the adverse effect on the bioavailability of micronutrients, especially minerals and trace elements. With regard to Ca, there is considerable evidence that phytate, which is associated with fibre in many foods, such as cereals and soya products, inhibits Ca absorption. However, there is some doubt as to whether fibres *per se* have an influence on Ca absorption.

Aim of the study: Therefore, the purpose of this study was to investigate the effect on Ca absorption of two cereal-based fibre extracts (wheat bran and barley hull). In addition, in order to distinguish between the effect of the fibre components *per se* and the associated phytate content in these fibre extracts, we investigated the effect of dephytinised wheat and barley fibre extracts and the effect of phytate, as sodium phytate at levels present in the fibre extracts, on Ca absorption. Ca absorption was assessed in Caco–2 cells, as a model for studying Ca absorption in humans.

Methods: The effect of wheat and barley fibre extracts, dephytinised wheat and barley fibre extracts, cellulose, and of sodium phytate on transepithelial ^{45}Ca transport and ^{45}Ca uptake was studied in differentiated Caco–2 cells grown on permeable filter supports. Wheat and barley fibre extracts were dephytinised with wheat phytase.

Results: Wheat fibre extract had a 3.2-fold higher phytate content (48.0 mmol/kg) than barley fibre extract (15.1 mmol/kg). Enzymatic dephytinisation of both fibre extracts reduced the phytate content to undetectable levels. The rate of transepithelial ^{45}Ca transport across Caco–2 cell monolayers and the uptake of ^{45}Ca into Caco–2 cells were unaffected by cellulose or barley fibre extract. On the other hand, inclusion of wheat fibre extract in the Ca transport buffer (50 g fibre/l) significantly reduced the rate of ^{45}Ca transport (by 17 and 19 % respectively) and the uptake of ^{45}Ca (by 24 and 25 % respectively) relative to a fibre-free buffer and a control fibre (cellulose) transport buffer. Increasing the phytate concentration of the transport buffer from 0 to 2 mM (a level close to that in the wheat fibre containing buffer) significantly reduced the rate of ^{45}Ca transport (by 16 %) and ^{45}Ca uptake (by 26 %). Dephytinisation of the wheat fibre extract removed its inhibitory effects on ^{45}Ca transport and e.g. ^{45}Ca uptake.

Conclusion: The results from the present study in Caco–2 cells suggest that it is the phytate in wheat fibre extract which is the major inhibitory factor of Ca absorption and that wheat fibre *per se* has little if any inhibitory effect on Ca absorption. In addition, the results of this study support the usefulness of Caco–2 cells for investigating the effects of dietary factors on the cellular uptake and transepithelial intestinal transport of Ca.

Key words Fibre – Phytate – Calcium absorption – Caco–2s

Received: 22 March 1999
Accepted: 2 February 2000

S. Kennefick · Dr. K. Cashman
Department of Nutrition,
University College,
Cork, Ireland
E-mail: K.Cashman@ucc.ie

Introduction

The daily consumption of dietary fibre in Europe is approximately 12–25 g [1], and strong and continued recommendations have been made to double or even triple the current intake among most populations. The increased awareness of the potential health benefits of dietary fibre among consumers has encouraged food manufacturers to develop fibre-enriched food products which contain plant fibre extracts as ingredients.

However, in spite of the strong evidence for the beneficial effects of dietary fibres, one of the potential nutritional disadvantages of high fibre diets is the adverse effect on the bioavailability of micronutrients, especially minerals and trace elements. With regard to Ca, there is considerable evidence that phytate, which is associated with fibre in many foods, such as cereals and soya products, inhibits Ca absorption in humans [2–4] and rats [5–7]. However, there is some doubt as to whether fibres *per se* have an influence on Ca absorption. For example, while some studies show a negative effect of fibre (predominantly cereal fibres) on Ca absorption [8,9,10,11,12], other studies have reported no effect [13,14,15,16]. It is apparent that the question of whether fibre adversely affects Ca absorption is far from clear and more research is warranted.

Therefore, the purpose of this study was to investigate the effect on Ca absorption of two cereal-based fibre extracts (wheat bran and barley hull). In addition, in order to distinguish between the effect of the fibre components *per se* and the associated phytate content in these fibre extracts, we investigated the effect of dephytinised wheat and barley fibre extracts and the effect of phytate, as sodium phytate at levels present in the fibre extracts, on Ca absorption.

Ideally, this research would be in the form of human studies. However, these studies are difficult, expensive, and provide limited data with each experiment [17]. While experimental animal studies are less expensive, they are somewhat limited by uncertainties with regard to differences in metabolism between animals and humans. In particular, the choice of the rat as a model may be questioned given the evidence for a much higher phytase activity (about 30-fold) in the rat small intestine compared to human [18].

The potential for using the human colon carcinoma cell line, Caco–2, as a model for studying Ca absorption in humans has been highlighted in several recent reports [19–21]. Guilliano and Wood used Caco–2 cells grown on permeable filter supports to study the mechanism of transepithelial intestinal Ca transport [19]. Their kinetic analyses support the existence, as shown for Ca transport in the intestine, of two transport processes: one which is saturable and transcellular, presumably carrier mediated and regulated by vitamin D, while the other is a non-saturable process which may reflect a diffusional or paracellular pathway [19]. Blias et al. simultaneously evaluated the contribution of these two processes to the total transepithelial transport of Ca across a Caco–2 cell monolayer and found that Ca predominantly uses the paracellular pathway [21]. This is similar to that observed *in vivo* where paracellular transport is considered to be the dominant route of Ca absorption under conditions of moderate to high Ca intakes [22]. Therefore, this relatively simple *in vitro* method appears to be a good model for predicting Ca bioavailability in humans.

Materials and Methods

Materials

Dietary fibre preparations produced from barley and wheat were obtained from Sofalia, Paris, France. Cellulose, in the form of Avicel microcrystalline cellulose, was obtained from FMC International Food and Pharmaceutical Products Division, Little Island, Cork, Ireland. Tissue culture materials, including Dulbecco's Modified Eagle's Medium (DMEM) (with L-glutamine and sodium bicarbonate), fetal bovine serum (FBS) and MEM non-essential amino acids were purchased from Gibco BRL Life Technologies, Paisley, Strathclyde, UK. Sodium phytate and phytase isolated from wheat (0.04 units/mg solid) were obtained from Sigma Chemical Co Ltd., Poole, Dorset, UK. ^{45}Ca (as ^{45}Ca in an aqueous solution of $CaCl_2$, specific activity 1.85 GBq/mg Ca) was purchased from Amersham International plc., Amersham, Bucks., UK.

Dephytinisation of fibres

Wheat and barley fibre slurries (5 g fibre extract/100 ml distilled water) were prepared and after pH adjustment to 5.1 (pH optimum of wheat phytase) with HCl, wheat phytase was added at a level of 250 mg (10 units)/100 ml slurry and the samples were incubated for 22 h at 55 °C in a shaking water bath. A control cellulose slurry was also prepared in the same manner. Samples were then freeze dried and the phytate and Ca contents determined.

Conditions of cell culture

The human colon adenocarcinoma, Caco–2, cell line was purchased from the European Collection of Animal Cell Cultures (Salisbury, Wilts., UK) and studied between the 25th and 50th passages. Cells were routinely grown in 75 cm^2 plastic flasks (Corning Glassworks, Corning, N. Y., USA) in DMEM supplemented with 1 % (v/v) non-essential amino acids and 10 % (v/v) FBS. Caco–2 cells were maintained at 37 °C in a 5 % CO_2–95 % air atmosphere. Cells were seeded at a density of 27 x $10^5/cm^2$ and were routinely passaged at a ratio of 1:3 when confluence was reached. Cells used in transepithelial Ca transport experi-

ments were seeded at a density of 2.5 x 10^5/cm^2 onto permeable Transwell filter inserts (24 mm in diameter, 3.0 μm pore size, Costar, Cambridge, Mass., USA). Cell culture media was changed on alternate days between 3 – 11 days and daily thereafter.

Confirmation of monolayer integrity

An epithelial volt-ohm meter with dual electrodes (World Precision Instruments, New Haven, Conn., USA) was used to measure transepithelial electrical resistance (TEER) of monolayers grown on membrane filters as described previously [23]. Filters were only used if the TEER of the monolayer exceeded 200 Ω/cm^2.

Ca transport studies

The technique for determining Ca transport in the present study is a modification of that of Fleet & Wood [24]. Transepithelial transport of Ca was studied with Caco–2 cells grown on permeable filter supports for 18 d by which time the cells had fully differentiated. On the day of an experiment, cell monolayers were rinsed three times with wash buffer (containing in mmol; 140 NaCl, 5 KCl, 0.5 $MgSO_4$, 0.5 $CaCl_2$, 4 glutamine, 25 glucose, and 20 HEPES, pH 7.4) at 37 °C and then the permeable filter inserts were transferred to fresh 6-well plates containing 2.5 ml serosal buffer (containing in mmol; 140 NaCl, 5.8 KCl, 0.8 $MgSO_4$, 0.44 KH_2PO_4, 0.34 Na_2HPO_4, 4 glutamine, 25 glucose, and 20 HEPES, pH 7.4). At time zero, 1.5 ml transport (Tx) buffer (wash buffer containing 1 μCi e.g. ^{45}Ca/ml and 500 μmol Ca/l as $CaCl_2$ with or without the test compound) were added to the upper wells. Following the addition of Tx buffers to the filters, the 6-well plates were covered and incubated at 37 °C in a shaking water bath for 60 min. At 30 and 60 min after addition of ^{45}Ca-labelled Tx buffers, duplicate aliquots were taken from the lower well. An equal volume of fresh buffer was returned to the lower well following the 30-min sampling. The ^{45}Ca content of fresh Tx buffers and of 30- and 60-min serosal buffer samples was determined by liquid scintillation counting using a Beckman LS 6500 multipurpose liquid scintillation counter (Beckman Instruments Inc., Fullerton, CA, USA). The rate of ^{45}Ca transport (representing the rate of total transepithelial Ca transport) was expressed as nmol Ca transported to serosal buffer per minute per well (nmol/min/well) during the 30- to 60-min time point. To evaluate uptake of ^{45}Ca by the Caco–2 cell monolayers at the end of an experiment, filters were rinsed three times with ice-cold Tx buffer, solubilised with 1.0 N NaOH and processed for scintillation counting. Uptake of Ca by cell monolayers was expressed as nmoles per well. In all studies, at least three wells were examined per treatment. Experiments were repeated three times.

Effect of dietary fibres, dephytinised fibres and sodium phytate on Ca transport

The effect of wheat and barley fibre extracts, dephytinised wheat and barley fibre extracts, and of sodium phytate on Ca transport was examined by adding these substances to the Ca Tx buffer and performing the transport studies as detailed above. Fibre extracts, dephytinised fibre extracts, cellulose and dephytinised cellulose were added to Ca Tx buffer at a level of 50 g/l. Tx buffer with no added fibre was used as a fibre-free control buffer. Sodium phytate was added at a concentration of 1 and 2 mmol per litre of cellulose-containing Tx buffer. The Ca content of the fibre preparations was taken into account in achieving a final Ca concentration of 500 μmol Ca/l of Tx buffer.

Experimental techniques

Ca content of fibre preparations Calcium in dietary fibre preparations was analysed in triplicate by atomic absorption spectrophotometry (Pye-Unicam Atomic Absorption Spectrophotometer, Model SP9, Cambridge, Cambs., UK) after dry ashing by the method of the Association of Official Analytical Chemists (AOAC) [25] and appropriate dilution with $LaCl_3$ (BDH Ltd., Poole, Dorset, UK). A range of Ca standards was used to obtain Ca calibration curves.

Phytate content of fibre preparations The phytate content of the fibre preparations was determined using the extraction and anion-exchange procedure previously described [26], followed by digestion and P determination by the method of the AOAC [25].

Statistical methods

^{45}Ca transport, ^{45}Ca uptake and TEER data were subjected to one-way ANOVA [27]. To follow-up the ANOVA, all pairs of means were compared by the method of least significant difference [27].

Results

Wheat fibre extract had a 3.2-fold higher phytate content (31.7 mg/g dry weight) than barley fibre extract (10.7 mg/g dry weight). Enzymatic dephytinisation of both fibre extracts reduced the phytate content to undetectable levels. The Ca content of the wheat and barley fibre extracts was 890 and 733 mg Ca/kg extract, respectively.

The effect of cereal fibre extracts, dephytinised cereal fibre extracts and phytate on transepithelial Ca transport across the Caco–2 monolayer and on Ca uptake into Caco–2 cells is shown in Table 1. The rate of transepithelial ^{45}Ca transport and the uptake of ^{45}Ca by Caco–2 cells

Table 1 Effect of fibres, dephytinised fibres, and phytate on transepithelial ^{45}Ca transport rates, ^{45}Ca uptake and transepithelial electrical resistance (TEER) in Caco–2 cells*. (Mean values and standard errors)

Transport Buffer	Phytate mmol/l	*n*	Ca transport rate+ (nmol/well/min) Mean	SEM	Ca uptake** (nmol/well) Mean	SEM	TEER** (Ω/cm^2) Mean	SEM
Control (Fibre-free)	0	36	0.66[a]	0.06	3.3[a]	0.6	205[a]	13
Cellulose (Control fibre)	0	24	0.68[a]	0.07	3.5[a]	0.5	205[a]	13
Barley	0.75	12	0.62[a]	0.07	3.8[a]	0.8	198[a]	14
Wheat	2.40	12	0.55[b]	0.03	2.5[b]	0.5	201[a]	13
Dephytinised Cellulose	ND	12	0.65[a]	0.05	3.4[a]	0.7	218[a]	14
Dephytinised Barley	ND	12	0.65[a]	0.07	3.3[a]	0.6	220[a]	15
Dephytinised Wheat	ND	12	0.67[a]	0.04	3.5[a]	0.6	207[a]	10
Cellulose + phytate	1.0	12	0.61[b]	0.05	2.7[b]	0.5	213[a]	12
Cellulose + phytate	2.0	12	0.57[b]	0.07	2.6[b]	0.4	213[a]	12

n is the total number of wells examined per treatment.
* For details of transport buffers and procedures, see Materials and methods section.
+ Transport rates were evaluated using the 30- to 60 min time points.
** Ca uptake and TEER were evaluated after 60 min.
[a,b,] Significant differences between groups are indicated by different superscripts within a column (P<0.05).

was unaffected by the inclusion of cellulose in the Ca transport (Tx) buffer, at a level of 50 g cellulose/l. Similarly, inclusion of barley fibre extract (50 g/l) had no effect on the rate of transepithelial ^{45}Ca transport or ^{45}Ca uptake relative to either the fibre-free Tx buffer or the cellulose containing (control fibre) Tx buffer.

Inclusion of wheat fibre extract in the transport buffer, at a level of 50 g/l, significantly ($P < 0.05$) reduced the rate of transepithelial ^{45}Ca transport by 17 and 19 % relative to the protein-free Tx buffer and the control fibre Tx buffer, respectively. Similarly, inclusion of wheat fibre extract significantly (P <0.05) reduced the uptake of ^{45}Ca by Caco–2 cells by 24 and 25 % relative to the fibre-free Tx buffer and the control fibre Tx buffer, respectively (Table 1).

The rate of transepithelial ^{45}Ca transport and the uptake of ^{45}Ca by Caco–2 cells were unaffected by the inclusion of dephytinised wheat or barley fibre extracts in the Ca Tx buffer, at a level of 50 g/l (Table 1).

Increasing the phytate concentration of the control fibre Tx buffer from 0 to 1 mM significantly (P <0.05) reduced the rate of transepithelial ^{45}Ca transport (by 10 %) and ^{45}Ca uptake by Caco–2 cells (by 23 %). However, when the phytate concentration was increased from 1 to 2 mM, no further decrease in ^{45}Ca transport or ^{45}Ca uptake was observed (Table 1).

Transepithelial electrical resistance (TEER) (an index of monolayer integrity) of Caco–2 cell monolayers was unaffected by the addition of cereal fibre extracts, dephytinised cereal fibre extracts, cellulose or phytate to the Tx buffer (Table 1).

Discussion

In the present study, the rate of transepithelial Ca transport across Caco–2 cell monolayers and the uptake of Ca into these Caco–2 cells was unaffected by cellulose or barley fibre extract. On the other hand, addition of wheat fibre extract to the Ca transport (Tx) buffer significantly reduced the rate of transepithelial Ca transport across Caco–2 cell monolayers (by 17 and 19 % respectively) and the uptake of Ca by Caco–2 cells (by 24 and 25 % respectively) relative to the fibre-free control and control fibre Tx buffers. This is the first report, to our knowledge, of the effect of dietary fibres on Ca absorption as determined in the human colon carcinoma cell line, Caco–2, model system.

The results of the present study are similar to those obtained in a study by Behall et al. on the effects on Ca balance of wheat bran compared to purified dietary fibres in human subjects [9]. They found that while apparent Ca absorption was reduced by a daily supplement of 30 g of wheat bran, it was unaffected by a daily supplement of 24 g of purified fibres, such as cellulose or carboxymethylcellulose. The authors suggested that a possible explanation for this was the high phytate content of the wheat bran compared to that of the purified fibres. There is considerable evidence that wheat bran is inhibitory to Ca absorption in humans and rats. As long ago as 1942, McCance and Widdowson reported that apparent Ca absorption in human subjects was lower from diets containing bread made from brown flour (92 % extraction rate) than from diets containing bread made from white flour (69 % extraction rate, and thus containing less fibre than the brown bread) [2]. These findings are supported by a number of other more recent studies. For example, human subjects have been reported to go into negative Ca balance when fed diets containing

50 % of calories from whole meal bread [8] or a daily supplement of 31 g wheat fibre [28]. Weaver et al. found that wheat bran (which is high in phytate), but not other wheat flour products, interferes with the absorption of co-ingested Ca in humans [11]. There is also evidence from animal studies, which agrees with the findings in humans. For example, Bagheri and Guéguen found that diets containing wheat bran at levels of 50, 100 and 150 g wheat bran per kg diet reduced Ca balance in weanling rats [29], while Donangelo and Eggum reported that inclusion of 80 g wheat fibre/kg diet significantly reduced Ca absorption in 5-week old rats [30].

The component(s) of wheat fibre responsible for the impairment of Ca absorption has not been identified with certainty, but fibre and/or phytate are most likely to be the major inhibitory factors. It has been suggested that it is difficult to clearly separate the effects of the two factors because their concentration in cereal products are correlated [31]. However, in the present study, where wheat and barley fibre extracts were dephytinised with wheat phytase, the phytate content of the fibre extracts was reduced to undetectable levels, and thus allowed for investigation of the effect on Ca absorption of the fibre components alone. Our results show that dephytinisation of the wheat fibre extract negated its inhibitory effects on the rate of transepithelial Ca transport and Ca uptake by Caco–2 cells, strongly suggestive of the fact that phytate and not the fibre components *per se* was responsible for the impaired Ca bioavailability. There is some evidence in the literature to support this hypothesis. McCance and Widdowson, for example, found that reduction of the phytate content of brown bread resulted in increased apparent Ca absorption in human subjects [32]. Morris and Ellis found that apparent Ca absorption in human volunteers was significantly greater from dephytinized bran muffins than from whole-wheat bran muffins [33].

Further support for the contention that phytate, rather than the fibre components *per se*, is the principal inhibitory component of cereal fibres is provided by our findings which showed that transepithelial Ca transport and Ca uptake by the Caco–2 cells was significantly reduced by phytate, as sodium phytate, when added at levels present in the two cereal fibre extracts. Increasing the phytate concentration of the cellulose transport buffer from 0 to 2 mmol/l (a level of phytate close to that in the wheat fibre containing buffer) reduced the rate of transepithelial Ca transport and Ca uptake by 16 and 26 %, respectively.

These findings *in vitro* agree with those from human studies that demonstrated an inhibition of Ca absorption by phytate. For example, McCance and Widdowson found that addition of sodium phytate to white bread had a marked inhibitory effect on Ca balance in human volunteers to the extent that faecal excretion of Ca was higher than Ca intake for many of the human subjects [2]. Morris and Ellis reported a dose dependent reduction of Ca balance by phytate in human subjects [3]. Addition of 0.5, 1.7 or 2.9 g of phytate to whole wheat muffins resulted in reduced Ca balances of 153, 94 or 23 mg/day, respectively. Heaney *et al.* compared the absorption of ^{45}Ca from soybeans with low (108 mg phytate/serving) and high (352 mg phytate/serving) phytate contents on Ca absorption from milk in women [4]. Fractional Ca absorption was 31 % from the high-phytate soybeans, 41 % from the low-phytate soybeans and 38 % from the milk.

The mechanism by which phytate reduces Ca absorption is believed to be through the formation of unabsorbable phytate-Ca complexes in the small intestine, which is the principle site of Ca absorption [34,35]. Lönnerdal et al. showed that the inhibitory effect of the inositol phosphates on Ca absorption in suckling rats was confined to the penta- and hexaphosphates (phytate) and that the tri- and tetra phosphates have no effect [6].

In conclusion, the results from the present study suggest that it is the phytate in wheat fibre which is the major inhibitory factor of Ca absorption and that wheat fibre *per se* has little if any inhibitory effect on Ca absorption. In addition, the results of this study support the usefulness of Caco–2 cells for investigating the effects of dietary factors on the cellular uptake and transepithelial transport of Ca in the intestine.

References

1 Cummings JH (1993) Dietary fibre intakes in Europe: an overview. In: Cummings J, Frolich W (eds) Food Technology, Dietary Intake in Europe survey of the Cost 92 Concerted Action on e–A "Metabolic and Physiological Aspects of Dietary Fibre in Food" Commission of the European Communities, DG XII, B–1049 Brussels, Rue de la Loi, 200, pp 11–19

2 McCance RA, Widdowson EM (1942) Mineral metabolism of healthy adults on white and brown dietaries. J Physiol 10: 44–85

3 Morris ER, Ellis R (1985) Trace element nutriture of adult men consuming three levels of phytate. In: Hills CF, Bremner I, Chester JK (eds) Trace Elements in Man and Animals, 5. Commonwealth Agricultural Bureaux, Slough, England, pp 443–452

4 Heaney RP, Weaver CM, Fitzsimmons MC (1991) Soybean phytate content: effect on calcium absorption. Am J Clin Nutr 53: 745–747

5 Taylor TG, Coleman JW (1979) A comparitive study of the absorption of calcium and the availability of phytate-phosphorus in the golden hamster (Mesorcricetus auratus) and the laboratory rat. Br J Nutr 42: 113–119

6 Lönnerdal B, Sandberg AS, Sandström B, Kunz C (1989) Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. J Nutr 119: 211–214

7 Kunz C, Lönnerdal B (1990) Calcium retention from human milk infant formula and preterm formula. FASEB J 4: A521

8 Reinhold JG, Faradji B, Abadi P, Ismail-Beigi F (1976) Decreased absorption of calcium, magnesium, zinc and phosphorus by humans due to increased fibre and phorphorus consumption as wheat bread. J Nutr 106: 493–503
9 Behall KM, Scholfield DJ, Lee K, Powell AS, Moser PB (1987) Mineral balance in adult men: effect of four refined fibres. Am J Clin Nutr 46: 307- 314
10 Turnlund JR (1987) Carbohydrate, fiber and mineral interactions. In: Levander OA (ed) Nutrition '87, American Institute of Nutrition, Bethesda, MD, pp 37–40
11 Weaver CM, Heaney RP, Martin BR, Fitzsimmons ML (1991) Human calcium absorption from whole-wheat products. J Nutr 121:1769–1775
12 Weaver CM, Heaney RP, Teegarden D, Hinders SM (1996) Wheat bran abolishes the inverse relationship between calcium load size and absorption fraction in women. J Nutr 126:303–307
13 Sandberg AS, Hasselblad C, Hasselblad K, Hultén L (1982) The effect of wheat bran on the absorption of minerals in the small intestine. Br J Nutr 48: 185–191
14 Van Dokkum W, Wesstra A, Schippers FA (1982) Physiological effect of fibre-rich types of bread, I. The effect of dietary fibre from bread on the mineral balance of young men. Br J Nutr 47: 451–460
15 Andersson H, Nävert B, Bingham S A, Englyst HN, Cummings JH (1983) The effects of breads containing similar amounts of phytate but different amounts of wheat bran on calcium, zinc and iron balance in man. Br J Nutr 50: 503–510
16 O'Brien KO, Allen LH, Quatromoni P, Sui Caldera ML, Vieira NE, Perez A, Holick MF, Yergey AL (1993) High fiber diets slow bone turnover in young men but have no effect on efficiency of intestinal calcium absorption. J Nutr 123: 2122–2128
17 Hansen M, Sanström B, Lönnerdal B (1996) The effect of casein phosphopeptides on zinc and calcium absorption from high phytate infant diets assessed in rat pups and Caco–2 cells. Pediatr Res 40: 547–552
18 Iqbal TH, Lewis Ko, Copper BT (1994) Phytase activity in the human and rat small intestine. Gut 35: 1233–1236
19 Guilliano AR, Wood RJ (1991) Vitamin D-regulated calcium transport in Caco–2 cells: unique in vitro model. Am J Physiol 260: G207-G212
20 Fleet JC, Bradley J, Reddy GS, Ray R, Wood RJ (1996) 1α,25-$(OH)_2$-vitamin D_3 analogs with minimal in vivo calcemic activity can stimulate significant transepithelial calcium transport and mRNA expression in vitro. Arch Biochem Biophys 329: 228–234
21 Blais, A, Aymard P, Lacour B (1997) Paracellular calcium transport across Caco–2 and HT29 cell monolayers. Pflügers Arch 434: 300–305
22 Bronner F (1987) Intestinal calcium absorption: mechanisms and applications. J Nutr 117: 1347–1352
23 Rindler MJ, Traber MG (1988) A specific sorting signal is not required for the polarised secretion of newly synthesised proteins from cultured intestinal cells. J Cell Biol 107: 471–479
24 Fleet JC, Wood RJ (1994) Identification of calbindin D–9k mRNA and its regulation by 1,25-dihydroxyvitamin D_3 in Caco–2 cells. Arch Biochem Biophys 308: 171–174
25 Association of Official Analytical Chemists (AOAC) (1984) Official Methods of Analysis of the AOAC [Williams S ed] The Association of Official Analytical Chemists Inc., Virginia
26 Harland B, Oberleas D (1986) Anion-exchange method for determination of phytate in foods: collaborative Study. J AOAC 69: 667–670
27 Snedecor GW, Cochran WG (1967) Statistical Methods Iowa State University Press, Ames Iowa
28 Cummings JH, Hill MJ, Jivraj T, Houston H, Branch WJ, Jenkins DJA (1979) The effect of meat protein and dietary fibre on colonic function and metabolism I. Changes in bowel habit bile acid excretion and calcium absorption. Am J Clin Nutr 32: 2086–2093
29 Bagheri, Guéguen L (1981) Influence of wheat bran diets containing unequal amounts of calcium magnesium phosphorus and zinc upon the absorption of these minerals in rats. Nutr Reports Int 32: 271–274
30 Donangelo CM, Eggum BO (1986) Comparitve effects of wheat bran and barley husk on nutrient utilization in rats II. zinc, calcium and phosphorus Br J Nutr 56: 269–280
31 Miller DD (1989) Calcium in the diet: Food sources recommened intakes and nutritional bioavailability. Adv Food Nutr Res 33: 103–156
32 McCance RA, Widdowson EM (1942) Mineral metabolism on dephytinised bread. J Physiol 101: 304–306
33 Morris ER, Ellis R (1985) Bioavailability of dietary calcium: effect of phytate on adult men consuming nonvegetarian diets. In: Kies e.g. C (ed) Nutritional Bioavailability of Calcium Symposium Series, 275, American Chemical Society, Washington DC, pp 63–72
34 Erdman Jr JW (1979) Oilseed phytates: nutritional implictions. J Am Oil Chem Soc 56: 736–740
35 Forbes RM, Weingartner KE, Parker HM, Bell RR, Erdman JW (1979) Bioavailability to rats of zinc magnesium and calcium in casein- egg- and soy protein-containing diets. J Nutr 109: 1652–1660